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The origins of thalamic inputs to grasp zones in frontal cortex of macaque monkeys

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Abstract

The hand representation in primary motor cortex (M1) is instrumental to manual dexterity in primates. In Old World monkeys, rostral and caudal aspects of the hand representation are located in the precentral gyrus and the anterior bank of the central sulcus, respectively. We previously reported the organization of the cortico-cortical connections of the grasp zone in rostral M1. Here we describe the organization of thalamocortical connections that were labeled from the same tracer injections. Thalamocortical connections of a grasp zone in ventral premotor cortex (PMv) and the M1 orofacial representation are included for direct comparison. The M1 grasp zone was primarily connected with ventral lateral divisions of motor thalamus. The largest proportion of inputs originated in the posterior division (VLp) followed by the medial and the anterior divisions. Thalamic inputs to the M1 grasp zone originated in more lateral aspects of VLp as compared to the origins of thalamic inputs to the M1 orofacial representation. Inputs to M1 from thalamic divisions connected with cerebellum constituted three fold the density of inputs from divisions connected with basal ganglia, whereas the ratio of inputs was more balanced for the grasp zone in PMv. Privileged access of the cerebellothalamic pathway to the grasp zone in rostral M1 is consistent with the connection patterns previously reported for the precentral gyrus. Thus, cerebellar nuclei are likely more involved than basal ganglia nuclei with the contributions of rostral M1 to manual dexterity.

Keywords

Intracortical electrical stimulation; Motor cortex; Ventral premotor cortex; Thalamocortical; Cerebellum; Basal ganglia

Introduction

Regions of the hand are represented multiple times in motor and premotor areas of the frontal cortex of non-human primates. In macaque monkeys, the hand representation in

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primary motor cortex (M1) spans the anterior bank of the central sulcus and the precentral gyrus. Corticospinal neurons with direct projections onto motoneurons of the spinal cord represent an evolutionary modification that are a distinguishing feature of caudal aspects of M1 (Rathelot and Strick 2009). In contrast, corticospinal projections from rostral aspects of M1 terminate primarily onto interneurons of the spinal cord, which in turn project onto motoneurons. This indirect connection pattern characterizes the predominant organization of corticospinal projections in a wide range of mammals, including non-human primates (Bortoff and Strick 1993; Nakajima et al. 2000; Nudo and Masterton 1988). Although the concentration of cortico-motoneuronal cells confers on caudal M1 an instrumental capacity for skilled movements, aspects of the hand representation in rostral M1 are also central to hand control. Indeed, neuro-physiological studies have shown that portions of the hand representation in rostral M1 are involved in grasping (McKiernan et al. 1998; Umiltà et al. 2007). In addition, the hand representation in rostral M1 has anatomical connections with frontal and parietal cortex (Dum and Strick 2005; Gharbawie et al. 2011b; Strick and Kim 1978) that support manual dexterity.

Accessibility of the grasp zone in rostral M1 makes it an attractive point of investigation for understanding how M1 controls manual dexterity. Inferring the anatomical infrastructure of this grasp zone from existing studies of the M1 hand representation is not feasible because the grasp zone, defined here as a cortical territory involved in executing finger movements for securing objects, occupies only a limited portion of the M1 hand representation (reviewed in Schieber 2001). Thus, we have refined an approach for investigating the anatomical organization of cortical areas involved in grasping. We use electrical stimulation to map the locations of grasp zones in the neocortex and then reveal their connections with tracer injections. This approach was instructive for our investigations of the cortico-cortical connections that support grasping in New and Old World monkeys (Gharbawie et al. 2011a, b). Here we chart the distribution of inputs from motor thalamus divisions to the grasp zone in rostral M1 in an effort to determine its accessibility to cerebellum and basal ganglia. Thalamocortical connections of the grasp zone in ventral premotor cortex (PMv) and the orofacial representation in M1 are also presented for direct comparison to the spatial organization of the connections of the M1 grasp zone.

Materials and methods

Animals

Five male macaque monkeys (3 *Macaca fascicularis*, 1 *Macaca mulatta*, 1 *Macaca radiata*) were used in the present study. Animals were 3–9 years old and weighed 3.5–10.5 kg. All procedures were approved by Vanderbilt University Animal Care and Use Committee and followed the National Institutes of Health guidelines for the care and use of laboratory animals.

Intracortical electrical stimulation

We used intracortical electrical stimulation to map the locations of grasp zones in primary motor (M1) and ventral premotor (PMv) cortex. We favored long trains (500 ms) of electrical stimulation over short trains of stimulation (60 ms) to evoke sequences of

movements and improve the likelihood of identifying grasping movements. We were particularly interested in sites that evoked flexion of the digits because of the direct relevance of those movements to grasping. Nevertheless, most microelectrode penetration sites in frontal cortex were also explored with short trains of stimulation.

Each monkey was anesthetized with ketamine hydrochloride (10–30 mg/kg, i.m.). The head was secured in a stereotaxic frame and a water blanket was positioned underneath the body for heating. Anesthesia during surgical procedures was maintained with 2 % isoflurane. A rectangular craniotomy was performed under aseptic conditions to expose areas within frontal and parietal cortex of the same hemisphere. The rostro-caudal extent of the opening included most of the intraparietal sulcus caudally and the arcuate sulcus rostrally. The medio-lateral extent of the opening included the M1 trunk representation medially and the face representation laterally. The dura was dissected and saline was consistently applied to the exposed cortex. Regions of interest were digitally photographed and a printout was used to record microelectrode penetrations. Anesthesia was switched at this point to ketamine hydrochloride mixed in physiological saline (20–40 mg/kg/h, i.v.) and continuously infused through the short saphenous vein. Small doses of xylazine (0.2–0.4 mg/kg, i.m.) were occasionally administered to control excessive muscle tone. Vital signs (heart rate, respiratory rate, expired CO₂ levels, and oxygen saturation in blood) were monitored and recorded every 10 min for the duration of anesthesia.

A tungsten microelectrode (1 M Ω impedance at 1 kHz) was lowered perpendicularly with a micromanipulator into the cortex to depths 1600–2000 μ m below the pia. Penetrations extended as deep as 3000 μ m near sulci. Inter-penetration distances were 0.5–1.0 mm, varying primarily to avoid vascular branches on the cortex. Interpenetration distances were smaller in and near the grasp zones. Long trains of electrical stimulation consisted of 150 biphasic pulses delivered over 500 ms. The duration of each phase was 0.2 ms at 300 Hz. Current intensity was increased from low levels (10 μ A) until a movement was reliably evoked (up to a maximum of 200 μ A). Ketamine was withdrawn once motor mapping was complete and anesthesia was maintained with 2 % isoflurane.

Anatomical tracer injections

Retrograde tracers were injected into frontal cortex in 5 hemispheres of 5 monkeys. A total of 8 injections met two criteria for inclusion in the present study. (1) Injection core was confined to the target zone and there was no evidence of white matter contamination. (2) Injection labeled a sufficient number of cells in thalamus (Table 2). Tracers were pressure injected with a 1 or 2 μ L Hamilton syringe fitted with a glass pipette beveled to a sharp tip. Tracers included Cholera toxin b-subunit (CTB; Molecular Probes, Carlsbad, CA; 1 % in distilled water), and the fluorescent tracers Diamidino Yellow (DY; Sigma–Aldrich, St Louis, MO, 2 % in distilled water), Fluoro Ruby (FR; Molecular Probes, 10 % in distilled water), and Fast Blue (FB; Polysciences, Warrington, PA; 2 % in distilled water). Two depths beneath the surface of the cortex (1200 and 800 μ m) were targeted at each injection site. The total volume for each injection site was 0.4 μ L for DY, FB, and CTB; and 1.0 μ L for FR.

After tracer injections, a layer of gelfilm was placed over the exposed cortex for protection. A skullcap prepared from dental acrylic was secured over the craniotomy. The scalp was sutured and covered with topical antibiotic. Monkeys were returned to the home cage and closely monitored until they completely recovered from anesthesia. A regimen of antibiotic injections every 24 h (Naxcel, 2.2 mg/kg, i.m.) and pain medication injections every 12 h (Buprenex 0.1 mg/kg, i.m.) was maintained for 72 h after surgery. Diet was adjusted in the initial days after surgery to include higher proportions of fruits and soft food to encourage eating. Approximately 10–12 days were allowed for tracer transport before the start of a terminal procedure that was conducted primarily for part of the project aimed at relating cortical connections to neuro-physiological maps (Gharbawie et al. 2011b).

Histology

A lethal dose of sodium pentobarbital (120 mg/kg, i.v.) was administered at the end of the terminal mapping procedure. Intracardial perfusion started with phosphate-buffered saline (PBS; pH 7.4), followed by 2 % paraformaldehyde in PBS, and then 2 % paraformaldehyde and 10 % sucrose in PBS. The brain was removed from the skull. Thalamus and brainstem were separated from neocortex and submerged for 36–48 h in 2 % paraformaldehyde and 30 % sucrose in PBS for additional fixation and cryoprotection. The thalamus was cut in the coronal plane at 40 μ m and saved in five series of adjacent sections. One series was not processed and was mounted onto glass slides for analysis of the distributions of fluorochrome-labeled cells. A second series was reacted for CTB immunohistochemistry and labeled cells were visualized with a nickel-intensified diaminobenzidine dihydrochloride (DAB) reaction (Bruce and Grofova 1992). Three non-successive series were stained for acetylcholinesterase (AChE), cytochrome oxidase (CO), and Nissl substance to identify architectonic borders of thalamus.

Data analysis

Distributions of labeled cells in the thalamus were plotted with a Leitz microscope (Leica Microsystems, Wetzlar, Germany) connected to an X–Y encoder. An experimenter manually marked the positions of labeled cells on a computer system running Neurolucida software (V. 5.05.4, MicroBrightField Inc., Chicago, IL). Cell plotting was primarily conducted using a 25 $\times$ magnification objective, but a 10 $\times$ magnification objective was also used. CTB-labeled cells were visualized under bright field illumination. FR-labeled cells were visualized with fluorescence illumination passed through a 530–560 nm wavelength filter, whereas a 360 nm wavelength filter was used for cells labeled with DY or FB.

Architectonic borders of thalamus were traced using a dissection microscope outfitted with a camera Lucida. Tracings were scanned and redrawn in Adobe Illustrator (CS4). Cell plotting files generated in Neurolucida were reformatted for compatibility with Adobe Illustrator. Tracings and plots from adjacent sections were imported into separate layers of an Adobe Illustrator file and then aligned according to blood vessels, fiber tracts, and tissue artifacts. Symbols marking the locations of labeled cells were adjusted for shape and color.

Results

Grasping movements

Long train electrical stimulation evoked several classes of movements that were previously described in multiple species of monkeys (Gharbawie et al. 2011a; Graziano et al. 2002; Stepniewska et al. 2005). Here we focus on movements that are involved in grasping, which were narrowly defined as flexion of any or all digits. The latter was often accompanied with wrist extension or forearm supination. Comparable movements have been illustrated for macaque monkeys (Graziano et al. 2002).

Organization of M1/PMv grasp zones

The organization of frontal cortex was explored to varying extents with microelectrodes in five macaque monkeys (Fig. 1). Microelectrode penetrations in M1 and PMv primarily targeted the dorsal convexity of the cortex. Long train electrical stimulation revealed M1 maps that included a medial trunk zone, a more lateral zone of proximal forelimb movements, an even more lateral zone of forearm supination and hand movements, and orofacial movements in the most lateral zone. Electrical stimulation sites that evoked any movements of the digits and/or wrist were included in the hand representation (Fig. 1). Sites that evoked grasping movements clustered into 1 or 2 zones within the precentral gyrus (M1) and a single zone within PMv. Grasp zones varied in size across cortical areas and cases, but consistently occupied less than 50 % of the pertinent hand representation.

A large portion of the M1 grasp zone was near the central sulcus. However, some electrical stimulation sites that evoked grasping were identified as far rostral as the border separating M1 from premotor cortex. Although the anterior bank of the central sulcus was not mapped, physiological and anatomical evidence suggests the existence of a contiguous representation of the digits in the same medio-lateral extent (Qi et al. 2000, 2010; Rathelot and Strick 2009). In three cases, electrical stimulation extended sufficiently rostral and lateral from the M1 grasp zone where a separate grasp zone was identified in PMv (Fig. 1b, c, e). Although the caudal bank of inferior arcuate sulcus was not mapped, anatomical and physiological evidence (Gentilucci et al. 1988; Gerbella et al. 2011) suggests the existence of a grasp zone that is even larger than the one we identified on the dorsal surface of PMv.

Borders separating M1, PMd, and PMv, were estimated from current thresholds of electrical stimulation, architectonics, and cortical landmarks. Current thresholds for evoking movements were lowest near the central sulcus. More rostral sites required incrementally higher currents. Thresholds were typically $>60 \mu\text{A}$ in premotor cortex. Sites that evoked trunk movements from ventral aspects of the superior arcuate sulcus were on the same medio-lateral level as the M1 grasp zone and confirmed the territory of PMd. The center of the grasp zone in PMv was approximately 3–5 mm lateral from the junction of the superior arcuate sulcus and inferior arcuate sulcus.

Thalamic nuclei borders

Thalamic nuclei relevant to the present study are shown in sections (Fig. 2) selected from case 10–29. It is instructive to describe in brief the present nomenclature for ventral lateral

(VL) divisions of motor thalamus (Table 1), which follows (Macchi and Jones 1997; Stepniewska et al. 1994a), and involves some subtle differences with the terms used in two common resources (Olszewski 1952; Jones 1985). Five main divisions of VL were readily identified here with AChE staining. (1) The most anterior is VL_a, which has been referred to as VL_o (Olszewski 1952) or VL_a (Jones 1985). (2) The largest division of VL is VL_p, which has been referred to as VL_o (Olszewski 1952) or part of VL_p (Jones 1985). We purposely used the term VL_p to reflect its classification as part of motor thalamus and to limit the possibility of including it with ventral posterior (VP) nucleus, which is part of the somatosensory thalamus. (3) The VL_x division is medial to VL_p and has been referred to as area X (Olszewski 1952) or recognized as a medial part of VL_p (Jones 1985). (4) The most dorsal division is VL_d, which has been referred to as VL_c (Olszewski 1952) or recognized as a dorsal part of VL_p (Jones 1985). (5) The most medial and ventral division (VL_m) has been referred to as VL_m (Olszewski 1952), or recognized as a separate nucleus from VL and referred to as VM (Jones 1985).

The motor thalamus also includes the ventral anterior nucleus (VA), most of which is rostral to VL. Large cells characterize the magnocellular division (VAmc) of VA, whereas smaller cells characterize the parvocellular (VApc) division. This terminology is consistent with both Olszewski (1952) and Jones (1985). There is general agreement that VL_p, VL_x, and VL_d are primarily connected with output nuclei of cerebellum, whereas VA divisions, VL_a, and VL_m are connected with output nuclei of the basal ganglia (reviewed in Sakai et al. 1996).

Nomenclature for parts of somatosensory thalamus are widely accepted (Kaas 2008). The lateral division of VP, which is the target of somatosensory inputs from below the face (via the cuneate and gracilis nuclei), is referred to here as VPL, but has been referred to as VPL_c (Olszewski 1952; Jones 1985). A medial division of VP, which receives most of the somatosensory input from the face, is referred to here as VPM. A separate division of the VP complex is VPM_{pc} (thalamic gustatory area). The anterior pulvinar (PA) is an integral component of the somatosensory system and has also been referred to as the oral division of pulvinar (PO) (Olszewski 1952; Jones 1985).

Anatomical tracers

Four different anatomical tracers were used in this study to facilitate the possibility of direct comparison of the anatomical connections of multiple cortical zones within the same case. In addition, an effort was made to rotate tracers among target zones and across cases to minimize the possibility of inadvertently introducing biases from properties of a given tracer. For example, the connections of the M1 grasp zone were investigated in five cases in the present study using four different tracers. Nevertheless, in considering all of the results in the present study, CTB was overrepresented as it was used in four of the eight tracer injections. The reliability of CTB for retrograde tracing of connections is the primary reason for its inclusion in multiple cases here. The connection patterns revealed with the two CTB injections in PM_v were similar to the patterns of thalamic inputs expected for PM_v, but the relatively large numbers of cells that were labeled (Table 2) were likely related to tracer efficacy. Conversion of the number of labeled cells into percentages as a means of

expressing the density of thalamic inputs, should have accounted for variations between cases that may have been due to tracer properties or injection size.

M1 grasp zone connections

In each monkey case, at least one grasp zone was identified in M1 of the precentral gyrus with electrical stimulation. A single injection of a tracer was delivered in part of the M1 grasp zone in each case. Variations in the relative location of the tracer injection within the target zone allowed us to comprehensively reveal the connections of the grasp zone. Sizes and locations of injection cores are presented in Fig. 1. Corresponding distributions of cortical connections were previously reported for four of the present five injections (Gharbawie et al. 2011b). Those results support the likelihood that injections were confined to the grasp zones in M1, which we identified with electrical stimulation. Distributions of cells labeled in thalamus from all five injections are presented in Table 2. The results indicate that thalamic inputs to the M1 grasp zone originated primarily in nuclei of motor thalamus. Moreover, the density of inputs from VL divisions associated with the cerebellothalamic pathway amounted to approximately three fold the density of inputs from thalamic divisions associated with the pallidothalamic pathway.

A CTB injection in lateral aspects of the M1 grasp zone (case 09–51, Fig. 1a) labeled more thalamic cells than any of the other M1 injections. Labeled cells were primarily in VL (72 %) and more than one third of those cells were in VLp (Fig. 3). Smaller proportions of labeled cells were rostral to VLp in VLa and medial to VLp in VLx. Even smaller proportions of labeled cells were ventral to VLp in VLm and dorsal to VLp in VLd. Beyond VL borders, most of the labeled cells were in VA or were more centrally located in MD, CL, and CM. The small concentrations of labeled cells rostral and caudal to VL were in VAp and PA, respectively.

A DY injection in lateral aspects of the M1 grasp zone (case 10–29; Fig. 1b) primarily labeled cells in VL (69 %). More than half of those cells were in VLp (Fig. 4). Smaller proportions of labeled cells were medial to VLp in VLx and rostral to VLp in VLa. Even smaller concentrations of labeled cells were ventral to VLp in VLm and dorsal to VLp in VLd. Beyond VL borders, most labeled cells were concentrated rostrally in VA. Smaller concentrations of cells were more centrally located in MD and CL.

A FR injection near the center of the M1 grasp zone (case 10–50, Fig. 1c) was relatively caudal to previous injections. The halo of tracer spread from the injection core encroached slightly into the adjacent forearm supination zone. Nevertheless, the injection primarily labeled cells in VL (73 %). Nearly half of those cells were in VLp (Fig. 5). Smaller proportions of labeled cells were medial to VLp in VLx and dorsal to VLp in VLd. Much smaller proportions of labeled cells were rostral to VLp in VLa. Beyond VL borders, most of the labeled cells were more centrally located in MD, CL, and CM. The small concentrations of labeled cells caudal to VL were in LP, PA, and PL.

A CTB injection in lateral aspects of the M1 grasp zone (case 10–14, Fig. 1d) was at the approximate rostro-caudal extent as the injection in case 10–50 (Fig. 1c). The CTB injection primarily labeled cells in VL (90 %) and more than one third of those cells were in VLp

(Fig. 6). The remainder of the labeled cells was primarily medial to VLp in VLx and rostral to VLp in VLd. Only small proportions of the cells labeled in VL were dorsal to VLp in VLd and ventral to VLp in VLm. Beyond VL borders, labeled cells were located more centrally in CL. Only a few labeled cells were caudal to VL and they were located in PA.

A FB injection in rostral aspects of the M1 grasp zone (case 09–20; Fig. 1d) primarily labeled cells in VL (92 %). Three quarters of those cells were in VLp (Fig. 7), which is denser than VLp inputs for other cases here and may be related to the relatively rostral location of the present injection. Nevertheless, a similar ratio of inputs from VLp has been reported from a tracer injection into the approximate location of the hand representation in M1 of the precentral gyrus (Holsapple et al. 1991). Much smaller proportions of labeled cells were rostral to VLp in VLd and dorsal to VLp in VLx. Even smaller proportions of labeled cells were medial to VLp in VLx and ventral to VLp in VLm. The small proportions of cells beyond VL borders were rostral to VLp in VAp and were also distributed among the intralaminar nuclei.

PMv grasp zone connections

Motor mapping extended sufficiently rostral and lateral from the M1 grasp zone in three cases. This facilitated identification of a grasp zone on the dorsal convexity of PMv, but the caudal bank of the inferior arcuate sulcus was not explored. Tracers were injected into the PMv grasp zone in two cases. Sizes and locations of injection cores are presented in Fig. 1. Corresponding distributions of cortical connections were previously reported for both injections (Gharbawie et al. 2011b). Those results support the likelihood that injections were largely confined to the grasp zones in PMv, but that there may have been slight encroachment into the PMv face representation in one case. Distributions of cells labeled in thalamus from both injections are presented in Table 2. Much like the M1 grasp zone, the results indicate that inputs to the PMv grasp zone originated primarily in nuclei of motor thalamus.

A CTB injection into the center of the PMv grasp zone (case 10–29, Fig. 1b) primarily labeled cells in VL (65 %). Approximately two thirds of those cells were equally distributed between VLd and VLm (Fig. 4). The remainder of the labeled cells was distributed between VLp and VLx. A concentration of labeled cells was rostral to VL in VA. A relatively large concentration of labeled cells was located more centrally in MD. Smaller concentrations of labeled cells were in CL, CM, and PA.

A large CTB injection filled the PMv grasp zone (case 10–50, Fig. 1c). The small size of this grasp zone and the pattern of cortical connections (Gharbawie et al. 2011b) suggest that the present injection may have included part of the orofacial representation in PMv. In addition, the possibility that the injection may have encroached into the crest of the inferior arcuate sulcus cannot be ruled out. The present injection primarily labeled cells in VL (71 %). More than half of those cells were in VLx (Fig. 5). The next densest concentration of labeled cells was rostral to VLx in VLd. The small remainder of labeled cells was lateral to VLx in VLp and ventral to VLx in VLm. Beyond VL borders, labeled cells were concentrated centrally in CM, CL, and MD. A separate concentration of labeled cells was rostral to VL in VAp. Only a small concentration of cells was caudal to VL in PA.

M1 orofacial zone connections

An orofacial zone was identified with electrical stimulation on the M1 dorsal convexity in all cases (Fig. 1). The M1 orofacial zone abutted lateral aspects of the M1 grasp zone. A FR injection in dorsal and caudal aspects of the M1 orofacial zone (case 10–29, Fig. 1b) was included here only to contrast the organization of the M1 grasp zone connections. The size and the location of the injection core is shown in Fig. 1b. Corresponding distributions of cortical connections were previously reported (Gharbawie et al. 2011b). Those results support the likelihood that injections were confined to the M1 orofacial zone, which we identified with electrical stimulation.

Distributions of cells labeled in thalamus are presented in Table 2. Labeled cells were primarily in VL (83 %). Approximately half of those cells were in VLx (Fig. 8). A smaller proportion of the cells labeled in VL was rostral and lateral to VLx in VLa. Only small proportions of labeled cells were ventral to VLx in VLm and lateral to VLx in VLp. Beyond VL borders, small concentrations of labeled cells were in VA and in the face nucleus of the ventral posterior thalamus (VPM). Small concentrations of labeled cells were also located centrally in MD, CL, and PC.

Summary of connections

A retrograde tracer was injected into the M1 grasp zone of the precentral gyrus in five hemispheres. Despite variations across cases in the location injected within the grasp zone and the distribution of cells labeled within the thalamus, some general patterns emerged from calculating the median density of thalamic inputs. VL contributed the largest proportion of inputs (73.17 ± 4.78 % of total) to the M1 grasp zone. Nearly half of those connections originated in VLp (35.44 ± 7.32 % of total). VLx provided the next largest contribution (15.66 ± 3.66 % of total) followed by VLa (9.69 ± 3.52 % of total). The density of the connections from VLm, VLd, and VA was small and variable. Thus, the M1 grasp zone of the precentral gyrus received most of its thalamic inputs (63.29 ± 5.18 % of total) from nuclei that are primarily connected with cerebellum and comparatively weaker thalamocortical inputs (26.58 ± 5.07 % of total) from nuclei that are primarily connected with basal ganglia (Fig. 9a, b). Beyond the motor thalamus borders, most thalamocortical inputs originated from central locations that included CL (5.70 ± 1.26 % of total), MD (3.35 ± 1.00 % of total) and CM (4.51 ± 1.25 % of total). Inputs from posterior thalamus were limited to weak connections with PA (2.93 ± 1.25 % of total).

A retrograde tracer was injected into the grasp zone of the PMv dorsal convexity in two hemispheres. Inputs to the PMv grasp zone originated primarily from more medial aspects of motor thalamus relative to the origins of inputs destined to the M1 grasp zone. Nevertheless, some overlap between the origins of the thalamic inputs of both grasp zones was observed at the border of VLp with VLx. General patterns emerged from calculating the mean density of the connections contributed from each division of motor thalamus despite variations across cases. VL contributed the largest proportion of inputs (68.02 ± 2.91 % of total). More than one third of those connections originated in VLx (24.54 ± 13.62 % of total). VLa provided the next largest contribution (18.10 ± 1.75 % of total). Smaller contributions were noted from VLm (13.84 ± 6.62 % of total) and from VLp (11.12 ± 2.48

of total). Inputs consistently originated from VA (9.13 ± 0.61 % of total) in both cases. Thus, the grasp zone on the PMv dorsal convexity receives comparable thalamocortical inputs from nuclei that are primarily connected with cerebellum (36.08 ± 11.29 % of total, Fig. 9a) and from nuclei that are primarily connected with basal ganglia (41.07 ± 7.76 % of total). Beyond the motor thalamus borders, most thalamocortical inputs originated from central locations that included MD (8.65 ± 5.50 % of total), CM (6.58 ± 4.93 % of total) and CL (2.59 ± 0.37 % of total). Inputs from posterior thalamus were limited to weak connections with PA (3.25 ± 2.04 % of total).

A retrograde tracer was injected into the M1 orofacial zone of a single hemisphere. Results from this case are included here only to contrast the spatial origins of the thalamocortical inputs of the M1 grasp zone. However, the origins of thalamic inputs of the M1 orofacial zone were generally medial to the origins of thalamic inputs destined to the M1 grasp zone.

Discussion

The present study is part of a larger project designed to determine the organization of the neural circuits that support grasping in non-human primates. Our strategy involves mapping the locations of grasp zones in the neocortex and investigating their anatomical connections. Injecting multiple retrograde tracers into individual hemispheres was the most productive approach for studying the cortico-cortical connections of multiple grasp zones (Gharbawie et al. 2011a, b). Here we report on the organization of thalamocortical connections that were labeled from tracer injections into the M1 and PMv grasp zones, which are arguably the main frontal nodes of the cortical network that mediates grasping. Our goal is to complete the profile of connections for those grasp zones. Divisions of motor thalamus were the main sources of input, but differential profiles characterized the M1 and PMv zones. The utility of studying the density of inputs from motor thalamus is the possibility of inferring the accessibility of both cortical zones to the cerebellothalamic and pallidothalamic pathways.

M1 grasp zone location

Long train electrical stimulation (500 ms) evoked grasping from a zone on the precentral gyrus, which is consistent with the previously defined location of a zone in M1 that is involved in object manipulation (Graziano et al. 2002). Short train microstimulation parameters (60 ms) in our study and others evoked twitches of the digits from the same location (Schieber 2001 for review). In addition, single unit recordings from similar locations showed strong correlations between neuronal activity and grasping (McKiernan et al. 1998; Umiltà et al. 2007). Thus, converging evidence indicates that our M1 grasp zone overlaps part of the hand representation that is involved in grasping. Although the anterior bank of the central sulcus was not systematically explored here, anatomical (Dum and Strick 1991; Rathelot and Strick 2009) and neurophysiological (Park et al. 2001, 2004; Qi et al. 2000, 2010; Schieber 2001) approaches reveal a substantial representation of the digits. Thus, long train electrical stimulation within this approximate territory of the anterior bank of the central sulcus could be expected to evoke grasping movements.

M1 grasp zone connections

Retrograde tracers were injected into different aspects of the grasp zone on the precentral gyrus. Inputs were primarily from motor thalamus and the largest proportion originated in VLp (VPLo in some other studies), which is consistent with previous work (Darian-Smith et al. 1990; Holsapple et al. 1991; Rouiller et al. 1994, 1999; Sakai et al. 1996; Schell and Strick 1984; Stepniewska et al. 1994b). Although the pattern of thalamic inputs that Matelli et al. (1989) reported from a single injection into rostral M1 (case IT 4–2) favors VLo over VPLo, the seeming differences between those results and the present findings as well as other work are diminished if inputs from the VLc–VPLo complex in Matelli et al. (1989) are grouped with the contribution from VPLo.

Nevertheless, there is some discrepancy among all of the studies, including our own, about the extent of converging inputs from divisions of motor thalamus onto the M1 hand representation. Discrepancies between studies could have emerged from differing characterizations of VL borders. For example, lumping VLp, VLx, and VLd into a single division such as VLp (Jones 1985) could have inflated the estimated contribution of VLp without influencing the interpretation of relative accessibility to cerebellum. Variations in the sizes and locations of tracer injections could have also contributed to the discrepancies in a number of ways. First, the hand representation in the precentral gyrus and the hand representation in the anterior bank of the central sulcus are differentially connected with divisions of motor thalamus (Holsapple et al. 1991; Matelli et al. 1989), which has implications for relative accessibility to the output nuclei of cerebellum and basal ganglia. Including thalamic connections of rostral and caudal aspects of the M1 hand representation in the same analysis could have contributed to inconsistencies. Filling both aspects of the M1 hand representation with the same tracer could have produced similar complications because such injections may have inadvertently favored one aspect of the M1 hand representation and biased the results accordingly. The problem would have been further exacerbated in the absence of neurophysiological guidance of tracer injections. Second, rostral and caudal aspects of the M1 hand representation mediate a wide range of hand functions. Differential thalamic inputs might facilitate their respective functional specializations. At least as defined with microstimulation, the M1 hand representation typically includes sites that evoke wrist and digits movements, even though they recruit different muscle synergies (Schieber 2001). Sites that evoke movements of proximal and distal aspects of the forelimb are also well represented on the precentral gyrus (Park et al. 2001). Tracing the connections of such a heterogeneous cortical representation could be expected to produce varying results. Thus, our strategy for injecting tracers into the neurophysiologically defined grasp zone in rostral M1 was designed in part to minimize the caveats discussed. Nevertheless, differences in injection locations within the grasp zone and challenges in the identification of thalamic borders, likely contributed to the variance in results across cases.

The profile of thalamic inputs to the grasp zone in rostral M1 of the precentral gyrus suggests that it is more accessible to the output nuclei of cerebellum as compared to the output nuclei of basal ganglia. Direct comparison of thalamic inputs to rostral and caudal M1 is not feasible here because we did not investigate the anatomical connections of caudal

M1. However, the input patterns that Holsapple et al. (1991) reported from two injections that were restricted to caudal M1 showed that inputs originated primarily in VLo (cf. Matelli et al. 1989), which is principally a target for the pallidothalamic pathway (Sakai et al. 1996; Stepniewska et al. 2003). The framework that emerges from considering the differential thalamic inputs to the hand representations in rostral and caudal M1 is that the cerebellothalamic pathway is more involved with “old” M1, which is considered evolutionarily conserved (Rathelot and Strick 2009). In contrast, the pallidothalamic pathway is more involved with “new” M1, which may have evolved substantially in Old World primates and Apes. It follows that the cerebellothalamic pathway may be more closely associated with the functions of corticospinal projections that terminate onto the intermediate zone of the spinal cord as opposed to the functions of corticospinal projections that terminate directly onto motoneurons of the ventral horn of the spinal cord. Nevertheless, differential connectivity of the hand representations of M1 with divisions of motor thalamus may reflect other functional specializations inherent to rostral and caudal M1 such as responsiveness to cutaneous and proprioceptive feedback (Picard and Smith 1992). Irrespective of interpretation, a transition zone may exist within the rostro-caudal extent of M1 where there is a gradual shift in thalamic inputs that reflect connectivity with the cerebellothalamic and pallidothalamic pathways.

We previously reported the organizations of the cortical connections from four of the present experiments on the M1 grasp zone (Gharbawie et al. 2011b). Frontal cortex motor regions contributed approximately 80 % of the connections and the remainder was from posterior (12 %) and anterior (7 %) parietal cortex. An analogous profile is apparent here where motor thalamus (VL and VA divisions) contributed 90 % of thalamic inputs to the M1 grasp zone. Similarly, weak inputs from anterior pulvinar to the M1 grasp zone parallel the density of connections of the M1 grasp zone with parietal cortex. Intralaminar nuclei project to a wide expanse of cortex (Jones and Leavitt 1974), which complicates drawing parallels between their relationship with the M1 grasp zone and its cortico-cortical connections.

PMv grasp zone location

Long train electrical stimulation revealed a grasp zone on the PMv dorsal convexity, which overlapped part of the hand representation previously identified with short train microstimulation parameters (Gentilucci et al. 1989; Godschalk et al. 1995). In other work, sites that evoked hand movements and sites that evoked concurrent movements of the face and shoulder/trunk were identified from the same approximate location as the current grasp zone (Maranesi et al. 2012). Single unit recordings from the same location showed correlations between neuronal activities and hand shaping for grasping (Murata et al. 1997; Raos et al. 2006; Rizzolatti et al. 1988). Deactivations of the same field impaired appropriate hand shaping for grasping (Fogassi et al. 2001). Thus, converging neurophysiological evidence shows that our PMv grasp zone overlaps a territory that has a widely accepted role in prehension. Nevertheless, many of the same studies have also shown that an even larger zone in the caudal bank of the inferior sulcus, which was not explored in our study, is intimately involved with grasping.

PMv grasp zone connections

Motor thalamus contributed the largest proportion (77 %) of thalamic inputs to the PMv grasp zone, which is consistent with previous reports (Matelli et al. 1989; Morel et al. 2005; Rouiller et al. 1999; Schell and Strick 1984). However, several factors complicate comparisons between our results and those studies. First, results varied between our two PMv cases. Although both injections targeted the center of the PMv grasp zone, connection patterns with motor thalamus favored divisions associated with basal ganglia in one case and cerebellar outputs in the second case. Those differences were in all likelihood related to dissimilarities in the size of the grasp zone and its proximity to the border of the architectonically distinct caudal (F4) and rostral (F5) aspects of PMv (Matelli et al. 1985). In case 10–50 in particular, the tracer injection probably encroached into the orofacial representation of PMv (Gharbawie et al. 2011b). Second, the lack of physiological control or absence of quantitative measures in previous studies also complicate comparison with our own work. Two of the cases in Matelli et al. (1989) are perhaps the exception, but even those results are only broadly comparable to the preset cases.

Comparison of M1 and PMv inputs

Our results suggest that the profile of thalamic inputs to the M1 and PMv grasp zones are only seemingly similar. First, inputs of the M1 grasp zone are more heavily biased towards the cerebellothalamic pathway than the pallidothalamic pathways, whereas those two pathways are more closely balanced in the PMv grasp zone. Second, although the cerebellothalamic pathway targets both cortical zones, M1 inputs originate from more lateral aspects of motor thalamus as compared to inputs of the PMv grasp zone. Differences in thalamic inputs to both grasp zones may support their related, yet different, roles in the grasp network.

Parallels between thalamic and cortical connections for the PMv grasp zone were not as apparent as was the case for the M1 grasp zone. For example, although most of the cortical connections of the PMv grasp zone originated in frontal cortex motor regions, those connections comprised a weaker proportion of the overall cortical connections as compared to the high ratio of thalamic inputs that originated in motor thalamus. Similarly, the PMv grasp zone was more densely connected than the M1 grasp zone with parietal cortex, but this pattern was not reflected in denser inputs from thalamic divisions that are associated with parietal cortex.

Alternative approaches

The utility of studying the density of inputs from motor thalamus to the M1 and PMv grasp zones is the possibility of inferring their accessibility to cerebellum and basal ganglia. However, such deductions could have been accomplished with more direct investigation. For example, transneuronal tracing of M1 connections revealed second order neurons in the output nuclei of cerebellum and basal ganglia (Hoover and Strick 1999). The same procedure revealed disynaptic connections between basal ganglia and cerebellum, which were otherwise considered components of independent thalamocortical loops (Hoshi et al. 2005). Another approach involves retrograde tracing of thalamic inputs to M1 and anterograde tracing of cerebellar and basal ganglia terminals in thalamus (Sakai et al. 1996).

Evaluating the overlap of cells and terminals labeled in motor thalamus revealed the relative accessibility of M1 to cerebellum and basal ganglia. The same approach facilitated identification of zones within motor thalamus that are exclusive to the cerebellothalamic pathway, or the pallidothalamic pathway, and transition zones that are accessible to both pathways. These alternative approaches have clear technical advantages, but are not without inherent challenges.

Conclusions

We propose that the M1 grasp zone of the precentral gyrus, which overlaps part of the hand representation in “old” M1, has a profile of thalamic inputs that facilitates its accessibility to output nuclei of cerebellum. Inputs from thalamic divisions that are primarily connected with basal ganglia constituted a much smaller proportion. This M1 grasp zone has proprioceptive characteristics (Picard and Smith 1992), connections with posterior parietal cortex (Gharbawie et al. 2011b), and projects to the intermediate zone of the spinal cord (Kuypers and Brinkman 1970; Rathelot and Strick 2006). Other work has shown that aspects of the hand representation that overlap “new” or more modified M1, located in the anterior bank of the central sulcus, has a profile of thalamic inputs that facilitates accessibility primarily to basal ganglia (Holsapple et al. 1991; Stepniewska et al. 2003). This part of the M1 hand representation has cutaneous characteristics (Picard and Smith 1992), weak connections with anterior parietal cortex, and projects directly onto motoneurons in the ventral horn of the spinal cord. We also propose that the grasp zone on the PMv dorsal convexity, which overlaps part of the hand representation in PMv, has a profile of thalamic connections that facilitates accessibility to both cerebellum and basal ganglia. This PMv grasp zone is densely interconnected with the M1 grasp zone of the precentral gyrus.

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References

- Bortoff GA, Strick PL. Corticospinal terminations in two new-world primates: further evidence that corticomotoneuronal connections provide part of the neural substrate for manual dexterity. *J Neurosci.* 1993; 13(12):5105–5118. [PubMed: 7504721]
- Bruce K, Grofova I. Notes on a light and electron microscopic double-labeling method combining anterograde tracing with *Phaseolus vulgaris* leucoagglutinin and retrograde tracing with cholera toxin subunit B. *J Neurosci Methods.* 1992; 45(1–2):23–33. DOI: 10.1016/0165-0270(92)90040-K [PubMed: 1283431]
- Darian-Smith C, Darian-Smith I, Cheema SS. Thalamic projections to sensorimotor cortex in the macaque monkey: use of multiple retrograde fluorescent tracers. *J Comp Neurol.* 1990; 299(1):17–46. [PubMed: 1698837]
- Dum RP, Strick PL. The origin of corticospinal projections from the premotor areas in the frontal lobe. *J Neurosci.* 1991; 11(3):667–689. [PubMed: 1705965]

- Dum RP, Strick PL. Frontal lobe inputs to the digit representations of the motor areas on the lateral surface of the hemisphere. *J Neurosci.* 2005; 25(6):1375–1386. doi:10.1523/JNEUR OSCI. 3902-04.2005. [PubMed: 15703391]
- Fogassi L, Gallese V, Buccino G, Craighero L, Fadiga L, Rizzolatti G. Cortical mechanism for the visual guidance of hand grasping movements in the monkey: a reversible inactivation study. *Brain.* 2001; 124(Pt 3):571–586. [PubMed: 11222457]
- Gentilucci M, Fogassi L, Luppino G, Matelli M, Camarda R, Rizzolatti G. Functional organization of inferior area 6 in the macaque monkey I. Somatotopy and the control of proximal movements. *Exp Brain Res.* 1988; 71(3):475–490. [PubMed: 3416964]
- Gentilucci M, Fogassi L, Luppino G, Matelli M, Camarda R, Rizzolatti G. Somatotopic representation in inferior area 6 of the macaque monkey. *Brain Behav Evol.* 1989; 33(2–3):118–121. [PubMed: 2758288]
- Gerbella M, Belmalih A, Borra E, Rozzi S, Luppino G. Cortical connections of the anterior (F5a) subdivision of the macaque ventral premotor area F5. *Brain Struct Funct.* 2011; 216(1):43–65. DOI: 10.1007/s00429-010-0293-6 [PubMed: 21132509]
- Gharbawie OA, Stepniewska I, Kaas JH. Cortical connections of functional zones in posterior parietal cortex and frontal cortex motor regions in New World monkeys. *Cereb Cortex.* 2011a; 21(9):1981–2002. DOI: 10.1093/cercor/bhq260 [PubMed: 21263034]
- Gharbawie OA, Stepniewska I, Qi H, Kaas JH. Multiple parietal-frontal pathways mediate grasping in macaque monkeys. *J Neurosci.* 2011b; 31(32):11660–11677. DOI: 10.1523/JNEUROSCI. 1777-11.2011 [PubMed: 21832196]
- Godschalk M, Mitz AR, van Duin B, van der Burg H. Somatotopy of monkey premotor cortex examined with microstimulation. *Neurosci Res.* 1995; 23(3):269–279. [PubMed: 8545075]
- Graziano MS, Taylor CS, Moore T. Complex movements evoked by microstimulation of precentral cortex. *Neuron.* 2002; 34(5):841–851. [PubMed: 12062029]
- Holsapple JW, Preston JB, Strick PL. The origin of thalamic inputs to the “hand” representation in the primary motor cortex. *J Neurosci.* 1991; 11(9):2644–2654. [PubMed: 1715388]
- Hoover JE, Strick PL. The organization of cerebellar and basal ganglia outputs to primary motor cortex as revealed by retrograde transneuronal transport of herpes simplex virus type 1. *J Neurosci.* 1999; 19(4):1446–1463. [PubMed: 9952421]
- Hoshi E, Tremblay L, Feger J, Carras PL, Strick PL. The cerebellum communicates with the basal ganglia. *Nat Neurosci.* 2005; 8(11):1491–1493. DOI: 10.1038/nn1544 [PubMed: 16205719]
- Jones, EG. *The thalamus.* Plenum Press; New York: 1985.
- Jones EG, Leavitt RY. Retrograde axonal transport and the demonstration of non-specific projections to the cerebral cortex and striatum from thalamic intralaminar nuclei in the rat, cat and monkey. *J Comp Neurol.* 1974; 154(4):349–377. DOI: 10.1002/cne.901540402 [PubMed: 4132969]
- Kaas, JH. The somatosensory thalamus and associated pathways. In: Gardner, EP.; Kaas, JH., editors. *The senses a comprehensive reference*, vol 6, Somatosensation. Elsevier; Oxford: 2008. p. 117–141.
- Kuypers HG, Brinkman J. Precentral projections to different parts of the spinal intermediate zone in the rhesus monkey. *Brain Res.* 1970; 24(1):29–48. DOI: 10.1016/0006-8993(70)90272-6 [PubMed: 4099987]
- Macchi G, Jones EG. Toward an agreement on terminology of nuclear and subnuclear divisions of the motor thalamus. *J Neurosurg.* 1997; 86(4):670–685. DOI: 10.3171/jns.1997.86.4.0670 [PubMed: 9120632]
- Maranesi M, Roda F, Bonini L, Rozzi S, Ferrari PF, Fogassi L, Coude G. Anatomic-functional organization of the ventral primary motor and premotor cortex in the macaque monkey. *Eur J Neurosci.* 2012; 36(10):3376–3387. DOI: 10.1111/j.1460-9568.2012.08252.x [PubMed: 22891812]
- Matelli M, Luppino G, Rizzolatti G. Patterns of cytochrome oxidase activity in the frontal agranular cortex of the macaque monkey. *Behav Brain Res.* 1985; 18(2):125–136. [PubMed: 3006721]
- Matelli M, Luppino G, Fogassi L, Rizzolatti G. Thalamic input to inferior area 6 and area 4 in the macaque monkey. *J Comp Neurol.* 1989; 280(3):468–488. [PubMed: 2537345]

- McKiernan BJ, Marcario JK, Karrer JH, Cheney PD. Corticomotoneuronal postspike effects in shoulder, elbow, wrist, digit, and intrinsic hand muscles during a reach and prehension task. *J Neurophysiol.* 1998; 80(4):1961–1980. [PubMed: 9772253]
- Morel A, Liu J, Wannier T, Jeanmonod D, Rouiller EM. Divergence and convergence of thalamocortical projections to premotor and supplementary motor cortex: a multiple tracing study in the macaque monkey. *Eur J Neurosci.* 2005; 21(4):1007–1029. DOI: 10.1111/j.1460-9568.2005.03921.x [PubMed: 15787707]
- Murata A, Fadiga L, Fogassi L, Gallese V, Raos V, Rizzolatti G. Object representation in the ventral premotor cortex (area F5) of the monkey. *J Neurophysiol.* 1997; 78(4):2226–2230. [PubMed: 9325390]
- Nakajima K, Maier MA, Kirkwood PA, Lemon RN. Striking differences in transmission of corticospinal excitation to upper limb motoneurons in two primate species. *J Neurophysiol.* 2000; 84(2):698–709. [PubMed: 10938297]
- Nudo RJ, Masterton RB. Descending pathways to the spinal cord: a comparative study of 22 mammals. *J Comp Neurol.* 1988; 277(1):53–79. DOI: 10.1002/cne.902770105 [PubMed: 3198796]
- Olszewski, J. The thalamus of *Macaca Mulatta*. Krager; Basel: 1952.
- Park MC, Belhaj-Saif A, Gordon M, Cheney PD. Consistent features in the forelimb representation of primary motor cortex in rhesus macaques. *J Neurosci.* 2001; 21(8):2784–2792. [PubMed: 11306630]
- Park MC, Belhaj-Saif A, Cheney PD. Properties of primary motor cortex output to forelimb muscles in rhesus macaques. *J Neurophysiol.* 2004; 92(5):2968–2984. DOI: 10.1152/jn.00649.2003 [PubMed: 15163675]
- Picard N, Smith AM. Primary motor cortical activity related to the weight and texture of grasped objects in the monkey. *J Neurophysiol.* 1992; 68(5):1867–1881. [PubMed: 1479450]
- Qi HX, Stepniewska I, Kaas JH. Reorganization of primary motor cortex in adult macaque monkeys with long-standing amputations. *J Neurophysiol.* 2000; 84(4):2133–2147. [PubMed: 11024101]
- Qi HX, Jain N, Collins CE, Lyon DC, Kaas JH. Functional organization of motor cortex of adult macaque monkeys is altered by sensory loss in infancy. *Proc Natl Acad Sci USA.* 2010; 107(7):3192–3197. DOI: 10.1073/pnas.0914962107 [PubMed: 20133738]
- Raos V, Umiltà MA, Murata A, Fogassi L, Gallese V. Functional properties of grasping-related neurons in the ventral premotor area F5 of the macaque monkey. *J Neurophysiol.* 2006; 95(2):709–729. DOI: 10.1152/jn.00463.2005 [PubMed: 16251265]
- Rathelot JA, Strick PL. Muscle representation in the macaque motor cortex: an anatomical perspective. *Proc Natl Acad Sci USA.* 2006; 103(21):8257–8262. DOI: 10.1073/Pnas.0602933103 [PubMed: 16702556]
- Rathelot JA, Strick PL. Subdivisions of primary motor cortex based on cortico-motoneuronal cells. *Proc Natl Acad Sci USA.* 2009; 106(3):918–923. DOI: 10.1073/pnas.0808362106 [PubMed: 19139417]
- Rizzolatti G, Camarda R, Fogassi L, Gentilucci M, Luppino G, Matelli M. Functional organization of inferior area 6 in the macaque monkey II. Area F5 and the control of distal movements. *Exp Brain Res.* 1988; 71(3):491–507. [PubMed: 3416965]
- Rouiller EM, Liang F, Babalian A, Moret V, Wiesendanger M. Cerebellothalamocortical and pallidothalamocortical projections to the primary and supplementary motor cortical areas: a multiple tracing study in macaque monkeys. *J Comp Neurol.* 1994; 345(2):185–213. DOI: 10.1002/cne.903450204 [PubMed: 7523459]
- Rouiller EM, Tanne J, Moret V, Boussaoud D. Origin of thalamic inputs to the primary, premotor, and supplementary motor cortical areas and to area 46 in macaque monkeys: a multiple retrograde tracing study. *J Comp Neurol.* 1999; 409(1):131–152. doi:10.1002/(SICI)1096-9861(19990621)409:1<131::AID-CNE10>3.0.CO;2-A. [PubMed: 10363716]
- Sakai ST, Inase M, Tanji J. Comparison of cerebellothalamic and pallidothalamic projections in the monkey (*Macaca fus-cata*): a double anterograde labeling study. *J Comp Neurol.* 1996; 368(2):215–228. doi:10.1002/(SICI)1096-9861(19960429)368:2<215::AID-CNE4>3.0.CO;2-6. [PubMed: 8725303]

- Schell GR, Strick PL. The origin of thalamic inputs to the arcuate premotor and supplementary motor areas. *J Neurosci*. 1984; 4(2):539–560. [PubMed: 6199485]
- Schieber MH. Constraints on somatotopic organization in the primary motor cortex. *J Neurophysiol*. 2001; 86(5):2125–2143. [PubMed: 11698506]
- Stepniewska I, Preuss TM, Kaas JH. Architectonic subdivisions of the motor thalamus of owl monkeys: Nissl, acetylcholinesterase, and cytochrome oxidase patterns. *J Comp Neurol*. 1994a; 349(4):536–557. [PubMed: 7860788]
- Stepniewska I, Preuss TM, Kaas JH. Thalamic connections of the primary motor cortex (M1) of owl monkeys. *J Comp Neurol*. 1994b; 349(4):558–582. [PubMed: 7532193]
- Stepniewska I, Sakai ST, Qi HX, Kaas JH. Somatosensory input to the ventrolateral thalamic region in the macaque monkey: potential substrate for parkinsonian tremor. *J Comp Neurol*. 2003; 455(3): 378–395. DOI: 10.1002/cne.10499 [PubMed: 12483689]
- Stepniewska I, Fang PC, Kaas JH. Microstimulation reveals specialized subregions for different complex movements in posterior parietal cortex of prosimian galagos. *Proc Natl Acad Sci USA*. 2005; 102(13):4878–4883. [PubMed: 15772167]
- Strick PL, Kim CC. Input to primate motor cortex from posterior parietal cortex (area 5) I. Demonstration by retrograde transport. *Brain Res*. 1978; 157:325–330. [PubMed: 102409]
- Umiltà MA, Brochier T, Spinks RL, Lemon RN. Simultaneous recording of macaque premotor and primary motor cortex neuronal populations reveals different functional contributions to visuomotor grasp. *J Neurophysiol*. 2007; 98(1):488–501. DOI: 10.1152/jn.01094.2006 [PubMed: 17329624]

Fig. 1.

Maps of movements evoked with long train electrical stimulation (each train is 150 biphasic pulses, 0.2 ms pulse width, delivered over 500 ms). The maps are aligned to outlines that were traced from digital photographs of the cortex after extraction and flattening (**a–e**). Microelectrode penetration sites are represented with different shapes according to the legend in (**f**) to reflect evoked movements. Functionally related responses are highlighted in *shades of gray*. Grasping, other digit movements, and wrist movements are included in the hand representation. Locations and sizes of injection cores are depicted in *yellow*. *Solid gray lines* mark the locations of the central sulcus (*CS*), arcuate sulcus (*ArS*), and intraparietal sulcus (*IPS*). *Dashed lines* mark the border between primary motor cortex (*M1*), dorsal premotor cortex (*PMd*), and ventral premotor cortex (*PMv*). Border locations were estimated from current thresholds for evoking movements, architectonics, and cortical landmarks. The right hemisphere was studied in case 10–14, but the illustration in **d** is reflected *horizontally* for consistency with other cases. **f** *White square* on the dorsolateral view of a macaque brain depicts the approximate area of cortex in **a–e**. *Scale bar* in (**b**) and applies to (**a–e**)

Fig. 2. Representative coronal sections (case 10–29) showing architectonic borders of motor thalamus. **a–b** Successive sections stained for acetylcholinesterase and cresyl violet, respectively. Section thickness is 40 μ m. **c** Architectonic borders reconstructed from sections in (**a**, **b**). **d–f** Follow the same format as **a–c**. Approximately 2160 μ m separate the sections in (**a**, **d**)

Fig. 3. Case 09–51. Coronal sections of thalamus show distribution of cells labeled from a cholera toxin b-subunit (CTB) injection in lateral aspects of the *M1* grasp zone. Each *gray circle* depicts a CTB-labeled cell. Thalamic borders were identified from adjacent sections stained for acetylcholinesterase, cresyl violet, and cytochrome oxidase. Sections are arranged from rostral to caudal. Section number and thickness (40 μ m) can be used for calculating the approximate distance between sections

Fig. 4.

Case 10–29. Coronal sections of thalamus show distributions of cells labeled from a *diamidino yellow* (DY) injection into lateral aspects of the *M1* grasp zone and a cholera toxin b-subunit injection into the *PMv* grasp zone. Other features follow conventions of Fig. 3. *DY* labeled a small number of cells as compared to the *CTB* injection. *DY*-labeled cells were in the main separate from the *CTB*-labeled population and occupied a relatively lateral position

Fig. 5.

Case 10–50. Coronal sections of thalamus show distributions of cells labeled from a *fluoro ruby* (FR) injection near the center of the *M1* grasp zone and a cholera toxin b-subunit into the *PMv* grasp zone. Other features follow conventions of Fig. 3. Although *FR* labeled a small number of cells as compared to the *CTB* injection, the *FR*-labeled cells were in the main separate from the *CTB*-labeled cells and occupied a relatively lateral position

Fig. 6.
Case 10–14. Coronal sections of thalamus show distributions of cells labeled from a *fluro-ruby* (FR) injection into lateral aspects of the *M1* grasp zone. Sections are from the right hemisphere, but were reflected horizontally for consistency with other cases. Other features follow conventions of Fig. 3. The FR-labeled cells were primarily concentrated in *VL* divisions of thalamus

Case 09-20

- M1 Grasp (FB)

Fig. 7. Case 09–20. Coronal sections of thalamus show distributions of cells labeled from a *fast blue* (FB) injection into rostral aspects of the *MI* grasp zone. Other features follow conventions of Fig. 3. The FB-labeled cells were primarily concentrated in *VL* divisions of thalamus

Fig. 8. Case 10–29. Coronal sections of thalamus show distributions of cells labeled from a *diamidino yellow* (DY) injection into lateral aspects of the *M1* grasp zone and a *fluoro ruby* (FR) injection into the *M1* orofacial representation. Other features follow conventions of Fig. 3. DY-labeled cells were in the main separate from the FR-labeled cells and occupied a relatively lateral position

Fig. 9.

a Scatter plot of the distributions of cells labeled in motor thalamus from five injections into the *M1* grasp zone and two injections into the *PMv* grasp zone. Motor thalamus divisions are grouped according to their relative accessibility to the output nuclei of cerebellum or basal ganglia. The general pattern that emerges from this presentation is that the *M1* grasp zone is more accessible to output nuclei of cerebellum, whereas the *PMv* grasp zone is near equally accessible to output nuclei of both cerebellum and basal ganglia. **b** Summary of the main thalamic inputs of the *M1* grasp zone. Line thickness is directly proportional to the mean ($n = 5$) density of inputs that originate from each structure

Table 1
List of abbreviations of thalamic nuclei

AV	Anterior ventral nucleus
AM	Anterior medial nucleus
CL	Central lateral nucleus
CM	Central median nucleus
IL	Intralaminar nuclei
LME	External medullary fold
LP	Lateral posterior nucleus
MD	Medial dorsal nucleus
PA	Anterior pulvinar
PC	Posterior division
PL	Lateral pulvinar
PM	Medial pulvinar
Ret	Reticular nucleus
VA	Ventral anterior thalamus
VApc	Parvocellular division
VAmc	Magnocellular division
VL	Ventral lateral thalamus
VL _a	Ventral lateral thalamus (anterior)
VL _d	Ventral lateral thalamus (dorsal)
VL _m	Ventral lateral thalamus (medial)
VL _p	Ventral lateral thalamus (posterior)
VL _{ps}	Ventral lateral p
VL _x	Area X
VP	Ventral posterior thalamus
VPI	Ventral posterior thalamus (inferior)
VPL	Ventral posterior thalamus (lateral)
VPM	Ventral posterior thalamus (medial)
VPMpc	Ventral posterior thalamus (pars centralis)
VPS	Ventral posterior thalamus (superior)

Table 2

Distributions of cells labeled from 8 injections into M1 and PMv

	M1 grasp			PMv grasp			M1 face		
	09-51 CTB 894	10-29 DY 258	10-50 FR 205	10-14 CTB 158	09-20 FB 256	10-29 CTB 1456	10-50 CTB 2660	10-29 FR 521	
Motor thalamus									
Cerebellum									
VLp	28.86	41.86	34.63	35.44	70.31	13.60	8.65	5.76	
VLx	15.66	12.40	17.07	26.58	3.91	10.92	38.16	42.42	
VLd	1.57	1.55	16.59	1.27	6.25	0.27	0.56	–	
Subtotal cerebellum	46.09	55.81	68.29	63.29	80.47	24.79	47.37	48.18	
Basal ganglia									
VLa	17.34	9.69	3.90	24.05	9.38	19.85	16.35	31.09	
VLm	8.61	3.88	0.98	2.53	1.95	20.47	7.22	3.84	
VA	5.70	21.32	–	–	2.34	8.52	9.74	3.65	
Subtotal basal ganglia	31.66	34.89	4.88	26.58	13.67	48.84	33.31	48.84	
Subtotal motor thalamus	77.74	90.70	73.17	89.87	94.14	73.63	80.68	86.76	
Ventral posterior									
VPL	–	–	–	–	–	0.21	0.19	–	
VPM	0.34	–	–	–	–	–	–	5.18	
VPMpc	0.45	–	–	–	–	0.14	–	–	
VPI	0.22	–	–	–	–	0.62	–	0.38	
VPS	–	–	–	–	–	–	–	–	
Subtotal ventral posterior	1.01	–	–	–	–	0.96	0.26	5.57	
MD	5.26	4.26	2.44	–	0.78	14.15	3.16	2.69	
LP	–	–	5.85	–	–	–	–	–	
Intralaminar									
CL	5.70	4.26	7.32	8.86	1.56	2.95	2.22	3.07	
CM	2.68	–	6.34	–	–	1.65	11.50	0.58	
LME	–	–	–	1.56	–	–	–	–	
PC	0.89	0.78	–	–	–	1.30	0.34	1.34	
Ret	–	–	–	–	1.95	–	0.15	–	

	MI grasp				PMv grasp				MI face	
	09-51 CTB 894	10-29 DY 258	10-50 FR 205	10-14 CTB 158	09-20 FB 256	10-29 CTB 1456	10-50 CTB 2660	10-29 FR 521		
Subtotal intralaminar	9.28	5.04	13.66	8.86	5.08	5.91	14.21	4.99		
Pulvinar										
PA	6.71	–	2.93	1.27	–	5.29	1.20	–		
PL	–	–	1.95	–	–	–	0.45	–		
PM	–	–	–	–	–	–	–	–		
Subtotal pulvinar	6.71	–	4.88	1.27	–	5.29	1.65	–		

Rows sequentially list the cortical zone of the injection, case number of each monkey, tracer injected, and total numbers of cells labeled in the thalamus. Thalamic nuclei are listed in the first column and successive columns contain the respective percentage of labeled cells. Divisions of motor thalamus are grouped according to their preferential connectivity with the output nuclei of cerebellum and basal ganglia. The total number of cells labeled in motor thalamus is highlighted in bold. The differential distribution of cells labeled in thalamic divisions associated with cerebellum and basal ganglia is readily gleaned from the subtotals highlighted in bold. Concentrations of cells less than 0.1 % are listed as “–”.