Direct Projections From the Dorsal Premotor Cortex to the Superior Colliculus in the Macaque (*Macaca mulatta*)

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ABSTRACT

The dorsal premotor cortex (PMd) is part of the cortical network for arm movements during reach-related behavior. Here we investigate the neuronal projections from the PMd to the midbrain superior colliculus (SC), which also contains reach-related neurons, to investigate how the SC integrates into a cortico-subcortical network responsible for initiation and modulation of goal-directed arm movements. By using anterograde transport of neuronal tracers, we found that the PMd projects most strongly to the deep layers of the lateral part of the SC and the underlying reticular formation corresponding to locations where reach-related neurons have been recorded, and from where descending tectofugal projections arise. A somewhat weaker projection targets the intermediate layers of the SC. By contrast, terminals originating from prearcuate area 8 mainly project to the intermediate layers of the SC. Thus, this projection pattern strengthens the view that different compartments in the SC are involved in the control of gaze and in the control or modulation of reaching movements. The PMD–SC projection assists in the participation of the SC in the skeletomotor system and provides the PMd with a parallel path to elicit forelimb movements. J. Comp. Neurol. 000:000–000, 2015.

INDEXING TERMS: dorsal premotor; corticotectal; eye–hand coordination; sensorimotor; reaching; AB_2336827; AB_2315331

Reaching and grasping are everyday activities for all dexterous mammals including humans. Research during the last decades in primates has revealed a widespread network of brain areas involved in various aspects of these actions. In the cerebral cortex, the main players are the primary motor cortex (M1), premotor cortex (PM), and posterior parietal cortex (area 5, AIP and MIP), with their cortical input from visual, somatosensory, and prefrontal cortex (Johnson et al., 1996; Hoshi and Tanji, 2007; Padberg et al., 2007; Kaas et al., 2012, 2013 and references therein). Based on electrophysiological recordings, microstimulation, and anatomical investigations, all these areas display topographic organization that, however, is somewhat crude in the premotor and posterior parietal cortex. This led to the concept of functional zones interconnected within and between relevant cortical areas, which would then together control the various sectors, joint angles, orientation, etc. of the forelimb during certain movements (Seelke et al., 2012; Kaas et al., 2012, 2013).

On functional and anatomical grounds, the premotor cortex (Brodmann area 6) can be divided in dorsal (PMd, F2, F7) and ventral (PMv, F4, F5) compartments (Matelli et al., 1985). Area PMv (F5) is mainly connected with the ventral portion of the dorsolateral prefrontal cortex (DLPC) and with the inferior parietal lobule, especially area AIP and PF (Luppino et al., 1999; Hoshi and Tanji, 2007; Borra et al., 2008; Gharbawie et al., 2011). Functionally, the PMv is involved in the preparation and execution of arm and hand movements related to grasping and contains not only neurons related to the subject’s own actions but to actions of others (mirror neurons).
NRp  n. reticularis parvocellularis
NRm  n. reticularis mesencephali
NRpN  n. reticularis pontis oralis

Abbreviations

Aaqueduct
A8  area 8
Amt  anterior mediotemporal sulcus
Ar  arcuate sulcus
BSC  brachium of the superior colliculus
Ca  calcarine sulcus
Ce  central sulcus
Ci  cingulate sulcus
DMZ  densely myelinated zone of the medial superior temporal area
Dpc  decussatio pedunculi cerebellaris
ec  ectococculus sulcus
FEF  frontal eye field
fM  fasciculus longitudinalis medialis
IC  inferior colliculus
io  infero-occipital sulcus
iop  intraparietal sulcus
la  lateral sulcus
LIP  lateral intraparietal area
II  lenuisculus lateralis
lu  lunate sulcus
M2  motor area 2
MIP  medial intraparietal area
MT  middle temporal area
NPo  n. pontis
NRm  n. reticularis mesencephali
NRp  n. reticularis parvocellularis
NRpN  n. reticularis pontis oralis
NPo  n. pontis

et al., 2014).

et al., 2012; Lehmann and Scherberger, 2013; Bonini et al., 2014).

By contrast, PMd is connected with the dorsal DLPC and the dorsal parietal lobe including area 5 and the MIP. Namely, the caudal part of the PMd (F2 dimple region and F2 ventrostral of Matelli et al., 1998; cPMd of Ghosh and Gattera, 1995) receives its main input from parietal areas PCe and PEip (F2 dimple), and the MIP and V6A (F2 ventrostral), respectively, as well as from frontal areas SMA, M1, and the cingulate motor areas (Ghosh and Gattera, 1995; Matelli et al., 1998). It is also involved in the preparation and execution of arm movements but shows preparatory activity and codes for direction, speed, and amplitude of the arm movement during reaching and eye–hand coordination (Barbas and Pandya, 1987; Colby et al., 1988; Fu et al., 1995; Tanné et al., 1995; Johnson et al., 1996; Matelli et al., 1998; Messier and Kalaska, 2000; Lupino et al., 2003; Churchland et al., 2006; Pesaran et al., 2006, 2010; Hoshi and Tanji, 2007; Yamagata et al., 2012).

Electrical microstimulation in the PMd leads to movements of the upper limb but at significantly higher thresholds than in the M1 (Weinreich and Wise, 1982; Preuss et al., 1996; Fujii et al., 2000; Raos et al., 2003). A systematic survey of the dorsal premotor cortex revealed a topographic order of evoked movements with simple movements of the contralateral shoulder mostly elicited from areas close to the precentral dimple, and simple movements of the contralateral distal forelimb mostly elicited more laterally close to the spur of the arcuate. In addition, neurons close to the dimple were mostly active during movements and somatosensory stimulation of the arm, whereas neurons close to the spur were active during movement and somatosensory stimulation of the distal forelimb (Raos et al., 2003). About half of the neurons were active during grasping, and about 37% were active during reaching. This forelimb-related region contains visual, somatosensory, purely motor, visually modulated, and visuomotor neurons (Fogassi et al., 1999; Raos et al., 2004). Electrical stimulation of the PMd resulted more often in suppression (50% of events) in upper limb muscles than stimulation of the SMA or M1 (20% of events) (Montgomery et al., 2013). With longer stimulation periods (500 ms), complex and more naturalistic movements of the arm could be evoked from the PMd (Graziano et al., 2002).

In recent years, reach-related activity has also been identified in neurons of the intermediate and deep layers of the midbrain superior colliculus (SC). These reach neurons are active before and during arm movements, and their activity is correlated with the direction of arm movements (Kutz et al., 1997) and/or with the electromyogram of proximal shoulder and arm muscles (Werner, 1993; Werner et al., 1997a, b; Stuphorn et al., 1999, 2000). Furthermore, microstimulation at recording sites of these reach neurons elicits arm movements (Philipp and Hoffmann, 2014).

The SC receives input from multiple cortical areas. The superficial layers are targeted mainly by early visual areas, whereas intermediate and deep layers receive input from the posterior parietal cortex including the lateral intraparietal area (LIP), forelimb representation...
of areas 2, 4, and 6, FEF, PMv, and prefrontal cortex (Finlay et al., 1976; Kuenzle et al., 1976; Leichnetz et al., 1981; Fries, 1984, 1985; Komatsu and Suzuki, 1985; Lynch et al., 1985; Lock et al., 2003; Borra et al., 2014; Cerkevich et al., 2014).

Only in some instances have these anatomical connections been characterized physiologically. The corticotectal projection originating from the primary visual cortex seems to be involved in the control of intracollicular visual processing (Finlay et al., 1976). The FEF projection to the SC mainly carries saccade-related and visual information about the central visual field (Segraves and Goldberg, 1987). By contrast, tectal projections from the LIP carry mostly visual saccade-related information from the peripheral field (Paré/C19 Ce and Wurtz, 1997; Gaymard et al., 2003). In contrast, projections from the dorsolateral prefrontal cortex transmit task-selective signals to the SC, i.e., neuronal signals selective for antisaccades that inhibit reflexive prosaccades (Johnston and Everling, 2006). To date, only little data for the premotor-tectal projections are available. Electrical microstimulation revealed excitatory as well as inhibitory influences of the PMd on forelimb muscles (Montgomery et al., 2013). We presume that at least part of the PMd output is mediated indirectly, e.g., via subcortical nuclei including the SC. In a preliminary study, antidromically identified premotor-tectal neurons were active during reach movements. In some of these cells the reach activity varied with the direction of gaze, which corresponds to the firing properties of a subset of SC reach neurons (Stuphorn et al., 1996).

In the present study we investigate the direct projections of the PMd to the SC to reveal the spatial location of anterogradely labeled terminals relative to recording sites of reach-related activity in the SC. This study is part of a project to further characterize the information transmitted from the PMd to the SC in the cortico-subcortical network for reaching.

**MATERIALS AND METHODS**

All experiments were approved by the local authorities (Regierungspräsidium Arnsberg, now LANUV) and the ethics committee, and were performed in accordance with the Deutsche Tierschutzgesetz of 7.26.2002, the European Communities Council Directive RL 2010/63/EC, and NIH guidelines for care and use of animals for experimental procedures.

**Animals**

Four adult male rhesus monkeys (*Macaca mulatta*) were used in the present investigation. All animals had participated in electrophysiological experiments prior to the tracer injections (Stuphorn et al., 2000; Kruse and Hoffmann, 2002; Kruse et al., 2002; Philipp and Hoffmann, 2014).

**Surgery**

After premedication with atropine sulfate (0.04 mg/kg) the animals were initially anesthetized with ketamine hydrochloride (10 mg/kg i.m., Braun, Melsungen, Germany). After local anesthesia with 10% xylocaine (Astra Zeneka, Wedel, Germany), they were intubated through the mouth, an intravenous catheter was introduced into the saphenous vein, and, after additional local anesthesia with bupivacaine hydrochloride 0.5%, the animals were placed into a stereotactic apparatus. Throughout the experiment the animals were artificially ventilated with nitrous oxide: oxygen as 3:1 containing 0.3%–1% halothane as needed. Deep analgesia was ensured by intravenous bolus and continuous application of fentanyl (3 μg/kg/h, Janssen, Neuss, Germany). Heart rate, SPO2, blood pressure, body temperature, and end-tidal CO2 were monitored constantly and kept at physiological levels. The corneae were protected with contact lenses.

**Injections and histological procedures**

In two of the animals, the injections were made through recording chambers that had previously been implanted for the electrophysiological experiments to probe the PMd for neurons antidromically activated from the SC. In the remaining two animals, the recording chamber was removed to provide access to a larger cortical area. We used biotin dextran amine (BDA) as an anterograde tracer. In addition, in case A8 (prearcuate oculomotor area 8), during the final electrophysiological experiment, we injected horseradish peroxidase.

**TABLE 1.** Summary of the Database, Tracers Used, Volume Injected, and Survival Time in the Different Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Tracer</th>
<th>Volume (μl)</th>
<th>Survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMd1</td>
<td>15% BDA MW3000 in 0.1 M citrate/NaOH pH 3.3</td>
<td>4</td>
<td>2 weeks</td>
</tr>
<tr>
<td>PMd2</td>
<td>15% BDA MW3000 in 0.1 M citrate/NaOH pH 3.3</td>
<td>2</td>
<td>2 weeks</td>
</tr>
<tr>
<td>PMd3</td>
<td>20% BDA MW3000 in 0.9% NaCl</td>
<td>3</td>
<td>2 weeks</td>
</tr>
<tr>
<td>A8</td>
<td>2.5% WGA-HRP in KCl/Tris/DMSO</td>
<td>0.3</td>
<td>48 hours</td>
</tr>
<tr>
<td>A8-2</td>
<td>10% BDA MW10000 in 0.9% NaCl</td>
<td>1</td>
<td>3 months</td>
</tr>
</tbody>
</table>
conjugated to wheat germ agglutinin (WGA-HRP) at the same location as the BDA injection (case A8-2). Table 1 summarizes the tracers, volumes, and survival times used in the present study.

After appropriate survival times, the animals received a lethal overdose of pentobarbital and were perfused through the heart with 0.9% NaCl containing 0.1% procaine hydrochloride followed by paraformaldehyde-

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**Figure 1.** Reconstructions of the left hemispheres of cases PMd1 (A), PMd2 (B), PMd3 (C), and A8 (D). The core of the tracer injections is shown in solid green, and the diffusion area in stippled green. For abbreviations see list. Scale bar = 5 mm.
lysine–periodate with 4% paraformaldehyde. After appropriate cryoprotection with 10% and 20% glycerol, the brains were shock frozen and stored at −70°C until processing.

The midbrains were cut at 40–50 μm on a vibratome or a cryostat in a plane angled 45° from posterior and thus approximately normal to the SC surface and parallel to the electrode penetrations. Frontal or sagittal sections of the injected cortical hemispheres were cut on a freezing microtome at 50 μm. Neuronal transport of BDA was visualized with the avidin-biotin method (ABC Elite, Vector, Burlingame, CA; 1:250, RRID: AB_2336827) with diaminobenzidine (DAB) as chromogen enhanced with ammonium nickel sulfate (cases

Figure 2. Examples of anterogradely labeled fibers and terminals after BDA injections into the PMd in case PMd1 (A–C) and case PMd3 (D–F). Gray dots in A mark labeled terminals, and black lines in A mark labeled fibers. The regions where the microphotographs shown in B and C were taken are marked in the reconstruction of the midbrain section (A). D: Darkfield photomicrograph of labeled fibers and terminals in the lateral SC of PMd3 visualized with TMB as a chromogen. The single asterisk marks the approximate location of the label shown in E, and the double asterisk marks the approximate location of F. E,F: Fibers and terminals in the deep lateral SC of PMd3. In B–D and E–F, labeled structures were visualized with enhanced DAB as a chromogen. Scale bar = 500 μm in D; 50 μm in B, C, E, F.
Figure 3. Camera lucida drawings of serial sections through the midbrain of case PMd1. Sections are arranged from posterior (upper left) to anterior (lower right). Intersection distance is 480 μm from the third section onward. Sections in the left row present the anatomical areas as seen in Nissl-stained sections, and green dashed lines indicate AChE-rich areas in the intermediate layers of the SC and the underlying midbrain. Anterogradely labeled fibers are shown in blue, and labeled terminals in red. In this case almost the entire anteroposterior and the entire mediolateral extent of the SC contains labeled terminals. Additional label is found in the pontine nuclei and in multiple locations within the mesencephalic reticular formation. For abbreviations see list. Scale bar = 2 mm.
PMd1, PMd2, and PMd3), or in alternate sections with tetramethylbenzidine (TMB) as chromogen (case PMd3; after Ding and Ellberger, 1995, van der Want, 1997). In case A8-2, WGA-HRP was visualized with TMB (after van der Want, 1997). A subset of midbrain sections was stained for acetylcholinesterase (AChE) histochemistry (Illing, 1988). Subsets of cortical sections were stained for Nissl, and myeloarchitecture (Gallyas, 1979, as modified by Hess and Marker, 1983).

In a further subset of cortical sections, the neurofilament-based chemoarchitecture of cortical areas was analyzed. Briefly, free-floating sections were washed in 0.1 M Tris-buffered saline (TBS), and treated for 30 minutes with 3% H2O2 and 0.2% Triton–X-100, respectively. Sections were incubated in an antibody against nonphosphorylated neurofilament protein for 48 hours at 4°C under gentle agitation (SMI-32, 1:1,000, Covance, Munster, Germany; cat# smi-32r; RRID: AB_2315331; Hof and Morrison, 1995). Then sections were washed thoroughly in TBS, and incubated overnight in the biotinylated secondary antibody at 4°C (sheep anti-mouse, 1:200, Amersham, GE Healthcare Life Sciences, Braunschweig, Germany; cat# RPN1001). After thorough washing, sections were incubated in ABC Elite (Vector, 1:250, RRID: AB_2336827) for 2 hours at room temperature, and antigenic sites were visualized with DAB. Controls omitting the primary antibody resulted in no labeling.

### Analysis

Labeled fibers and terminals in midbrain sections were drawn with a camera lucida under a microscope (Zeiss Axioplan) coupled to a reconstruction system (AccuStage MDPot v5), and cortical connections were plotted with the reconstruction system. Cortical areas were identified based on cyto-, myelo-, and neurofilament-based chemoarchitecture (Barbas and Pandya, 1987; Blatt et al., 1990; Colby et al., 1993; Hof and Morris, 1995; Hof et al., 1995; Preuss et al., 1997; Gabernet et al., 1999; Rozzi et al., 2006; Belmahi et al., 2007; Takahara et al., 2012) largely following the criteria summarized and demonstrated by Lewis and van Essen (2000). The histological sections were photographed under a photomicroscope (Zeiss Axioplan) equipped with a Canon EOS 600D camera, and brightness and contrast were adjusted with Adobe Photoshop (RRID: SciRes_000161, version 5.5).

### RESULTS

The present study is based on three tracer injections into the dorsal premotor cortex. For comparison, the prearcuate oculomotor cortex (area 8) was injected in an additional animal. The location of the injection sites is presented on the reconstructions of the lateral view of the left cortical hemispheres in Figure 1. All data are presented as left hemispheres to facilitate comparison.

### Case PMd1

In case PMd1, the tracer injections into the PMd followed the electrophysiological recordings from cortical neurons that projected to the SC as identified by antidromic stimulation. Three tracer injections (green areas) were placed into the anterior part of the PMd (F2) rostral to the precentral dimple (pcd) (Fig. 1A) but clearly not encroaching on area 8 in the prearcuate gyrus or FEF in the anterior bank of the arcuate sulcus. Examples of the anterogradely labeled fibers and terminals are depicted in Figure 2B and C, and the location of the labeled structures in the SC and underlying mesencephalic reticular formation is given in Figure 2A. Anterogradely labeled terminals (Fig. 2, red dots in Fig. 3) and fibers (Fig. 2, blue marks in Fig. 3) were located in the intermediate and predominantly in the deep
layers of the ipsilateral SC throughout its entire antero-posterior and mediolateral extent (Fig. 3). As a label for the intermediate layers, we used AChE histochemistry (green structures in the left drawings of Fig. 3); the other collicular layers were determined in neighboring sections stained for cytoarchitecture. The PMd terminals were located predominantly below the AChE-rich patches. In addition, labeled terminals were found in the mesencephalic reticular formation close to the peri-aqueductal gray and in the pontine nuclei. To further characterize and compare the injected area between cases, we also analyzed the cortical connections revealed by the injections. Here we largely follow the criteria and nomenclature of Lewis and van Essen (2000). Figure 4 demonstrates examples of the neurofilament-based cytoarchitecture of various areas of interest. The border between area 4 and the PMd was recognized by the larger abundance of large layer V pyramidal cells and a decrease of immunoreactivity in supragranular layers (Fig. 4A; Preuss et al., 1997; Gabernet et al., 1999). In the parietal cortex (Fig. 4B), areas LIP, VIP, MIP, and 5V could be identified (Hof and Morrison, 1995; Hof et al., 1995; Lewis and van Essen, 2000). Intracortical projections were limited to a few areas, largely confirming earlier studies (Johnson et al., 1996; Matelli et al., 1998; Leichnetz, 2001; Luppino et al., 2003; Burman et al., 2014). In the parietal cortex, retrogradely labeled cells were mainly located in the medial bank of the intraparietal sulcus including areas MIP, VIP, and ventral area 5, as well as in area V6A on the medial aspect of the parietal lobe. Frontally, numerous retrogradely labeled neurons were present in...
Figure 6. Camera lucida drawings of serial sections through the midbrain of case PMd2. Intersection distance is 800 μm. For other conventions see Figure 3. The injection in PMd2 resulted in anterograde labeling restricted to the deep layers of the lateral part of the SC, to the nucleus ruber, and to multiple sites within the mesencephalic reticular formation. For abbreviations see list. Scale bar = 2 mm.
the precentral motor cortex (area 4), within dorsal area 6 anterior to the injection site and posterior in the spur of the arcuate sulcus, and in area M2. Medially, fewer neurons were located in areas 23 and 24 in the cingulate sulcus (Fig. 5).

Case PMd2

The tracer injection in case PMd2 was located more posterior than PMd1, between the anterior half of the pcd and the spur of the arcuate sulcus (Fig. 1B). Anterogradely labeled terminals in the SC were exclusively located in the deep layers of the lateral SC far away from the AChE patches. There was not a continuous band of labeled terminals from lateral to medial as in case PMd1. Additionally, labeled terminals were found in the nucleus ruber and were widespread in the mesencephalic reticular formation (Fig. 6). Cortical projections were restricted to the medial bank of the intraparietal sulcus including area MIP/ventral area 5, to the precentral area 4, and to area 6V. In contrast to case PMd1, area M2 was far less heavily labelled; areas 23 and 24 on the medial aspect of the brain were largely devoid of label (Fig. 7).

Case PMd3

Case PMd3 represents the caudalmost injection site in this study lying between the posterior tip of the precentral dimple and the posterior tip of the spur of the
Figure 8. Camera lucida drawings of serial sections through the midbrain of case PMd3. Intersection distance is 320 μm. For other conventions see Figure 3. Anterogradely labeled terminals are present mainly in the deep layers of the lateral SC and in the underlying mesencephalic reticular formation. For abbreviations see list. Scale bar = 2 mm.
arcuate (Fig. 1C). Examples of the resulting anterograde labeling are shown in Figure 2D–F. Labeled terminals were again concentrated in the deep layers predominantly in the lateral SC and in the underlying mesencephalic reticular formation (Fig. 8). In the parietal cortex, retrogradely labeled neurons were mostly found in the anterior part of the medial bank of the intraparietal sulcus (area 5V) with additional label on the suprapparietal gyrus (area 5), the infraparietal gyrus (area 7a), and in the medial bank of the lateral fissure. Fewer neurons were labeled in the posterior bank of the intraparietal sulcus, including area LIP. In the frontal cortex, labeled cells were concentrated in area 4 on the precentral gyrus, area 6V, and M2. Labeled cells in M2 were much denser on the medial aspect of the brain than on the crown of the cortex (Fig. 9).

**Case A8**

As a control and for comparison, further injections were placed into the oculomotor area on the prearcuate gyrus (area 8) in the representation of the peripheral visual field (Komatsu and Suzuki, 1985) (Fig. 1D). In this case, in addition to the BDA injection, WGA-HRP was injected at the same location, resulting in a complete overlap of the two injection sites and the resulting labeling. In contrast to the PMd projections, terminal labeling from both tracer injections into area 8 was predominantly found in the intermediate layers of the medial SC; the lateral SC was notably devoid of label (Fig. 10). Intracortical transport in this case was rather limited. In the frontal cortex, a few patches of labeled neurons were located in area M2 and area 6D (Fig. 11). Unfortunately, the posterior cortex was not available for analysis in this case.

**Figure 9. A–E: Frontal sections through the left cerebral cortex of case PMd3 showing the intracortical projections resulting from the tracer injection into the PMd. For other conventions see Figure 5. For abbreviations see list. Scale bar = 5 mm.**
Figure 10. Camera lucida drawings of serial sections through the midbrain of case A8. The plots demonstrate the WGA-HRP labeling. Inter-
section distance is 300 μm. For other conventions see Figure 3. In contrast to the PMd injections, the area 8 injection resulted in terminal 
labeling predominantly but not exclusively in the intermediate layers of the medial SC. For abbreviations see list. Scale bar = 2 mm.
Thus, the injection in PMd1 located in the anterior PMd and possibly encroaching on area 6Dr (F7; Luppino et al., 2003) resulted in terminal labeling in the intermediate and deep layers of the entire ipsilateral SC. After injections into more posterior parts of the PMd only the deep lateral part of the SC was labeled. By contrast, output from our area 8 injection mainly terminated more superficially compared with the PMd in the intermediate layers, sparing the most lateral SC.

**DISCUSSION**

In our experiments we were able to show that area PMd (F2) directly projects moderately to the intermediate and more strongly to the deep layers of the ipsilateral SC. These projections were particularly heavy in the lateral part of the SC representing the ventral visual field, and coincided with the location of reach-related neurons in the SC (Werner et al., 1997b) as well as tectofugal neurons described in other studies (Castiglioni et al., 1978; Nudo et al., 1993; Rathelot and Strick, personal communication).

**Corticotel projection from PMd**

The existence of a direct connection between the PMd and SC was suggested by earlier retrograde tracing studies (Leichnetz et al., 1981; Fries, 1984, 1985; but see Lock et al., 2003); however, the density of this projection seemed rather low. Our anterograde data indicate that the PMd projects primarily to the deep layers of the SC, below the AChE-rich patches in the intermediate layers. The most complete labeling was achieved by the anteriormost injection (PMd1), which coincided with the area where neurons could be antidromically activated from the SC in related electrophysiological experiments. Based on the location and the cortical connections, i.e., input from the V6A and MIP in the parietal cortex and from cingulate motor areas, case PMd1 includes the ventrorostral part of area F2 (Matelli et al., 1998; Luppino et al., 2003). It also corresponds to the region from where simple and complex...
movements of the distal forelimb can be elicited by intracortical microstimulation (Raos et al., 2003) and where grasp-related neurons are located (Raos et al., 2004). More posterior injections mainly labeled the lateral part of the SC. Both PMd2 and PMd3 were located in the region between the precentral dimple and the spur of the arcuate, with little or no input from the cingulate cortex (Matelli et al., 1998; Luppino et al., 2003). The core of the PMd2 injection was closer to the dimple in the region where microstimulation leads to movements of the proximal forelimb (Raos et al., 2003). PMd3 included the entire distance between the dimple and spur, thus including both proximal and distal forelimb regions (Raos et al., 2003). Therefore, all our PMd injections were in the part of area F2 that controls arm reaching movements. The differences in our SC labeling could well be due to topographical differences between the injected regions, but could also be the result of different sizes of injections. The tracer volume injected and the resulting injection site was slightly larger in PMd1 than in PMd2 and PMd3 (Table 1, Fig. 1). Similarly, topographic differences have also been reported for the projections from the basal ganglia to rostral and caudal regions of F2 (Saga et al., 2011).

Interestingly, corticotectal projections from the PMv (F5) and other areas of the cortical network for grasping, e.g., ventral prefrontal areas 46 and 12, parietal area AIP, but also from saccade-related area LIP, also terminate in the deep and intermediate layers mainly of the lateral SC (Lynch et al., 1985; Selemon and Goldman-Rakic, 1988; Borra et al., 2010, 2014). The same SC region also receives direct input from the oro-facial primary motor cortex (Tokuno et al., 1995). Injections into area 6DC (supplementary eye field) at the crown of the cerebral cortex also revealed projections to the intermediate layers of the lateral SC (Shook et al., 1990), thus placing this part of the SC in an even wider context.

Our control injection into prearcuate area 8 confirms findings of previous studies. It resulted in labeling of the intermediate layers of the SC that was stronger in the medial than in the lateral part of the SC, which may be due to the topography in the corticotectal projection from the prearcuate oculomotor cortex. Similar results were achieved by comparable injections in other studies (Komatsu and Suzuki, 1985; Lynch et al., 1985; Stanton et al., 1988; Shook et al., 1990; Tokuno et al., 1995); the labeling of the entire SC shown by Leichnetz

Figure 12. Schematic summary of the relationship of the cortical input to the SC from the PMd (red) and prearcuate oculomotor cortex (area 8, black) with the location of tectofugal neurons projecting to the cervical spinal cord (green, data taken from Castiglioni et al., 1979) and indirectly to the shoulder muscles, e.g., the spinodeltoid muscle (blue, Rathelot and Strick, personal communication). Cortical input from the PMd largely overlaps with the tectospinal output in the deep layers of the SC.
and coworkers may again be due to the larger injection site (Leichnetz et al., 1981).

Unfortunately we could not analyze the cortical connections with the posterior cortex in our area 8 case, but labeling in the posterior bank of the intraparietal sulcus including the LIP as well as in visual and polymodal extrastriate areas has been demonstrated in other studies (Leichnetz et al., 1981; Schall et al., 1985), which confirms that even our largest and most anterior PMd injection shows an intracortical projection pattern distinct from that of the preoculomotor cortex.

Figure 12 summarizes the spatial relationship of cortical projections from the PMd (red dots) and area 8 (black dots) to the SC (this study) relative to the location of tectofugal neurons retrogradely labeled from the cervical spinal cord (Castiglioni et al., 1978, green areas) or transsynaptically from the spinodeltoid muscle (Rathelot and Strick, personal communication, blue areas). For this chart the labeled SC regions from all our PMd cases were combined. There is a striking overlap of input from PMd and the SC output to the cervical spinal cord and the shoulder muscles, demonstrating the putative neuronal substrate for modulating head and arm movements via the SC.

Functional considerations

The optic tectum of nonmammalian vertebrates and the SC of mammals is traditionally viewed as a key structure for orienting behaviour, especially of the body, head, eyes, and pinnae (Akert, 1949; Alstermark and Isa, 2012). With the discovery of arm-movement–related activity in the SC, this scheme was extended to limb movements also in monkeys in agreement with earlier studies in the cat (Courjon et al., 2004). Reach neurons are located in the intermediate and deep layers predominantly in the lateral part of the monkey SC and in the underlying mesencephalic reticular formation (Werner 1993; Werner et al., 1997a, b; Stuphorn et al., 1999, 2000) and are active before and during reach movements mainly but not exclusively of the contralateral arm. They reside in a region where descending tectofugal projections arise (Castiglioni et al., 1978; Grantyn and Grantyn, 1982; Olivier et al., 1991; Nudo et al., 1993; Rathelot and Strick, personal communication).

Recent microstimulation experiments revealed that arm movements can indeed be elicited by microstimulation at recording sites of collicular reach neurons (Philipp and Hoffmann, 2014), and that ongoing reach movements can be perturbed by simultaneous SC stimulation in the cat (Courjon et al., 2004). Another subpopulation of SC neurons has a clear somatosensory component, as these neurons are active when the hand touches and pushes a target but are inactive during the reach phase (Nagy et al., 2006). They were also located in the intermediate and deep layers of the SC, between 1.2 and 4 mm below the collicular surface (median = 3.1 mm), i.e., in the same depths as the reach neurons (median = 2.8 mm, range = 1.4–4 mm). The coincidence of the anatomical location of SC reach and somatosensory-motor neurons with the cortical afferents originating from the dorsal premotor (this study) and ventral premotor cortex and related cortical areas (Borra et al., 2014) suggests that the SC is strongly involved in arm and hand movements in addition to the well-known gaze movements. The convergence of the reach-to-grasp system (i.e., the PMv) and the reach-to-destination system (the PMd) onto the same regions close to the gaze system in the SC puts this structure in a unique position to modulate reach and grasp movements in various behavioral contexts involving eye-hand coordination.

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CONFLICT OF INTEREST STATEMENT

The authors state they have no conflicts of interest.

ROLE OF AUTHORS

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: KPH and CD, acquisition of data: KPH and CD, analysis and interpretation of data: CD and KPH, drafting of the manuscript: CD and KPH.

LITERATURE CITED


