Cortical Connections of Parietal Field PEc in the Macaque: Linking Vision and Somatic Sensation for the Control of Limb Action

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The cortical projections to the caudal part of the superior parietal lobule (area PEc) were studied in 6 cynomolgus monkeys using fluorescence tracers. Significant numbers of labeled cells were found in a restricted network of parietal, mesial, and frontal areas. Quantitative analysis demonstrated that approximately 30% of the total projection neurons originated in the adjacent areas of the dorsocaudal part of the superior parietal lobule (areas PE and V6A). The medial bank of the intraparietal sulcus, inferior parietal lobule, and frontal lobe (mainly the dorsocaudal part of premotor area F2) each contributed approximately 15% of the projection neurons. About 15% of the labeled neurons were located in the posterior cingulate area (PEci) and another 10% in other areas of the mesial surface of the hemisphere. Based on these data, we suggest that PEc processes information about the position of the limbs. The specific anatomical links between PEc and motor and premotor areas that host a representation of the lower limbs, together with the link with vestibular cortex and with areas involved in the analysis of optic flow and spatial navigation, imply a role for PEc in locomotion and coordinated limb movement in the environment.

Keywords: arm reaching, leg representation, locomotion, posterior parietal cortex, proprioception

Introduction

In a reappraisal of classical anatomical studies (Brodmann 1909; Vogt and Vogt 1919; von Bonin and Bailey 1947), Pandya and Seltzer (1982) defined cytoarchitectonically the region in the caudal pole of the superior parietal lobule of rhesus monkeys as area PEc. As shown in Figure 1, this area occupies the caudalmost third of the exposed cortex of the superior parietal lobule and extends onto the mesial surface of the hemisphere where it borders area PGm/7m (Pandya and Seltzer 1982; Cavada and Goldman-Rakic 1989). The unique architectural profile of PEc was recently confirmed in the cynomolgus monkey (Luppino et al. 2005), in a study that also described the criteria for the demarcation of the caudal border of PEc with adjacent visual area V6A (Galletti et al. 1999), in the anterior bank of the parieto-occipital sulcus.

Recent physiological studies have yielded insights on the sensory and motor properties of cells in PEc. Many neurons in this area respond to moving visual stimuli such as moving bars and optic flow signals (Squatrito et al. 2001; Raffi et al. 2002; Breveglieri et al. 2008), whereas others respond to tactile stimulation and passive single-joint rotation, mainly of the upper limbs (Breveglieri et al. 2006); bimodal activation occurred in about 20% of the cells (Breveglieri et al. 2008). In the above studies, no clear retinotopy or somatotopy was discerned. Within the cytoarchitectural territory of PEc, visual responses become predominant caudally, toward V6A, whereas somatosensory responses become predominant rostrally, toward area 5/PE (Breveglieri et al. 2006). Behavioral paradigms such as oculomotor and reaching tasks drive PEc neurons, with several cells further influenced by eye-position information (Fattori et al. 2000; Battaglia-Mayer et al. 2001; Ferraina et al. 2001; Raffi et al. 2008). All these findings challenged the traditional view of the superior parietal cortex as a somatic associative region (Hyvärinen 1982; Pandya and Seltzer 1982), suggesting its involvement in visuomotor behavior (Johnson et al. 1996; Battaglia-Mayer et al. 2001) and in the internal perception of oneness (Breveglieri et al. 2006).

Earlier studies tracing the cortical connectivity pattern of the caudal superior parietal lobule did not discriminate between areas PE and PEc (Peele 1942; Pandya and Kuypers 1969; Jones et al. 1978; Lynch 1980). More recent papers have reported that PEc projects to the parietal and dorsolateral premotor cortices (Pandya and Seltzer 1982, Petrides and Pandya 1984; Matelli et al. 1998; Tanne-Gariepy et al. 2002). The only study that specifically focused on the retrograde connectivity of PEc (Marconi et al. 2001) was published before the anatomical organization of the areas that border PEc caudally was clarified (see Luppino et al. 2005). Thus, we investigated the sources of cortical projections to the cytoarchitecturally defined area PEc. The current findings demonstrate that PEc is linked to a restricted network of somatosensor-related parietal, mesial, and frontal areas, implying a role in locomotion and coordinated limb movement in the environment.

A preliminary account of the present data has been presented in abstract form (Bakola et al. 2008).

Materials and Methods

Fluorescent retrograde tracers were injected or applied as crystals in area PEc in 7 hemispheres of 6 cynomolgus monkeys (Macaca fascicularis, 3–7 kg). All experimental protocols were approved by the Bioethical Committee of the University of Bologna and complied with the European Directive 86/609/EEC on the care and use of laboratory animals.

The animals were pretreated with atropine (0.04 mg/kg, i.m.) and ketamine hydrochloride (15 mg/kg, i.m.) and, after 30 min, anesthetized with sodium thiopental (8 mg/kg, i.v.). In order to avoid edema, mannitol was administered intravenously (1 g/kg). Heart rate, blood pressure, respiratory depth, and body temperature were constantly monitored by veterinary staff. Surgical procedures took place in standard aseptic conditions.

When deep anesthesia was achieved, the animals were secured to a stereotaxic frame, and a craniotomy was performed over the medial posterior parietal cortex. The dura mater was cut and retracted to 1 side, to expose the dorsal surface of the superior parietal lobule. In some cases, fluorescent compounds were injected using a Hamilton microsyringe that had a glass micropipette attached to its needle. Tracers included dianidinyloxy-labeled Alexa fluor 488 (CTB green) conjugated with sodium thiopental (8 mg/kg, i.v.). In order to avoid edema, mannitol was administered intravenously (1 g/kg). Heart rate, blood pressure, respiratory depth, and body temperature were constantly monitored by veterinary staff. Surgical procedures took place in standard aseptic conditions.

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Fast Blue (FB) and DY were applied as crystals (Rosa et al. 2005) by visual inspection of the exposed cortex. A summary of case types, of amount of tracers injected, and of plane of sectioning is provided in Table 1. In the 3 cases in which tracers were injected via a microsyringe (Table 1), the tip of the attached micropipette was lowered 1.5 mm into the cortex, and a small amount of tracer was slowly released at that depth. Then, the micropipette was slowly withdrawn in steps of one or few hundred micrometers, with small amounts of tracer being released at each location up to about 1-mm depth. Overall, these injections lasted approximately 30--40 min (the figures listed in Table 1 refer to the total amount of tracer released at various sites).

During surgery, selection of injection sites was guided by direct visualization of the intraparietal and parieto-occipital sulci and consideration of the typical extent of area PEC in this species (Breveglieri et al. 2006). Thus, we aimed at the cortex located between the midline and the lip of the intraparietal sulcus, no more than 7 mm rostral to the lip of the parieto-occipital sulcus. After completing the injection procedure, the cortex was covered with surgical foam, the bone was fixed in place, and the dura mater, muscles, and skin were sutured. When animals regained consciousness, they were returned to their cage. Analgesics (ketorolac, 1 mg/kg, i.m.) and antibiotics (erythromycin, 1--1.5 mL/10 kg) were provided postoperatively.

After a survival period of 14 days, the animals were preanesthetized with ketamine hydrochloride (15 mg/kg, i.m.) followed by a lethal dose of sodium thiopental i.v. They were perfused transcardially first with 3 L of saline and then with 5 L of 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4, and 4 L of 5% glycerol in the same buffer. Brains were cryoprotected by immersion into buffered solutions containing 10% buffer, pH 3.7) to determine cortical cytoarchitecture, areal borders, and location of injection sites.

**Data Analysis**

Fluorescent material was analyzed under a Zeiss Axioscope equipped with ×10 and ×20 objective. Figure 2 illustrates photomicrographs of representative injection sites in PEC (Fig. 2A,D) and examples of retrogradely labeled cells (Fig. 2C,E). For all sections examined, the inner limit of layer VI and the outer limit or layer I, the outlines of the injection sites, and the location of labeled cells were charted using custom-made software with the aid of X/Y transducers mounted on the microscope stage. Labeled neurons were typically plotted every 600 μm (1 section every 2, Gamberrini et al. 2009). In all sections analyzed, the entire cortex was examined for retrograde labeling. Two- and 3-dimensional reconstructions of cortex were produced with CARET software (http://www.nitrc.org/projects/caret/; Van Essen et al. 2001), from midthickness section contours, according to the procedures described previously (Galletti et al. 2005). The same software was employed to prepare the density maps of labeled neurons (see Figs. 4 and 5) by projecting the location of each neuron to the nearest midthickness contour. For each map, the number of labeled neurons was calculated in area units of 600 × 600 μm superimposed on the flattened reconstruction. The area unit that contained the highest number of labeled cells was considered as reference, and the density of projection cells was expressed as a percentage of this maximum unit value (Rosa et al. 2009).

The borders of area PEC were defined in Nissl-stained sections following established cytoarchitectonic criteria (Pandya and Seltzer 1982; Pandya and Seltzer 1982; Luppino et al. 2005). In the instances where cortical organization was disrupted due to methodological factors (such as tracer injection), boundaries were drawn at the average distance between the recognized boundaries in the previous and following sections. To obtain an objective estimate of the average extent of PEC, the surface of PEC in each hemisphere was calculated on flattened maps of the superior parietal lobule. Surfaces from individual hemispheres were then averaged to produce the reference contour of the region.

When possible, labeled neurons were assigned to specific areas on the basis of cytoarchitectonic descriptions of the areas existing in literature. For neighboring areas PE, PECi/SSA (supplementary somatosensory area, Murray and Coulter 1981), and PGM, we relied on the report by Pandya and Seltzer (1982); for area V6A on Luppino et al. (2005). Somatosensory area 2 was designated according to Jones et al. (1978). The inferior parietal lobule was parcellated according to Pandya and Seltzer (1982) and Gregoriou et al. (2006). Labeled cells in the upper bank of the lateral fissure were attributed to PGop according to Pandya and Seltzer (1982) and to area Ri of Robinson and Burton (1980). For the identification of the areas of the cingulate sulcus, we consulted several sources (Matelli et al. 1991; Morecraft and Hoesen 1992; Morecraft et al. 2004; Vogt et al. 2005). In particular, our designation of area 23 in the ventral bank of the caudal cingulate sulcus includes the dysgranular region (23d) of Vogt et al. (2005), and our designation of midcingulate area 24d includes much of Vogt’s et al. (2005) area p24d. The premotor cortex was subdivided following the criteria of Matelli et al. (1991) and Geyer et al. (2000). In a few cases (e.g., when the plane of sectioning was not favorable), assignment
relied primarily on location relative to sulcal landmarks, using the above reports as a guide.

Because the type and amount of different tracers result in variable numbers of labeled cells, the distribution of labeled cells in each area was expressed as a fraction of total retrograde labeling in individual cases, as previously reported (Galletti et al. 2001; Gamberini et al. 2009). Quantified data from case A3R were not included for reasons discussed below (see Results). The estimation of the laminar pattern of projection neurons was determined by calculating the percentage of labeled cells in supragranular layers with respect to the total number of labeled cells in each area (%SLN, Barone et al. 2000).

To calculate the distribution of labeled cells in different laminae, the plot of fluorescent labeling was superimposed with the adjacent Nissl-stained section (scaled in the same magnification) using the camera lucida. Digital images were acquired with a Zeiss AxioCam and the AxioVision v.4.4 software (Carl Zeiss, Oberkochen, Germany). Final figures were prepared with Adobe Illustrator and Photoshop (Adobe Systems Inc, San Jose, CA). This involved adjusting brightness/contrast, scaling of original drawings to appropriate size, and compiling individual figures to construct composite illustrations. Raw data were not altered by these manipulations.

Results

We describe the cortical distribution of projection neurons to area PEc based on 8 cases of retrograde fluorescent tracer injections or crystal applications. Figure 3 shows injection sites and sites of crystal application on 2-dimensional maps. To help compare the locations of injection sites in different cases, these have been projected onto a single “reference” brain, along with the average outline of the cytoarchitectural border of PEc. A summary of the strength of retrograde labeling after PEc injections in all the studied hemispheres is presented in Table 2.

Large PEc Injections

To obtain information about the regional distribution of projection neurons to PEc, we placed multiple crystals of a single tracer (DY) in 2 hemispheres of different animals (A5R

Figure 2. Photomicrographs of PEc injection sites and of retrogradely labeled cells in representative target areas. (A) DY crystal applied in the intermediate part of PEc (case MF20L). (B) Injection of CTB green in the caudal part of PEc (case A2R). Scale bar in A and B (shown in B) = 500 μm. (C) DY-labeled cells in area PE after a crystal application in case MF20L. Scale bar = 300 μm. Inset: (D) Higher magnification (×20) of DY-labeled cells in area PE after placing a DY crystal in case MF20L. Scale bar = 200 μm. (E) CTB green-labeled cells in layer III of area 23 after injection in case A2R. Scale bar = 100 μm.

Figure 3. Injection sites. In panels (A--C), the injection sites of different cases are reported on 2D reconstructions of the caudal part of the superior parietal lobule of a macaque reference brain (shown on the left). Dashed contours in the 3D reconstruction of reference brain and in flattened maps represent the average cytoarchitectural border of PEc. Scale bar for the 2D maps, shown in (C), = 3 mm. ips, intraparietal sulcus; Is, lunate sulcus; A, anterior; and M, medial. Other abbreviations as in Figure 1.
and A4R, Fig. 3A,B, Table 1). The results are presented in the flat maps and in representative sections in Figures 4 and 5.

In the parietal lobe, significant numbers of labeled cells arose from area PE, immediately rostral to the injection sites (Figs. 4C,D and 5A–D). In the intraparietal sulcus, projection neurons were located in the caudal half of the medial bank (Figs. 4A,B and 5B,C). Projection neurons from this region were quite distinct in terms of density from those originating more ventrally in the medial bank. Thus, despite the lack of a clear architectural boundary, we adopted the designation dMIP to refer to the densely labeled region in the dorsal part of the medial bank (see Figs. 4A,B and 5C). We retained the designation MIP for the more ventral cortex in the caudal half of the medial bank, in agreement with the original report by Colby et al. (1988; see also Fig. 1). Caudal to the injection sites, labeled neurons were observed in the anterior bank of the parieto-occipital sulcus, within the dorsal part of area V6A (V6Ad in Fig. 1, on the flat maps of Figs. 4 and 5, and in Fig. 5A,B).

On the lateral surface of the hemisphere, moderate numbers of labeled neurons originated in somatosensory area 2 on the exposed postcentral gyrus (Figs. 4D and 5B–D). In case A5R, but not A4R, labeled cells were observed in the inferior parietal lobule (PG, Fig. 4B,C), possibly because injection sites in the latter case covered mainly the rostral half of PEC (Fig. 3B). A similar finding has been reported before (Pandya and Seltzer 1982). Both cases with large injections in PEC revealed retrograde labeling in the caudal upper bank of the lateral fissure (PGop, Figs. 4B,C and 5E,F). Further anterior in the depths of the lateral fissure, projection neurons were found in the retroinsular area (RI, Figs. 4C and 5E) and the caudal part of the insula (IG, Figs. 4E and 5E,F). These regions have been indicated as parietoinsular vestibular cortex (PIVC, Guldlin and Grusser 1998).

On the mesial cortex, large numbers of projection neurons originated in somatosensory area PECi (Figs. 4B,C and 5A–C), at the caudal tip of the cingulate sulcus, and in area 23, in the ventral bank of the sulcus (Figs. 4D,E and 5A,B). Fewer labeled cells were found in the transitional somatosensory area of the upper cingulate bank (TSA, flat map of Figs. 4 and 5, and Fig. 5B) and, at more anterior locations, in areas 24d (Figs. 4G and 5A,B).

![Figure 4](image_url)

**Figure 4.** Cortical distribution of labeled cells after placing DY crystals in most of the extent of PEC. (Left) 7 representative coronal sections (A–G) taken at the level shown in the brain figure. Black dots on sections represent labeled cells. D, dorsal and L, lateral. (Right) 2D reconstruction illustrating the distribution of labeled cells. Color scale indicates the relative density of labeled cells, counted within 800 × 600-μm units, as a percentage of the maximum unit value. The dashed line represents the transition between the lateral surface and the mesial wall of the hemisphere. Abbreviations as in Figures 1 and 3.
and 24c (flat maps of Figs. 4 and 5, and Fig. 5A). In addition, labeled neurons were evident in areas PGm (Figs. 4A and 5A) and 31 (flat maps of Figs. 4 and 5 and Fig. 4B, C).

In the frontal lobe, the majority of neurons projecting to PEc originated in premotor area F2 (superior area 6), mainly in its dorsocaudal part (Figs. 4F, G and 5B, C), with fewer labeled neurons in the ventral part of F2, in particular, within the caudal bank of the arcuate sulcus (Figs. 4G and 5D). Labeled neurons were also observed in the dorsomedial part of the primary motor cortex (F1, Figs. 4E and 5B, C) and in the caudal part of area F3/SMA-proper (Figs. 4F and 5A). In case A5R, a few labeled cells were observed in the rostral area pre-SMA (area F6) and in the inferior limb of the arcuate sulcus (see flat map of Fig. 4).

To summarize, after injections that involved large parts of PEc, significant numbers of retrogradely labeled cells were observed in the adjacent posterior parietal cortex, within and around the cingulate sulcus (mainly in its caudal part), in the caudal inferior parietal lobule, and in frontal motor cortex.

**Small PEc Injections**

Although demonstrating in general the presence of retrograde labeling in sensorimotor cortices, the cases described above address the possibility that different subregions of this area may have different emphases in their pattern of projection neurons. Thus, we have also placed a series of smaller injections in different PEc locations.

In cases A1R, A2R, and A3R, injections were administered at caudal PEc locations (Table 1, Fig. 3C). The most caudal injection (A3R) involved only the superficial layers and resulted in small numbers of labeled cells. In addition, the halo of the injection site invaded area V6Ad, at the caudal border of PEc. Thus, although its pattern of retrograde labeling was qualitatively similar to the other 2 cases with caudal injections (Table 2), results of case A3R were excluded from the quantitative analyses.

The upper part of Figure 6 shows the distribution of retrogradely labeled cells in a graphic 3D reconstruction of case A2R (see also Gamberini et al. 2009). The overall density of labeled cells was weaker in comparison to that resulting from multiple injections (Figs. 4 and 5), but the topography of projection neurons was largely the same. For example, labeled cells were numerous in superior parietal areas PE and V6Ad, as well as in the caudal part of the medial bank of the intraparietal sulcus. In the inferior parietal lobule, labeled cells were observed in area PG but not in the parietal operculum or the insula. Similar to the cases with larger injections, several
patches of labeled cells were found on the mesial surface of the hemisphere, within and around the caudal and middle portions of the cingulate sulcus (areas 23, 24d, and 31). In the frontal cortex, modest numbers of projection neurons were observed in premotor area F2, where approximately equal numbers of labeled cells occupied the posterior arcuate bank and the dorsocaudal convexity. A small proportion of labeled cells were shown in mesial areas F3/SMA-proper and in the anterior part of superior area 6 (area F7). Contrary to the 2 cases with large PEC involvement described in the previous section, the primary motor cortex and the postcentral somatosensory areas were devoid of labeled cells.

In another hemisphere (case M20L), 2 injections placed around the central portion of PEC, one (DY) medially and another (FB) laterally (Table 1, Fig. 3C), showed similar distributions of labeled cells (Table 2). The middle part of Figure 6 illustrates the results obtained in one of these cases (M20L/DY). The regional distribution of labeled cells followed the general pattern of PEC connectivity, with dense concentrations of labeled neurons in the posterior parietal (PE and V6Ad) and caudal mesial (PECi, 23) areas. Large numbers of projection neurons were concentrated in the dorsalmost part of the medial bank of the intraparietal sulcus (dMIP), whereas only sparse labeled cells were found ventrally (MIP). Labeled neurons were also found in area PG and, contrary to the more caudal cases, in the upper bank of the lateral fissure (PGop) and the insula. In addition, projection neurons were observed in area 2, in the anterior parietal somatosensory cortex. In the rostral parts of the cingulate sulcus, labeled neurons were located in areas 24d and 24c. In the frontal lobe, area F2 was heavily labeled in the dorsal exposed surface of the hemisphere, around and medial to the precentral dimple, whereas a weaker proportion of labeled cells arose from the caudal bank of the posterior part of arcuate sulcus. Low numbers of labeled neurons were found in the primary motor cortex (F1) and in area F3. A few scattered cells were noted in area F7 and around the principal sulcus.

The lower part of Figure 6 shows a case (A5L) in which one FB crystal was placed in a rostromedial part of PEC (Table 1, Fig. 3C). Representative coronal sections of this case illustrate in greater detail the regional topography of retrograde cells (Fig. 7). Similar to the previously reported cases, a big proportion of labeled cells originated in areas PE (Fig. 7C,D) and PECi (Fig. 7B–D). The dorsal third of the caudal medial bank of the intraparietal sulcus (dMIP) revealed moderate numbers of labeled cells (Fig. 7A–C), and few cells were also noted in the
ventral middle third, presumably in area MIP (Fig. 7C). Labeled neurons were found in area 2 (see bottom 3D map of Fig. 6) and in the areas of the upper bank of the lateral fissure (PGop, Fig. 7C, Ri, Fig. 7C, D) and the insula (Ig, Fig. 7E). In contrast, no projection neurons were found in area PG on the exposed surface of the inferior parietal lobule. The mesial cortex revealed labeled cells in areas 31 (Fig. 7C), 23 (Fig. 7E), 24d (Fig. 7H), and 24c (Fig. 7H). On the frontal cortex, projection neurons were located on the exposed part of superior premotor area F2 (Fig. 7G), as well as in the primary motor cortex (F1, Fig. 7E) and in area F3 (Fig. 7F).

Taken as a whole, the present results indicate a trend in the topography of labeled cells (see also Table 2). Caudal parts of PEc are targeted by neurons located in the dorsal part of the visual parieto-occipital area V6A (V6Ad), whereas rostral parts of PEc by neurons located in somatosensory as well as motor cortices. In addition, when injections involved the anterior part of PEc, the majority of labeled cells in the premotor area F2 (superior area 6) occupied a region within and medial to the precentral dimple, whereas after injections in the caudal part of PEc, projection neurons were also observed in the F2 sector located in and around the posterior bank of the superior arcuate sulcus (e.g., compare the 3 cases shown in Fig. 6).

Quantitative Analysis

Figure 8A illustrates the cortical fields that contained the most significant numbers of projection neurons, that is, those corresponding to >1% of the total number of projection neurons, based on data from 7 cases (excluding case A3R). Although the number of labeled neurons in each area varied with the type and amount of tracer, the topography of labeled cells was consistent among our cases (see also Table 2). Projection neurons from the adjacent areas of the superior parietal lobule (V6Ad and PE) formed the main component of retrograde labeling to PEc, accounting for approximately 30% of total labeled cells. The caudal part of the medial bank of the intraparietal sulcus contributed for about 15% of total projection neurons, with the majority of labeled cells located in the dorsalmost part of the sulcus (dMIP). Approximately equal proportions of projection neurons originated in lateral parietal cortex, including areas 2, PG, PGop, and in vestibular cortex (PIVC), which comprises the retroinsular area and the posterior insula. Among the areas of the mesial cortex, the largest proportion of labeled cells (about 25%) originated in areas PEci and 23. In the frontal lobe, the highest numbers of labeled neurons (about 10% of the total labeled population) were found in superior area 6 (F2). Areas that contributed minor percentages of labeled cells (<1%, not shown in Fig. 8) included the somatosensory cortex on the dorsal bank of the cingulate sulcus (TSA), area 24c, and the rostral part of mesial, superior, and inferior premotor cortex (areas F6, F7, and F5, respectively).

Analysis of the laminar origin of projection neurons in the 7 cases with quantified data returned a wide range of %SLN values (37–80%, Table 2; see also Fig. 8B). Grant and Hilgetag (2005) proposed a model according to which, projections with %SLN values from 0 to 33 were classified as feedback, between 39 and 69 as lateral, and >76 as feedforward. Intermediate %SLN values could not be attributed with confidence to one of the adjacent types of projections. The laminar patterns of labeled neurons in most of the areas and regions were of the lateral type, characterized by %SLN values between 39 and 69. None of the areas with labeled neurons showed the feedback type of projections, whereas areas PG and PGop (%SLN 37) could not be unambiguously assigned to the feedback or lateral type. Visual inspection of the %SLN histogram of Figure 8B shows that the distribution of labeled neurons in different laminae should be best described as graded rather than be forced into distinct categories. Among all areas examined, the 3 areas and regions that displayed the lowest %SLN values (37–43) were around the inferior parietal lobule (PG, PGop, and

Figure 7. Cortical distribution of labeled cells after injection in a rostral PEc location. Representative coronal sections were taken at the levels shown in the brain figurine. The same case is shown in the bottom part of Figure 6 as 3D reconstruction. ias, inferior limb of arcuate sulcus; sas, superior limb of arcuate sulcus. Other abbreviations as in Figures 1 and 3.
PIVC), possibly suggesting a different level of processing from the rest of parietal areas. At the opposite end, frontal areas displayed relatively high %SLN values. Although surprising, this finding has been repeatedly observed before (Jones et al. 1978; Shipp et al. 1998; Barone et al. 2000; Burman et al. 2006; Gamberini et al. 2009). The functional significance of these observations is difficult to reconcile with current models of information flow in the monkey brain (Felleman and Van Essen 1991). It might be that through feedforward processing, the frontal cortex informs high-order parietal areas of an impending motor action so as to facilitate perceptual stability.

Discussion

According to the revised cytoarchitectural organization of the caudal part of the macaque superior parietal lobule (Luppino et al. 2005), this cortical region houses 2 areas: V6a, in the anteromedial part of Brodmann’s area 19 (anterior bank of the parieto-occipital sulcus; see Fig. 1), and PEc in the caudalmost part of Brodmann’s area 5 (exposed surface of the superior parietal lobule; see Fig. 1). Functionally, both areas have been reported to participate in reaching movements (Gallelli et al. 1997; Battaglia-Mayer et al. 2001; Fattori et al. 2001, 2005; Ferraina et al. 2001; Breveglieri et al. 2006). Hodologically, it has been recently reported (Gamberini et al. 2009) that the spatial distribution of labeled cells after injections in the dorsal part of V6A (V6Ad) fits well with the proposed visuomotor role for this area (Gallelli et al. 2003). The present paper investigates the cortical topography and densities of projection neurons after injections in nearby area PEc and attempts to evaluate their functional significance.

Sensory Projections

Reflecting expectations based on the response properties of PEc neurons (Breveglieri et al. 2006, 2008), the highest numbers of labeled cells projecting to PEc originated in somatosensory-related cortex, that is, areas PE/5 and PEci/SSA (Duffy and Burchfiel 1971; Sakata et al. 1973; Mountcastle et al. 1975; Murray and Coulter 1981; Andersen et al. 1990). In the caudal part of the medial bank of the intraparietal sulcus, labeled cells were mainly found in the dorsal part of the bank, a region termed here dMIP, whereas only minor retrograde

Figure 8. (A) Average percentages of labeled neurons in different cortical fields after injections in area PEc, pooled from data of 7 cases. Only cortical areas and regions that contained significant (≥1%) percentages of labeled cells are shown. Areas and regions have been grouped according to brain location indicated on the left. (B) Laminar organization of projection neurons. Areas and regions (represented in boxes) that contained labeled neurons have been positioned according to their %SLN values. Those included within the 2 dashed lines (%SLN values between 39 and 69) displayed lateral patterns of projections (Grant and Hilgetag 2005). Abbreviations as in Figures 1 and 3, and Table 2.
labeling was observed within the traditional limits of area MIP (Colby et al. 1988). Considering the existence of a dorsoventral, somatosensory-to-visual, gradient in the medial wall of the intraparietal sulcus (Colby and Duhamel 1991; Savaki et al. 1993), the location of labeled cells again reflects dominance by the somatosensory modality. Finally, we observed projection neurons in the postcentral area 2 and the somatosensory-vestibular cortex in the depths of the lateral fissure. These findings have not been previously highlighted in the literature, though there has been at least one report of an injection in macaque somatosensory area 2 that led to labeling in the caudal intraparietal sulcus, possibly within the borders of PEc (Pons and Kaas 1986). In addition, connections between the ventral somatosensory areas and superior parietal lobule have been described in New World monkeys (Akbarian et al. 1988).

The main visual input to PEc originated in the medial parieto-occipital area V6a, where more than half of neurons show visual responses (Galletti et al. 1999). Given that labeled cells originated in the dorsal part of V6a, where somatosensory cells are concentrated and where visual neurons are scarcer compared with the ventral part of the area (Fattori et al. 1999), this finding too underlines the importance of somatosensory information for defining the function of PEc. After injections in PEc, we did not find labeled neurons in visual areas VIP, AIP, MST, and V4, in contrast with an earlier study that described projections to caudal parts of PEC from these fields (Marconi et al. 2001). Our findings are in line with previous studies where retrograde tracers were placed in those cortical areas and no labeled neurons were found in PEC (Boussaoud et al. 1990; Lewis and Van Essen 2000; Borra et al. 2008; Ungerleider et al. 2008). Because the study of Marconi et al. (2001) predated the current knowledge of areal boundaries in the caudalmost part of the superior parietal lobule, the discrepancies with their results could be explained by the possible involvement of nearby areas in the caudal injection site of Marconi et al. (2001). In fact, areas adjacent to the caudal border of PEC (PGm and V6Ad) have been reported to be connected with VIP and MST (Cavada and Goldman-Rakic 1989; Shipp et al. 1998; Leichnetz 2001; Gamberini et al. 2009), and V6Ad is also targeted by area AIP (Gamberini et al. 2009).

The predominance of proprioceptive responses in PEC (Breveglieri et al. 2006), as well as in the parietal areas that project to it (Mountcastle et al. 1975; Kaas et al. 1979; Murray and Coulter 1981; Taoka et al. 2000), likely highlights a network of areas that code sensory events related to the position of body parts in space. The projection from parietal area 2 in particular, a field that appears to be present only in those primates that demonstrate skillful use of their hands (Padberg et al. 2007), is consistent with the more sophisticated use of limbs in macaques that allows them to grasp objects with both hands and feet. These suggestions are complemented by the rich anatomical links of parietal cortex with the areas of the posterior mesial cortex (see also Pandya and Seltzer 1982; Petrides and Pandya 1984; Marconi et al. 2001; Morecraft et al. 2004) associated with movement in the environment (Saito et al. 1986; Wall and Smith 2008).

Frontal Motor Projections
The present data are congruent with the previous finding that area F2 (part of superior area 6) forms the main motor connection of PEC (Chavis and Pandya 1976; Petrides and Pandya 1984; Matelli et al. 1998; Marconi et al. 2001; Tanne-Gariety et al. 2002). In our study, the majority of F2 projection cells (~75%) were found in the exposed dorsocaudal part of the area, around the precentral dimple, which is rich in somatosensory-related neurons, whereas fewer projection neurons were located in the visually responsive portion of F2 in and around the caudal bank of the arcuate sulcus (Fogassi et al. 1999). The only other premotor area that showed a significant, albeit smaller, concentration of labeled cells was mesial area F3/SMA-proper (see also Petrides and Pandya 1984; Marconi et al. 2001). Similar to PEC (Breveglieri et al. 2006), a high proportion of neurons in F3 respond to passive manipulation of the limbs (Humelsheim et al. 1988; Matsuzaka et al. 1992) and only a small fraction to visual stimulation (Matsuzaka et al. 1992). Overall, these data emphasize the specific anatomical link between PEC and those caudal premotor structures (areas F2 and F3) that process somatosensory information and which, via their direct connections with the spinal cord and motor cortex, are directly associated with movement execution (Barbas and Pandya 1987; Luppino et al. 1993; Belmalih et al. 2007).

Somewhat to our surprise, labeled neurons were also noted in primary motor cortex, in an anterior location where neurons with proprioceptive properties have been recorded (Tanji and Wise 1981). Projection neurons from this cortical field violate the notion that rostral area PE is connected to motor cortex, whereas caudal area PE (presumably PEC) to premotor cortex (Jones et al. 1978). In the same line, no connections with motor cortex were reported by previous studies involving injections in PEC (Petrides and Pandya 1984; Marconi et al. 2001). However, our results were consistent in all cases with tracer injections in relatively anterior parts of PEC (see Table 2). One possibility is that tracer in our injections invaded area PE, but even when injection sites were small and quite distant from the cytoarchitectonic border of PE (cases A5L, M20L/FB, M20L/DY; see Figs. 6 and 7), the projection from the primary motor cortex was evident. On the other hand, previous tracing of precentral connectivity produced labeling that extended to the caudalmost part of the superior parietal lobule (Künzle 1978), and injections of bidirectional tracers in the dorsolateral precentral gyrus revealed labeling up to the caudal end of the intraparietal sulcus (Leichnetz 1986).

PEC Somatotopy
The present results show that PEC is more heavily targeted by neuronal populations located in the dorsocaudal part of premotor area F2. The somatotopic organization of F2 has been inferred by the distribution of corticospinal projections (Dum and Strick 1991; He et al. 1993), microstimulation (Godschalk et al. 1995; Raos et al. 2003), and electrophysiological recordings (Kurata 1989; Raos et al. 2003). These findings converge to demonstrate that the precentral dimple marks the approximate border between proximal leg representation, medially, and proximal arm representation, laterally. Therefore, the location of projection neurons in F2 suggests that PEC hosts a representation of the limbs, particularly the hindlimb. A number of additional observations point to the same direction. The specific locations of primary motor and sensory cortices that project to PEC correspond to leg fields (Jankowska et al. 1975; Nelson et al. 1980; Tanji and Wise 1981; Kaas 1983; Hatanaka et al. 2001). Leg representation fields have also been described in other locations that project monosynaptically to PEC: the caudal part of mesial area F3/SMA-proper (Mitz and Wise 1987; Luppino et al. 1991), the caudal
ventral bank of the cingulate sulcus (CMAv, Hutchins et al. 1988; Dum and Strick 1991), and the dorsal bank of the cingulate sulcus (CMAd, Dum and Strick 1991; 24d, Luppino et al. 1991). In contrast, there is a paucity of labeled cells from areas dedicated to arm/hand movements, such as the anterior part of cingulate area 24 (24c, Luppino et al. 1991) and the inferior premotor cortex (Muakkassa and Strick 1979; Gentili et al. 1988; Rizzolatti et al. 1988).

The abundance of projection neurons in zones that represent the lower limbs contrasts with the reported involvement of PEc in manual tasks (Fattori et al. 2000; Battaglia-Mayer et al. 2001; Ferraina et al. 2001; Breveglieri et al. 2006; Evangeliou et al. 2009) as well as with the reported somatosensory overrepresentation of the upper limbs in PEc (Breveglieri et al. 2006). Such a contrast could be due to a methodological bias: Experiments in behaving primates are usually conducted in animals trained to sit quietly, and it may be simply the case that the types of tasks likely to reveal a role in control of legs (posture, locomotion, etc.) have not yet been tested in detail. It could also be due to a more limited neuronal sampling in studies to date from anterior PEc locations, where lower limbs are more represented (Breveglieri et al. 2006). In line with the latter view, the present data demonstrate that after injections in PEc, the areal distribution of retrogradely labeled cells is not uniform, with the anterior part of PEc being more strongly targeted by somatosensory and motor areas than the caudal part. This is reminiscent of the asymmetrical connections of area 2, with foot representation displaying wider connections with the motor fields than arm representation (Pons and Kaas 1986).

If we take into account the relative emphasis of leg-field projections to PEc (present results) and the relative emphasis of arm-field projections to adjacent superior parietal area V6Ad (Gamberini et al. 2009), it could be suggested that the caudal pole of the superior parietal lobe, taken as a whole, contains the neuronal machinery to help control of both lower and upper limb movements. Indeed, it has been argued that the ancestral mammalian condition involved a posterior parietal field that was architecturally heterogeneous but formed a complete body representation (Manger et al. 2002). For macaques, interactions between these fields would probably be very important during locomotion through complex, patterned environments, where coordination between arms and legs is essential.

**Concluding Remarks**

As outlined in Figure 9, after injections in PEc, the most prominent concentrations of labeled cells were found in the somatosensory and somatomotor areas of the parietal lobe and the dorsolateral premotor cortex. These results indicate that PEc is functionally related to skeletonmotor control. In marmosets, the distribution of labeled neurons after injecting the cortical region at the border between the anterior and posterior parietal strip (likely homologue of PEc) largely resemble our results in that retrograde labeling was confined to mesial, ventrolateral, and frontal fields (Burman et al. 2008, compare their Fig. 9C with our Fig. 4). The notion that the current findings are closely paralleled by those reported for New World monkeys suggests that main features of posterior parietal organization are relatively conserved among primates.

In macaques, PEc receives the bulk of its visual inputs from high level visual areas, through a specific pathway that involves area V6Ad, and is further influenced by inputs from the areas of the caudal mesial cortex that are involved in the execution of eye movements (Olson et al. 1996; Dean et al. 2004), in the analysis of optic flow (Wall and Smith 2008) and self-motion (Kovacs et al. 2008) signals, and in navigation in the environment (Grön et al. 2000; Sato et al. 2006). It has also been reported that the activity of PEc neurons is modulated by saccades (Raffi et al. 2008) and by moving visual stimuli (Squartrito et al. 2001; Breveglieri et al. 2008), in particular optic flow stimuli (Raffi et al. 2002). Together, both the connectional and the physiological properties of PEc neurons lead us to suggest that, along the sensorimotor pathway linking the superior parietal with the frontal cortex, area PEc might have a specialized role in locomotion and in coordinated movement in the environment.

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**References**


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