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Conflict of interest

The authors declare that they have no conflict 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: MG, CG. Acquisition of data: DI, MG, LP, MR. Analysis and interpretation of data: DI, MG, CG. Drafting of the manuscript: DI, MR, CG. Critical revision of the manuscript for important intellectual content: MR. Obtained funding: MR, CG. Administrative, technical, and material support: LP. Study supervision: CG.

Abstract (240/250 words)

The exposed surface of the superior parietal lobule in macaque brain contains two architectonically defined areas named PEc and PE. The aim of the present study is the characterization of thalamic afferents of these two areas. For this purpose, retrograde neuronal tracers were injected, or placed in crystal form, in areas PEc and PE. We found that the two areas show a similar pattern of thalamic inputs, mainly originating from Lateral Posterior (LP), Pulvinar (Pul), Ventral Posterior Lateral (VPL), and Ventral Lateral (VL) nuclei, all structures known to be involved in visual, somatosensory, and/or sensorimotor processing. Minor afferents were observed from the Centromedian/Parafascicular complex (CM/PF), Central Lateral (CL), Ventral Anterior (VA) and Medial Dorsal (MD) nuclei. LP and VL were more strongly connected to PEc than to PE, while the other main thalamic inputs to the two areas showed slight differences in strength. The part of the Pulvinar mostly connected with areas PEc and PE was the Medial Pulvinar. No labeled cells were found in the retinotopically organized Lateral and Inferior Pulvinar. In the somatotopically organized VPL and VL nuclei, labeled neurons were mainly found in regions likely to correspond to the trunk and limb representations (in particular the legs). These findings are in line with the sensory-motor nature of areas PEc and PE, and with their putative functional roles, being them suggested to be involved in the preparation and control of limb interaction with the environment, and in locomotion.

Keywords: Thalamus; Connectivity; Macaque; superior parietal lobule; Somatosensory; Sensory-motor input; RRID: SCR_006260

Introduction

In macaques, the exposed surface of the superior parietal lobule contains two cyto-architectural areas, named PEc and PE (Pandya and Seltzer, 1982), which are functionally distinct. Here we describe the thalamic sources of projections to these areas, using fluorescent tracer injections.

Area PEc, which overlaps with the most caudal and medial part of Brodmann's area 7 (Brodmann, 1909; Luppino et al., 2005; Gamberini et al., 2015), forms an incomplete map of the body, principally focused on the limbs, without any evident sign of topographical organization (Breveglieri et al., 2006, 2008). PEc neurons respond to visual and tactile stimuli, as well as to passive single-joint rotations (Squatrito et al., 2001; Raffi et al., 2002; Breveglieri et al., 2006, 2008), and some neurons are capable of bimodal responses (Breveglieri et al., 2008). PEc neurons are also known to show arm and eye movement-related activity (Battaglia-Mayer et al., 2001; Ferraina 01; Piserchia et al., 2017), including sensitivity to the direction and depth of movement (Bhattacharyya et al., 2009; Hadjidimitrakis et al., 2015). In contrast, area PE, which overlaps with Brodmann's area 5 (Brodmann, 1909), contains a rough topographical representation of the body, with over-representation of the arms and hands (Taoka et al., 1998, 2000; Padberg et al., 2007; Krubitzer and Disbrow, 2008; Seelke et al., 2012). PE neurons are mainly activated by proprioceptive stimulation, although some respond to tactile stimulation (Duffy and Burchfiel, 1971; Sakata et al., 1973; Mountcastle et al., 1975). PE neurons are involved in the preparation and control of limb movements (Burbaud et al., 1991; Ferraina and Bianchi, 1994; Lacquaniti et al., 1995; Kalaska, 1996; Ferraina et al., 2009; Bremner and Andersen, 2012), and become active during skil ions (Maimon and Assad, 2006; Chen et al., 2009; Shi et al., 2013).

In summary, PEc is a bimodal area, albeit with predominantly somatosensory inputs, whereas PE is essentially a high-order somatosensory area. Both areas over-represent the limbs, whether according to a crude somatotopic map (PE), or non-topographically (area PEc), and their functional properties strongly suggest that both areas are involved in the control of limb

movements. The cortico-cortical connections of these areas are well established (PEc: Pandya and Seltzer, 1982; Tanné et al., 1995; Matelli et al., 1998; Marconi et al., 2001; Tanné-Gariépy et al., 2002; Bakola et al., 2010; PE: Jones et al., 1978; Johnson et al., 1996; Matelli et al., 1998; Bakola et al., 2013), but their subcortical connections have not been investigated with the same level of detail. Previous studies have shown that the main thalamic afferents to the exposed surface of the superior parietal lobule arise from the Lateral Posterior (LP), Pulvinar (Pul), Ventral Posterior Lateral

ral Lateral (VL) nuclei (Yeterian and Pandya, 1985; Schmahmann and Pandya, 1990; Cappe et al., 2007; Padberg et al., 2009), but it has remained unclear whether PE and PEc

previous studies have been based mostly on the analysis of single or few injections, leaving unexplored the issue of possible variations in the pattern of connections, according to location of the injection sites. Here we describe in detail the thalamo-cortical

as PEc and PE, based on the analysis of retrograde tracer injections that cover, together, almost the whole extent of the two areas.

Materials and Methods

Experimental protocols followed the guidelines of the European Directive 86/609/EEC and the revised Directive 2010/63/EU for the Care and Use of Animals for Scientific Purposes.

Retrograde neuronal tracers were released into the cortex of six hemispheres of five male adult monkeys (*Macaca fascicularis*, 2.0-5.3 kg). The tracer cholera toxin subunit B (CTB; conjugated with Alexa Fluor® 488 [CTB-green], 1.7-2.0 µl, 1% in phosphate buffer solution, or with Alexa Fluor® 594 [CTB-red], 1.7 µl, 1% in phosphate buffer solution; Molecular Probes, Inc., Eugene, OR, USA) was injected through Hamilton micro-syringes fitted with a glass micropipettes attached to the needles. Fast Blue (FB; $C_{20}H_{17}N_5O$. HCl; Polysciences, Europe GmbH, Eppelheim, Germany) and Diamidino Yellow (DY; Diamidino Yellow dihydrochloride; Sigma-Aldrich Logistik GmbH, Schnelldorf, Germany) were inserted into the cortex as crystals with the aid of a

sa et al., 2005; Palmer and Rosa, 2006). The injections were directed to the exposed surface of the superior parietal lobule based on visual inspection. The attribution of each injection site to a specific cortical area was based on *post mortem* analysis of cyto- and myelo-architectural material, according to criteria described by Luppino et al. (2005) and Bakola et al. (2010, 2013). This analysis indicated that 3 of the injections were within the limits of area PEc, and 5 within those of area PE. Table 1 presents the details of each injection, and Figure 1 shows the extent and location of injection sites relative to the histological boundaries of cortical areas, projected onto a flat map reconstruction of a reference macaque brain obtained with the software CARET (Computerized Anatomical Reconstruction and Editing Toolkit, RRID: SCR_006260; Van Essen et al.,). To appreciate the location of the injection sites into the cortical thickness, coronal (for cases 1 and 2) and parasagittal (for cases from 3 to 8) sections are shown. For each injection, the core (dark spot) and the halo zone (colored region around the core) are shown.

Figure 1 near here

Table 1 near here

Surgical procedures

A detailed description of the experimental procedures is available in previous publications. Briefly (for details see Bakola et al., 2010, 2013), the surgeries were performed under aseptic conditions and full anesthesia, with the animal's head held in a stereotaxic frame. The animals were pretreated with atropine (0.05 mg/kg, i.m.), pre-anesthetized with ketamine hydrochloride (12 mg/kg, i.m.) and, after 30 minutes, anesthetized with sodium thiopental (8 mg/kg, i.v. with supplemental doses as required). To avoid edema, mannitol was administered intravenously (1 g/kg). The injections were placed in the cortex following craniotomy and durotomy. At the end of the surgical procedures, the dura mater was sutured, and the surgical site covered with surgical foam; the bone flap was positioned back in place, and the wound sutured. Analgesics (Ketorolac, 1 mg/kg, i.m., for 2-3 subsequent days) and antibiotics (erythromycin, 1-1.5 ml/10 kg) were administered postoperatively. The veterinary staff of the University of Bologna assisted to the surgery, monitoring physiological parameters, as well as the animal's recovery in the subsequent days.

Histological procedures

Fourteen days after the tracer injections, the animals were treated with ketamine hydrochloride (15 mg/kg, i.m.). Following loss of consciousness, they received a lethal dose of sodium thio l (i.v.) and, upon cardiac arrest, were perfused with 3 liters of normal saline solution, followed by 5 liters of 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4, and 4 liters of 5% glycerol in the same buffer. The brains were removed from the skulls, photographed from all views, and cryo-protected by immersion in 0.1 M phosphate buffer solutions containing glycerol d 20% for all cases). The brains were then snap-frozen and stored at minus 80°C. Sections (60 µm of thickness) were obtained using a freezing microtome. In most cases, the brain was sectioned in parasagittal plane. This choice was dictated by the need to determine the

histol daries between areas PEc and PE, which are better recognizable in this plane of section, as show in Figure 1c. Five series of sections were obtained, one of which was always stained for Nissl substance and another for myelin (Gallyas, 1979). The other series were left unstained, and one of these was used for analysis of fluorescent tracers. All sections were cover-slipped with DPX after quick steps of dehydration in 100% ethanol, and cleared with xylene.

Data analysis

The unstained sections were examined for labeled neurons using a Zeiss microscope (Axioscope s) equipped with 10x and 20x objectives. In each case, the entire hemisphere ipsilateral to the injection site was processed. Section outlines and locations of labeled neurons were plotted at 600 µm intervals (1 in 10 sections) using a computerized system linked to X/Y transducers mounted on the microscope stage. Photomicrographs of labeled cells were obtained using a digital camera connected to the microscope (Axiovision software, version 4.4; Carl Zeiss). Figure 2 illustrates examples of labeled cells.

Figure 2 near here

The assignment to each injection site to area PEc or PE was made taking into account the architectonic subdivision of the exposed surface of the superior parietal lobule proposed by Pandya and Seltzer (1982). To identify the thalamic nuclei, the atlases of Olszewski (1952), for coronal sections, and Ilinsky and Kultas-Ilinsky (1987), for parasagittal sections, were used. To harmonize the names and abbreviations of thalamic nuclei across these atlases we took into account the conclusions of Mai and Forutan (2012), who reviewed previous studies of the primate thalamus in light of recent improvements made possible by neuroimaging technologies. With respect to the lateral region of the thalamus, these authors concluded that the most accurate nomenclature was the one proposed by Ilinsky and Kultas-Ilinsky (1987).

Table 2 shows the terminology adopted in the present work.

Table 2 near here

A *camera lucida* was used to bring into register the stained histological sections and locations of labeled cells. The borders of thalamic nuclei were reconstructed using sections stained with Nissl method. In some cases, the sections stained with Gallyas method were used to distinguish borders that were not well evident with Nissl method. In order to facilitate the identification of the thalamic nuclei, the cases in which parasagittal sections were obtained were resliced in coronal plane, using the software CARET. Figure 3 shows a comparison between sections reported in Olszewski (1952) atlas (Figure 3a,b) and our reconstructions of thalamic nuclei obtained from coronal sections taken at similar levels (Figure 3e,f). There was a good correspondence between our observations (actual or digitally reconstructed) and the atlas. Similarly, there was a good fit between Ilinsky and Kultas-Ilinsky (1987) atlas (Figure 3c,d) and our observations in parasagittal sections (Figure 3g,h).

Figure 3 near here

To obtain the overall maps of the distribution of labeled neurons in LP, Pul, VPL, and VL thalamic nuclei (Figure 8), we first reconstructed these nuclei in each animal by aligning the coronal sections according to the Olszewski (1952) atlas, as shown in Figure 3 for the whole thalamus. As mentioned above, if a case was sectioned in sagittal plane, coronal sections were obtained with the re-slicing tool f CARET. Then, we superimposed on a template obtained from the Olszewski (1952) atlas the reconstructions of each nucleus of each case, and the labeled cells found within that nucleus (see left and central columns in Figure 8).

Results

It is well known that subcortical neurons represent a small fraction of the overall number of cells projecting to a cortical area (Markov et al., 2011), and our results confirm this general rule. The number of labeled neurons in cortex and thalamus differed between cases (see Table 1) likely because of the different type of tracer used, the different uptake of the tracer in different cases, and/or the different cortical layers involved by the injection site. On average, labeled cells after PEc tracer injections were 1.8% ± 0.8% of the total labeled cells, and after PE injections they were 4.1% ± 2.6%.

Thalamic afferents to area PEc

Figure 4 shows the results of a representative case of thalamic labeling after PEc injection (Case 3, see injection site in Figure 1). Four parasagittal sections through the thalamus are shown, together with a reconstruction of a medial view of the thalamus, which shows the most densely labeled thalamic nuclei (colored polygons) obtained by overlapping outlines deriving from all sections available. As visible in both single sections and reconstruction, labeled cells were concentrated in the dorsal part of the thalamus, including the Lateral Posterior (LP, green), Pulvinar (Pul, blue), Ventral Posterior Lateral (VPL, purple) and Ventral Lateral (VL, red) nuclei. The proportions of thalamic afferents in different nuclei are shown in Figure 5a.

Minor afferents to PEc were found in two out of three cases (from 2.3% to 5.4% of the total label), and originated from Central Lateral (CL) nucleus in cases 1 and 2, and from the Medial

d Ventral Anterior (VA) nuclei, in case 2.

Figure 4 near here

Figure 5 near here

Thalamic afferents to area PE

Figure 6 shows the thalamic labeling following one of the PE injections (Case 8, see injection site in Figure 1). Five parasagittal sections and a reconstruction of a medial view of the thalamus are illustrated. These illustrations show that labeled cells were, as for area PEc injections, mainly distributed in the dorsal part of the thalamus. However, the distribution of labeled cells was more widespread, particularly in the dorso-ventral dimension. Figure 5b shows that the thalamic nuclei that were strongly labeled in cases with PE injections were the same as those that were strongly labeled after PEc injection (see Figure 5a), i.e. the LP, Pul, VPL, and VL nuclei. Minor afferents to area PE, observed only in some cases, originated from the MD and VA nuclei, and from the CM/PF complex.

Figure 6 near here

Comparison between thalamic connections to areas PEc and PE

Figure 7a shows the distribution of the thalamic afferents to areas PEc and PE according to the thalamic subdivision proposed by Mai and Forutan (2012). The superior and periventricular regions did not show any labeled cells. Only a low percentage of labeled cells were observed in the medial region (PEc: 1.1% ± 1.6%; PE: 1.8% ± 2.2%) and in the intralaminar formation (PEc: 2.8% ± 2.0%; PE: ± 4.8%). The highest numbers of labeled cells were observed in the lateral (PEc: 52.7% ± 8.8%; PE: 43.0% ± 18.7%) and posterior (PEc: 42.5% ± 5.3%; PE: 49.3% ± 17.5%) nuclear groups of the thalamus. According to this analysis, differences between PEc and PE were not statistically significant (unpaired Student's t test).

Mai and Forutan (2012) suggested that the lateral region of the thalamus can be subdivided in two regions, which they named "motor" and "sensory" based on functional properties, and we analyzed the distribution of the labeled cells among these two subdivisions. According to Mai and Forutan (2012), the "motor" thalamus includes the VA and VL nuclei, while the "sensory" thalamus comprises the VM, VPI, VPL, and VPM nuclei. As shown in Figure 7b, the sensory thalamus

projections were stronger than the motor projections to both cortical areas, with this trend being particularly clear following injections in area PE.

Figure 7 near here

To ribution of labeled cells

As reported above, the main thalamic nuclei projecting to the cortical areas PEc and PE are LP, Pul, VPL, and VL. Figure 8 shows the spatial distributions of labeled cells within these nuclei. In Figure 8, we reconstructed each of these nuclei by superimposing coronal sections from all cases available; brains originally sectioned in parasagittal planes were first re-sliced into coronal views, following 3D reconstructions in CARET.

Figure 8a shows the distribution of labeled cells in the LP nucleus. Cells were distributed in the late of LP, whether PEc or PE was injected. The labeling after PEc injections appeared to cover a larger proportion of this nucleus, compared to PE.

The Pulvinar nucleus is traditionally subdivided into four parts: Medial, Lateral, Anterior, zewski, 1952; Snider and Lee, 1961; Grieve et al., 2000). Figure 8b shows that both PEc and PE mainly receive from the Medial Pulvinar. Area PEc, in addition, may receive a numerically small projection from the Anterior Pulvinar. Cells projecting to PE were distributed more dorsally, with respect to those projecting to PEc.

Figure 8c shows that cells projecting to area PEc are strictly segregated to the dorsal part of the VPL nucleus, whereas those projecting to PE are more widely distributed. According to Rausell and coworkers (1998), VPL represents the whole body except the head (see Figure 8c right), which is represented in VPM. Labeled cells projecting to PEc are located in the parts of VPL that most likely represent the trunk and the proximal portions of the limbs (in particular, the legs). Cells projecting to PE, in addition, appeared to also be located in the representations of more distal parts of the limbs. The VPM nucleus did not project to PEc or PE.

Figure 8d shows the distribution of labeled neurons in the VL nucleus. Projections to PEc and PE are very similar, involving the dorsal-most part of the nucleus and, far more sparsely, the ventral part. Comparison with the somatotopic map proposed by Vitek and coworkers (1994) suggests that the labeled cells are located in parts of VL mostly representing the trunk and legs, although the ventral group of cells appears to overlap with the region of face representation.

Figure 8 near here

Discussion

The present study defined the thalamo-cortical connections of the posterior parietal areas PEc and PE (Pandya and Seltzer, 1982). We have found that these areas receive major thalamic afferents from the posterior and lateral regions of the thalamus (namely, the VL, VPL, LP, and Medial Pulvinar nuclei), and minor afferents from the medial and intralaminar regions. There have been previous studies investigating the thalamic connections of the superior parietal lobule (Yeterian and Pandya, 1985; Schmahmann and Pandya, 1990; Cappe et al., 2007; Padberg et al., 2009). The present study refined and extended the observations of these earlier studies by making use of a larger series of injection sites, which allowed us to study PEc and PE separately, while considering the entire extents of these areas.

Major thalamic afferents

Areas PEc and PE receive the majority of their thalamic afferents from the posterior and lateral regions of the thalamus (Mai and Forutan, 2012). The posterior thalamus is dominated by the Pulvinar complex, which account for about a quarter of its total mass (Grieve et al., 2000; Mai and Forutan, 2012), and it is traditionally subdivided into four sectors, each with specific functional properties (Olszewski, 1952; Snider and Lee, 1961; Grieve et al., 2000; Mai and Forutan, 2012; see Figure 8b). The Anterior Pulvinar is reported to have somatosensory functions (Grieve et al., 2000); the Lateral and Inferior nuclei contain visually responsive cells, which are organized retinotopically (Kaas and Lyon, 2007), and the Medial Pulvinar contains visual cells which are not retinotopically organized (Mathers and Rapisardi, 1973; Grieve et al., 2000), as well as cells responding to reaching activity (Acuña et al., 1990) and auditory stimuli (Yirmiya and Hocherman, 1987). The Medial Pulvinar also seems to be involved in directing attention and in recognizing visual salience (A Laberge and Buchsbaum, 1990; Mesulam, 1990; Romanski et al., 1997). Immediately anterior to the Medial Pulvinar is the Lateral Posterior (LP) nucleus. Given the difficulty in establishing a reliable anatomical boundary between the Medial Pulvinar and the LP,

these two nuclei are often considered as part of a single complex (Van Buren and Borke, 1972; Cooper et al., 1974; Percheron, 2004); indeed, the few functional studies investigating LP in the macaque found similar functional characteristics in comparison with the Medial Pulvinar (Acuña et al., 1986, 1990; Cudeiro et al., 1989).

Our results show that both the Medial Pulvinar and the LP form major projections to areas ough area PE tends to receive comparatively less numerous afferents from LP (Figure 5). The strong Medial Pulvinar inputs are in line with the role attributed to these areas in preparation/execution of reaching actions (Burbaud et al., 1991; Ferraina et al., 2001; Bremner and Anders Hadjidimitrakis et al., 2015; Piserchia et al., 2017). The reason for the comparatively weaker LP inputs to PE is unclear. Recent studies on the dopaminergic innervation (Sanchez-Gonzalez, 2005; García-Cabezas et al., 2007, 2009) have demonstrated that the LP nucleus is heavily innervated by dopaminergic fibers, while the Medial Pulvinar is only mildly innervated. Since also the primary motor cortex and the nuclei of the "motor" thalamus receive strong dopaminergic input, these studies suggested that the LP nucleus is involved in the control of motor actions.

In a recent study, it has been found that area V6A, a visuo-motor area located further caudally, adjacent to PEc (see Figure 1), is strongly connected to the LP nucleus, and less so to the Medial Pulvinar (Gamberini et al., 2016), further emphasizing the view that different balances in the thalamic inputs contribute to the functional differences among superior parietal lobule areas. Thus, the thalamic input from the LP nucleus becomes comparatively more significant from rostral to caudal (i.e. from area PE to area V6A), while that from Medial Pulvinar progressively decreases. Interestingly, the LP input increases according to the incidence of visually responsive cells in its cortical target: such cells are virtually absent in PE (Mountcastle et al., 1975), form approximately 40% of the population in PEc (Breveglieri et al., 2008) and 65% in V6A (Gamberini et al., 2011). Based on these observations, and taking into account the observations discussed in the paragraph above, we suggest that LP input mainly contributes to visuo-motor information.

Nuclei in the lateral region of the thalamus are strongly connected with both areas PEc and PE. Our results show that, for area PE, the inputs coming from the "sensory" subdivision of the lateral thalamus (Mai and Forutan, 2012) are more numerous than those from the "motor" subdivision, while for area PEc, they appear to be more balanced (Figure 7). This finding is in line with the functional properties of the two cortical areas, which suggest that PEc controls the interaction of the four limbs with the environment (Bakola et al., 2010), for which an integration between motor and sensory (visual and somatic) information is required, whereas area PE is involved in the preparation of limb movement (Burbaud et al., 1991; Bremner and Andersen, 2012), a function that requires a strong somatosensory input, in particular proprioception, to control the posture to accomplish a correct limb movement.

The VPM and VPL are two of the nuclei composing the "sensory" thalamus. Together, they contain a complete and topographically organized representation of the body, with the head represented in VPM and the trunk and limbs in VPL (Rausell et al., 1998). We found that neither PEc nor PE received thalamic inputs from VPM, while receiving strong afferents from the portion of VPL which represents the trunk and the proximal parts of the limbs. Interestingly, the portion of VPL representing the distal part of the limbs projected only to area PE. These observations agree with the somatosensory representation in areas PEc and PE, in that PEc represents only the trunk and the proximal parts of the four limbs (Breveglieri et al., 2006) and PE also the hands and feet (Padberg et al., 2007; Krubitzer and Disbrow, 2008).

The "motor" sector of the lateral thalamus is formed by the VA and VL nuclei (Mai and Forutan, 2012). VA formed only minor projections, which were not constantly present in all cases we studied. In contrast, the VL nucleus is strongly connected with both PEc and PE. According to Vitek and coworkers (1994), VL contains a motor topographical map of the whole body, including the head. After PEc and PE injections labeled cells in VL were mainly located in the dorsal part of the nucleus, likely overlapping with the representations of the trunk and legs, but a few cells were also observed in the ventral part of the nucleus, which represents the face (Figure 8d). No labeled

cells were found in the putative arm representation. This cell distribution is somewhat surprising, given that in both PEc and PE cells are responsive to forelimb movements (PEc: Bhattacharyya et al., 2009; Hadjidimitrakis et al., 2015; Piserchia et al., 2017; PE: Burbaud et al., 1991; Ferraina et al., 2009; Bremner and Andersen, 2012), and to tactile and proprioception stimulations of forelimbs (PEc: Breveglieri et al., 2006; PE: Padberg et al., 2007; Krubitzer and Disbrow, 2008). Our tracer injections covered the entire extent of area PEc, and the vast majority of the extent of area PE, in particular the antero-lateral part of the area where the forelimb is represented (Krubitzer and Di Therefore, we expected to find many labeled cells in the sectors of VL representing arm and hand, but this was not apparent in our data. A similar situation was observed by Bakola et al. (2010, 2013), who reported an emphasis of somatosensory and premotor/motor leg-field cortical projections to PEc and PE. It could be that both thalamo-cortical and cortico-cortical networks are involved in the control of movements performed with the four limbs, typical of non-human primates moving in natural habitat. A cortico-thalamo-cortical loop could be engaged in the control of more stereotyped movements, as those activated in locomotion, that mainly involve the legs, while an alternative cortico-cortical network would be mainly activated when the grasping of an object is requested. Alternatively, this apparent discrepancy may reflect the difficulty in comparison across studies which used different methods.

Minor thalamic afferents

In addition to the major thalamic afferents described above, recognized in all our cases, we found minor and variable afferents from the MD, VA, CL, and CM/PF nuclei. The MD nucleus, which sends minor afferents to both areas PEc and PE, is reported to be involved in the control of saccades (Watanabe and Funahashi, 2004) and in learning and decision-making functions (Mitchell, 2015). Saccadic activity has been reported in PEc (Raffi et al., 2008), but to our knowledge not in area PE, and nothing is known about a possible involvement of PEc and/or PE in learning and decision-making processes. The VA nucleus, which sends a few afferents to both areas PEc and PE,

is described as a node of a loop involved in the induction, execution, and control of principal aspects of voluntary movements, in particular when multiple alternatives are possible (Mushiake and Strick, 1995; Middleton and Strick, 2000; Sommer, 2003). The CL nucleus and CM/PF complex send few afferents to areas PEc and PE, respectively. CL is possibly involved in the execution of cognitive functions (Van Der Werf et al., 2002), and CM/PF seems to have a role in movement regulation (Mai and Forutan, 2012).

Comparison with previous studies

Previous studies focused on the thalamic connections of superior parietal lobule were based on few injections, which in most cases did not encompass the complete extent of a cytoarchitectonically-defined area (Yeterian and Pandya, 1985; Schmahmann and Pandya, 1990; Cappe et al., 2007; Padberg et al., 2009). Table 3 shows a comparison of the present observations (column 5) with those of previous studies (columns 1-4). In Table 3, we only show data from injections of retrograde tracers (as those used in this work) located in a specific cortical area of the superior parietal lobule, avoiding data from injections of anterograde tracers and/or that involved more than one area. The nomenclature adopted in older studies was harmonized with that used in the present work (see Table 2).

Table 3 shows that the thalamo-cortical afferents we observed for area PEc were very similar to those of Yeterian and Pandya (1985), although specific differences (absence of labeled neurons in the R nucleus, and their presence in the VPL nucleus) were observed. Our conclusions differ more substantially from those of Schmahmann and Pandya (1990), possibly due to the more comprehensive sample obtained in the present study.

With respect to the thalamic afferents of area PE, our results differ from the previous literature in several ways (see Table 3). For example, we did not observe afferents from the Anterior Pulvinar, which were reported by earlier studies. Other aspects of our study reflect earlier observations, such as the presence of major afferents from the Medial Pulvinar, and the LP and VPL

nuclei. Overall, our conclusions are in closer agreement with those of Cappe et al. (2007). Although some of the discrepancies could be due to the fact that earlier studies did not cover the entire extent of area PE, other factors, such as the use of different criteria for parcellation of the thalamus, are likely to also play a role in explaining such differences.

Table 3 near here

In humans, several studies that use DTI and resting-state fMRI techniques allowed subdividing the thalamus in clusters, each comprising various nuclei (Mastropasqua et al., 2015; O'Muircheartaigh et al., 2015; Yuan et al., 2016; Hwang et al., 2017; Kumar et al., 2017). These different clusters are connected with different cortical regions, and confirm the present and previous works on the macaque monkey in showing that the clusters that include VL, VPL, LP, and Pul are connected with the posterior parietal cortex (PPC). However, the limits of the neuroimaging techniques in discerning the border of cortical and subcortical architectonic subdivisions do not allow a direct comparison of the thalamo-cortical connections of areas PEc and PE in macaques and humans. Furthermore, the great difference in extent and location of areas 5 and 7 in macaques and humans (Brodmann, 1909; Amunts and Zilles, 2015) would make this comparison unreliable.

Conclusions

The thalamic inputs to areas PEc and PE reported here confirm the sensory-motor integration nature of these posterior parietal areas (Mountcastle et al., 1975; Burbaud et al., 1991; Breveglieri et al., 2006, 2008; Padberg et al., 2007; Krubitzer and Disbrow, 2008; Bremner and Andersen, 2012). The thalamic afferents to these areas are largely similar, in that they both originate mainly from regions of the thalamus which represent trunk, upper limbs and lower limbs, particularly the legs and the proximal parts of both limbs, but also show differences. These

observations well agree with the functional roles proposed for PEc and PE, with the first suggested to control the interaction of the four limbs with the environment (Bakola et al., 2010), and the second to be involved in the preparation/execution of limbs movement (Burbaud et al., 1991; Ferraina and Bianchi, 1994; Lacquaniti et al., 1995; Kalaska, 1996; Ferraina et al., 2009; Bremner and Andersen, 2012). The thalamic inputs to PEc and PE also suggest the existence of cortico-thalamo-cortical circuits supporting a certain degree of motor automatism, particularly important in locomotion.

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Abbreviations of Thalamic nuclei and tracts

AD	Anterior Dorsal
AM	Anterior Medial
AV	Anterior Ventral
bsc	brachium of superior colliculus
Cdc	Central densocellularis
CL	Central Lateral
Clc	Central latocellularis
CM/PF	Centromedian/Parafascicular
Cn.Md	Centromedian
eml	External medullary lamina
ITP	Inferior thalamic peduncle
LD	Lateral Dorsal
LG	Lateral Geniculate
LP	Lateral Posterior
MD	Medial Dorsal
MDdc	Medial Dorsal, pars densocellularis
MDmc	Medial Dorsal, pars magnocellularis
MDmc/pc	Medial Dorsal, pars magnocellularis/parvocellularis
MDmf	Medial Dorsal, pars multiformis
MDpc	Medial Dorsal, pars parvocellularis
MGmc	Medial Geniculate, pars magnocellularis
MGpc	Medial Geniculate, pars parvocellularis
ot	optic tract
Pa	Paraventricular
Pf/PF	Parafascicular
Pg	Pregeniculate
Pul	Pulvinar
Pul.i	Pulvinar, inferior subdivision
Pul.l	Pulvinar, lateral subdivision
Pul.m	Pulvinar, medial subdivision
Pul.o	Pulvinar, oral (anterior) subdivision
R	Reticular
Re	Reuniens
Sg	Suprageniculate
VA	Ventral Anterior
VAdc	Ventral Anterior, pars densocellularis
VAmc	Ventral Anterior, pars magnocellularis
VApc/dc	Ventral Anterior, pars parvocellularis/densocellularis
VL	Ventral Lateral
VLc	Ventral Lateral, pars caudalis
VLo	Ventral Lateral, pars oralis
VLps	Ventral Lateral, pars postrema
VM	Ventral Medial
VPI	Ventral Posterior Inferior
VPL	Ventral Posterior Lateral
VPLc	Ventral Posterior Lateral, pars caudalis
VPLo	Ventral Posterior Lateral, pars oralis
VPM	Ventral Posterior Medial
VPMpc	Ventral Posterior Medial, pars parvocellularis

Table 1. Injection sites and neuronal tracers employed in the experiments

Case							
Present study	Gamberini et al., 2017	Bakola et al., 2010 & Bakola et al., 2013	Cutting Plane	Injected area	Tracer	Amount and concentration of tracer	Number of cortical/thalamic labeled cells
1[a]	1	A5L	Coronal	PEc	FB[d]	1 crystal	8933/256
2[a]		A5R	Coronal	PEc	DY[e]	7 crystals	36899/725
3	3	A4R	Parasagittal	PEc	DY[e]	4 crystals	17175/102
4[b]	4	2	Parasagittal	PE	CTB-green[f]	1.7 µl; 1% in PBS[i]	17315/498
5[b]	5		Parasagittal	PE	CTB-red[g]	1.7 µl; 1% in PBS[i]	604/40
6[c]	6	1	Parasagittal	PE	FB[d]	1 crystal	13925/138
7[c]	7	6	Parasagittal	PE	CTB-green[f]	2 µl; 1% in PBS[i]	3567/84
8	9	4	Parasagittal	PE	CTB-green[f]	1.7 µl; 1% in PBS[i]	3124/244

[a] Same animal
[b] Same hemisphere
[d] Fast Blue, Polysciences Europe
[e] Diamidino Yellow, Sigma Aldrich
[f] Cholera Toxin subunit B-green, Molecular Probes
[g] Cholera Toxin subunit B-red, Molecular Probes
[h] Fluoro Ruby, Invitrogen – Molecular Probes
[i] Phosphate Buffered Saline solution

Table 2. Correspondence of nomenclature of the thalamic nuclei involved in this study

Thalamic Regions	Olszewski (1952)	Ilinsky & Kultas-Ilinsky (1987)	Present study
Medial	MDdc	MDdc	MD
	MDmc/pc	MDmc/pc	MD
	MDmf	MDmf	MD
	MDpc	MDpc	MD
Lateral			
Motor	VA	VA	VA
	VAdc	VAdc	VA
	VAmc	VAmc	VA
	VApc/dc	VApc/dc	VA
	VLo	VAdc	VA
	VLc	VL	VL
	VLps	VL	VL
	VPLo	VL	VL
Sensory	VPLc	VPL	VPL
Intralamin	CL	CL	CL
	Cn.Md	CM	CM
	Pf	PF	PF
Posterior	LP	LP	LP
	Pul.i	Pul.i	Pul
	Pul.l	Pul	Pul
	Pul.m	Pul	Pul
	Pul.o	Pul	Pul

For the extended nomenclature of the thalamic nuclei, see the list of abbreviations.

Table 3. Comparison with previous studies on the macaque

	PEc			PE				
	1	2	5	1	2	3	4	5
Anterior Pul	+	+++	+	++	+++	+	+++	-
Lateral Pul	-	+	-	+++	+	-	-	-
Medial Pul	+++	-	+++	++	-	++	-	+++
CL	+	+	+	-	++	+	-	-
M/PF	-	+	-	-	++	+	-	+
LP	+++	+++	+++	+++	+++	+++	+++	++
MD	++	+	+	-	+	++	-	+
R	++	-	-	++	-	-	-	-
VA	-	-	+	-	-	-	-	+
VL	+++	+	+++	+++	+	-	+	++
VPI	-	-	-	-	-	-	+	-
VPL	-	+	+++	+++	++	+++	+++	+++

1: Yeterian and Pandya, 1985 (retrograde)
2: Schmahmann and Pandya, 1990 (anterograde/retrograde)
3: Cappe et al., 2007 (anterograde/retrograde)
4: Padberg et al., 2009 (retrograde)
5: Present study (retrograde)
-: no connections
+: weak connections
++: moderate connections
+++: high connections
For the extended nomenclature of the thalamic nuclei, see the list of abbreviations.

Legends

Figure 1. Summary of injection site locations. a, b: Injection sites in five animals are illustrated on a two-dimensional reconstruction (b) of the caudal superior parietal lobule of the right hemisphere of a reference macaque brain shown on the left (a). For each injection, the core (dark spot) and the halo zone (colored region around the core) are shown. The dashed contours indicate the average cyto-architectonic border of areas PEc and PE. The location of the injection sites in the cortical thickness is shown on coronal (cases 1 and 2) and parasagittal (cases from 3 to 8) sections. c: Drawing of a parasagittal section centered on the anterior wall of the parieto-occipital sulcus. The brain silhouette shows the level of the parasagittal section shown below. The grey boxes indicate the location of two high-magnification views shown in the panels on the right. Abbreviations: ars, arcuate sulcus; cal, calcarine sulcus; cin, cingulate sulcus; cs, central sulcus; ips, intraparietal sulcus; lf, lateral fissure; ls, lunate sulcus; pcd, post-central dimple; pos, parieto-occipital sulcus; ps, principal sulcus; sts, superior temporal sulcus; C, caudal; D, dorsal; L, lateral; M, medial; R, rostral. V6A, PEc, PE, PEci, area 2: areas V6A, PEc, PE, PEci, 2.

Figure 2. Examples of labeled cells in the thalamus. Top: dorsal view of a reference macaque brain; the dashed circle represents the approximate location and extent of the thalamus. a: thalamic section of a PEc injection case. b-c: medium- and high-power photomicrographs, respectively, of DY labeled cells taken at 10x and 20x magnifications. d: thalamic section of a PE case. e-f: medium- and high-power photomicrographs, respectively, of CTB-green labeled cells taken at 10x and 20x magnifications. For the nomenclature of thalamic nuclei, see the list of abbreviations. Other details and abbreviations as in Figure 1.

Figure 3. Thalamic nuclei. Top: dorsal view of a reference macaque brain; the dashed circle represents the location and extent of the thalamus. a-b: typical brain sections showing the thalamic nuclei, taken from Olszewski (1952) atlas. e-f: sections of Case 1 taken at the same approximate level of atlas sections a-b. c-d: typical brain sections showing the thalamic nuclei, taken from the Ilinsky & Kultas-Ilinsky (1987) atlas. g-h: sections of Case 4 taken at the same approximate level of

atlas sections a-c. For the nomenclature of thalamic nuclei and tracts, see the list of abbreviations.

Ot abbreviations as in Figures 1 and 2.

Figure 4. Typical case with thalamic afferents to area PEc. Four parasagittal sections from Case 3 are reported. The white circles represent the labeled cells. At the center, a reconstruction of the most involved thalamic nuclei is shown, obtained by overlapping all sections at our disposal. The thalamic nuclei that contain labeled cells are highlighted with various colors: green for LP, blue for Pul, red for VL, and purple for VPL. For the nomenclature of thalamic nuclei, see the list of abbreviations. Other details and abbreviations as in Figures 1-3.

Figure 5. a: Thalamic afferents to area PEc. Percentage of labeled cells in the thalamic nuclei after injections confined within the cyto-architectonic limits of area PEc. Only labeling that represented on average > 1% of thalamic afferents are reported. b: Thalamic afferents to area PE. Percentage of labeled cells in the thalamic nuclei after injections confined within the cyto-architectonic limits of area PE. Only labeling that represented on average > 1 % of thalamic afferents are reported. For the nomenclature of thalamic nuclei, see list of abbreviations and Table 2.

Figure 6. Typical case with thalamic afferents to area PE. Five parasagittal sections from Case 8 are reported. For the nomenclature of thalamic nuclei and tracts, see the list of abbreviations. Other

viations as in Figure 4.

Figure 7. Regional subdivision of thalamic afferents to areas PEc and PE. a: Average percentages of thalamic cells labeled in the six thalamic regions described by Mai & Forutan (2012) after injections in areas PEc and PE. b: Average percentages of labeled cells in the Lateral region of the thalamus, subdivided in “Motor” and “Sensory” thalamus according to Mai & Forutan (2012). Vertical bar: SD; ** $p < 0.01$.

Figure 8. Distribution of labeled cells in LP, Pul, VPL and VL nuclei. a-d: To the left, the outline of the most external limit of the thalamus in a typical coronal section, with a reconstruction of LP, Pul, VPL, VL nuclei (enlarged at the center), and their subdivision (on the right) according to Grieve et al., 2000 (Pul), Rausell et al., 1998 (VPL), and Vitek et al., 1994 (VL) are shown. The

subdivisions of Pul, VPL and VL are also reported at the center of the figure (black contour) where they are morphed on the shape of specific thalamic nucleus in order to facilitate the allocation of labeled cells. Yellow and green dots represent labeled cells sending projections to PE and PEc, respectively. er details and abbreviations as in Figures 1-3.