

The cortical connections of area V6: an occipito-parietal network processing visual information

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Abstract

The aim of this work was to study the cortical connections of area V6 by injecting neuronal tracers into different retinotopic representations of this area. To this purpose, we first functionally recognized V6 by recording from neurons of the parieto-occipital cortex in awake macaque monkeys. Penetrations with recording syringes were performed in the behaving animals in order to inject tracers exactly at the recording sites. The tracers were injected into the central or peripheral field representation of V6 in different hemispheres. Irrespective of whether injections were made in the centre or periphery, area V6 showed reciprocal connections with areas V1, V2, V3, V3A, V4T, the middle temporal area/V5 (MT/V5), the medial superior temporal area (MST), the medial intraparietal area (MIP), the ventral intraparietal area (VIP), the ventral part of the lateral intraparietal area (LIP_v) and the ventral part of area V6A (V6A_v). No labelled cells or terminals were found in the inferior temporal, mesial and frontal cortices. The connections of V6 with V1, and with all the retinotopically organized prestriate areas, were organized retinotopically. The connection of V6 with MIP suggests a visuotopic organization for this latter. Labelling in V6A and VIP after either central or peripheral V6 injections was very similar in location and extent, as expected on the basis of the nonretinotopic organization of these areas. We suggest that V6 plays a pivotal role in the dorsal visual stream, by distributing the visual information coming from the occipital lobe to the sensorimotor areas of the parietal cortex. Given the functional characteristics of the cells of this network, we suggest that it could perform the fast form and motion analyses needed for the visual guiding of arm movements as well as their coordination with the eyes and the head.

Introduction

Area V6 is a cortical visual area recently identified in behaving animals on the basis of functional criteria (Galletti *et al.*, 1996, 1999b). It is located medially in the parieto-occipital region of the brain, distinguished from neighbouring visual areas V2, V3 and V6A. It is partly coextensive with area PO, an extrastriate visual area first defined on the basis of myeloarchitectural organization by Colby *et al.* (1988).

As shown in Fig. 1, area V6 occupies a 'C'-shaped belt of cortex located between the occipital and parietal lobes and arranged in a roughly coronal plane. The upper branch of the 'C' is located in the depth of the parieto-occipital sulcus (POS) and the lower one in the depth of medial parieto-occipital sulcus (POM), with the mesial surface of the brain as a junction zone between the two branches. Area V6 borders on V3 posteriorly and V6A anteriorly, and contains a topographically organized representation of the contralateral visual field up to an eccentricity of at least 80° (see Galletti *et al.*, 1999b). The lower visual field representation is located dorsally, in the ventral part of POS, and the upper field ventrally, in the dorsal wall of POM. The representation of the peripheral visual field occupies a long strip of cortex, extending from the dorsal wall of POM to the anterior bank of POS, whereas the central 20° of the visual field are represented in a relatively small cortical region located in the most medial aspect of

the annectant gyrus, in the most lateral part of the posterior bank of the POS (Galletti *et al.*, 1999b).

The aim of this work was to describe the cortical connections of central and peripheral field representations of area V6. As V6 is hidden in a highly convoluted cortical region, located 10–15 mm away from the brain surface, it was extremely difficult to succeed in reaching it with the needle of an injecting syringe basing the penetration only upon the stereotaxic coordinates. Conversely, it was quite easy to recognize V6 by recording single cells in awake, behaving monkeys (see Galletti *et al.*, 1996, 1999a, 1999b). To overcome the problem of V6 localization, and to ensure injection of the tracer into parts of V6 representing different parts of the visual field, we developed a specially devised recording syringe that allowed us to inject tracers exactly at recording sites and in behaving animals.

Present results show that area V6 is reciprocally and retinotopically interconnected with the striate cortex as well as with several extrastriate areas of the 'dorsal visual stream'. All together, these cortical areas represent an occipito-parietal network able to encode and elaborate visual information that are likely to support frontal premotor activity. Preliminary results have been published in abstract form (Galletti *et al.*, 1999c).

Materials and methods

Three macaque monkeys (two *M. fascicularis*, one *M. nemestrina*; 3.3–7.1 kg) were used in these experiments. In two animals, single unit recordings from the cortex of the parieto-occipital region of the

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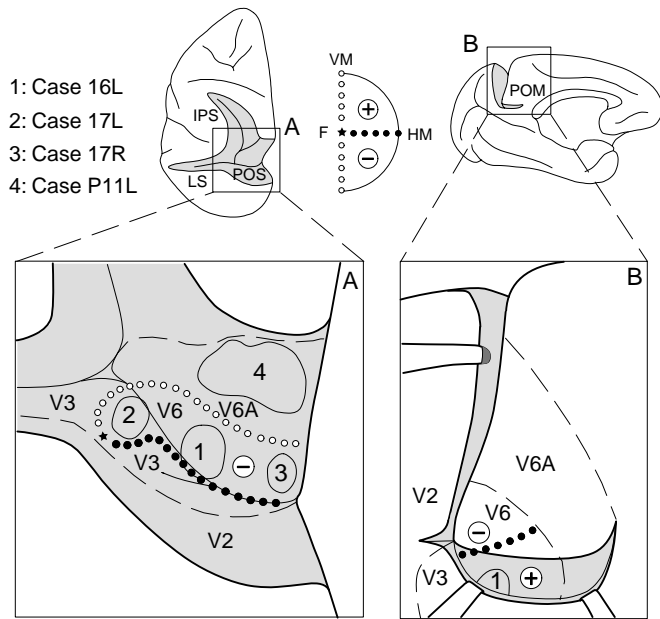


FIG. 1. Summary of tracer injections. Left: Dorsal view of the brain. The POS, LS and IPS are shown open to reveal the cortex buried within them (grey area). In the enlargement shown in inset (A), filled and empty circles indicate the representation of the horizontal (HM) and vertical (VM) meridians of area V6, respectively; the star, the centre of gaze (F). The minus sign indicates the lower visual field representation. Dashed lines indicate the borders between different cortical areas. Locations and topography of the areas are according to the present as well as previous results (see Galletti *et al.*, 1999a, 1999b). Encircled numbers (1–4) indicate the approximated locations of tracer injections in four cases (see top left). Right: Medial view of the hemisphere. The POM is shown partially open. In the enlargement shown in inset (B), the cortex of the cuneate gyrus is pulled back and down to show part of the dorsal wall of POM, where the upper visual field is represented (+ sign). The encircled number 1 indicates the involvement of upper visual field in case 16L. Other details as in A.

brain were carried out for several months in behaving animals in order to study the functional characteristics of neurons located in both banks of POS (see Galletti *et al.*, 1999a, 1999b). At the end of the recording sessions, neuronal tracers were injected by a specially devised recording syringe into the functionally defined area V6 (according to Galletti *et al.*, 1999b) to study its cortical connections. In a third animal, the neuronal tracer was injected under barbiturate anaesthesia into the dorsal part of the anterior bank of POS (area V6A; Galletti *et al.*, 1999a). Experimental protocols were approved by the Bioethical Committee of the University of Bologna and were complied with the National and European laws on the care and use of laboratory animals. Details of training, surgical and recording procedures, as well as of visual stimulation, anatomical reconstruction of recording sites and animal care are reported elsewhere (Galletti *et al.*, 1995, 1999b; Matelli *et al.*, 1998). The following is a brief description of them and a full account of procedures not previously reported.

Recording sessions

During recording sessions, the animals sat in a primate chair facing a large ($80^\circ \times 80^\circ$) tangent screen. They performed a fixation task, with the head restrained, whereas glass-coated Elgiloy microelectrodes (Suzuki & Azuma, 1976) were advanced through the intact dura with a remote-controlled microdrive daily installed on a recording chamber fixed to the skull. Penetrations were spaced

about 1 mm apart one from another. Eye positions were recorded by an infrared oculometer (Dr Bouis, Germany; Bach *et al.*, 1983). The sample rate for action potentials was 1 kHz and that for eye position 100 Hz.

Standard visual stimuli rear-projected on the tangent screen in front of the animal were used for testing the visual responsiveness of cells in record. The visual receptive field (RF) of single cells was mapped by using visual stimuli of different form, colour, size, orientation, direction and speed of movement (Galletti *et al.*, 1999a, 1999b). Occasionally, the RFs of small clusters of cells were also mapped.

The sensitivity of the cells to oculomotor and skeletomotor activities, as well as to passive somatosensory stimulations, were tested (Galletti *et al.*, 1995, 1997; Fattori *et al.*, 1999b).

Injections of neuronal tracers

After several months of recording sessions which revealed the location of area V6 (see Galletti *et al.*, 1999b), penetrations were made with a specially devised recording syringe in order to inject neuronal tracers into different parts of V6 (see Fig. 1). The recording syringe was a 1- μ L Hamilton syringe with an Elgiloy microelectrode (Suzuki & Azuma, 1976) attached to the needle. The electrode tip protruded about 300 μ m with respect to the tip of the needle. Penetrations with this modified syringe were carried out through the intact dura in the awake animal by the same remote-controlled microdrive used to advance the microelectrode in previous recording sessions. The needle of the syringe was carefully advanced into the brain to reach area V6. Then, it was advanced through V6 as far as the electrode entered into the white matter or in a different cortical area. Finally, the syringe was slowly withdrawn in order to place the tracer at the selected cortical depth.

A major problem of deep brain injections is the leakage of tracer along the needle track. We tried to overcome this problem by avoiding the presence of the tracer on the tip of the needle whereas advancing through the cortical tissue, and by withdrawing the needle from the brain completely devoid of tracer. To this purpose, the recording syringe was first filled with 0.3 μ L of paraffin oil, then with the exact amount of tracer that had to be injected and, finally, with 0.10 μ L of paraffin oil. A 0.08 μ L aliquot of oil was injected into the saline solution of the recording chamber before passing through the dura. The remaining 0.02 μ L of oil, occupying the very tip of the needle, was injected into area V6 a few hundred μ m below the selected injection site.

The tracer injections [wheatgerm agglutinin conjugated with horseradish peroxidase (WGA-HRP) 4% in distilled water: 0.12 μ L in case 16L, 0.10 μ L in case 17L; fast blue (FB) 3% in distilled water: 0.20 μ L in case 17R] were carried out by releasing several small amounts of tracer at a distance of 100–200 μ m from one another, around the centre of the cortical extent of V6. Each release took about 2 min, and about 5 min elapsed before the position of the needle was changed. Five min after the last release of neuronal tracer, the needle was drawn back for another 100 μ m and a small amount of paraffin oil was injected finally. The needle was left in place for 25–30 min and then withdrawn from the brain, very slowly (2 μ m/s) for the first 1000 μ m, and then more rapidly.

In one animal (case P11L) WGA-HRP (4% in distilled water; 0.48 μ L) was injected under barbiturate anaesthesia in two separate locations in the dorsal part of the anterior bank of POS. Injection sites were chosen by using POS, lunate sulcus (LS), intraparietal sulcus (IPS) and the interhemispheric fissure as anatomical landmarks. The tracer was pressure injected slowly through a glass micropipette (tip diameter 50–100 μ m) attached to a 1 μ L Hamilton syringe. The WGA-HRP was delivered at various depths below the exposed

cortical surface: 2.5 mm (0.1 μ L), 4 mm (0.1 μ L) and 5.5 mm (0.08 μ L) in the medial injection; 2.5 mm (0.1 μ L) and 4.5 mm (0.1 μ L) in the lateral one. Injections took about 30 min (medial) and 15 min (lateral). In both cases, the needle was left in place about 30 min before withdrawal.

After appropriate survival periods (14 days for FB and 2 days for WGA-HRP), each animal was anaesthetized with ketamine hydrochloride (15 mg/kg i.m.) followed by a lethal injection of sodium thiopental i.v. The animals were perfused through the left cardiac ventricle with the following solutions: 0.9% sodium chloride; 3.5% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4; and 5% glycerol in the same buffer. The brains were then removed from the skull, photographed and placed in 10% buffered glycerol for 3 days and in 20% glycerol for 4 days. Finally, they were cut, on a freezing microtome, in parasagittal (cases 16L, 17L, 17R) or coronal (case P11L) planes at 60 μ m. In cases 16L, 17L and P11L, one section of every five was processed for HRP histochemistry with tetramethylbenzidine as chromogen (Mesulam, 1982). In case 17R one section of each five was mounted, air-dried and quickly coverslipped for fluorescence microscopy. In all cases, one section of every five adjacent to HRP-processed material or fluorescent material was Nissl stained.

The tracks of recording needles were easily reconstructed on the Nissl-stained sections on the basis of the lesions they left on neuronal tissue and the location of tracer injection. The tracks of previous microelectrode penetrations and the approximate location of each recording site were reconstructed on the basis of marking lesions and several other cues, such as the coordinates of the penetrations within the recording chamber, the functional characteristics of cortical areas passed through, the location of boundaries between white and grey matter, and the distance of recording site from the surface of the hemisphere (see Galletti *et al.*, 1999a, 1999b).

Analysis of anatomical data

The HRP-processed material was studied under both bright field and dark field illumination at low magnification. The fluorescent material was viewed at 400 \times , with a Zeiss Universal epi-fluorescence microscope equipped with a narrow band excitation filter (BP 365/11), a dichroic mirror (FT 395), and a barrier filter (LP 395).

The extent of the WGA-HRP injection site was taken to be the densely stained region adjacent to the needle track (core) and the surrounding halo. Also the FB injection site had two concentric zones; an inner zone, sharply delineated around the needle track and that appeared necrotic and intensely fluorescent, and an outer zone, less sharply delineated, which was less brilliantly fluorescent, and where almost all of the neurons and many of the glial cells showed strong fluorescence. The outer border of the second zone was considered to be the border of the injection site.

In each section every 600 μ m, the outer and inner cortical borders and the location of each labelled neuron were plotted with the aid of inductive displacement transducers mounted on X and Y axes of the microscope stage. The transducer signals were digitized and stored by using software that allows the visualization of section outlines, of grey-white matter borders, and of labelled cells and terminals (Matelli *et al.*, 1998).

In order to visualize the distribution of cortical labelling, two-dimensional reconstructions of various sectors of the cortical mantle were obtained by using the same software. The procedure was the following. In each plotted section, the cortex was subdivided into columnar bins by drawing lines perpendicular to the cortical surface and connecting the outer and inner cortical contours. In order to minimize the distortion caused by cortical curvature, the cortex was

then unfolded at the level of a line connecting the midpoints of all the perpendicular lines, that is approximately at the level of layer IV. The unfolded sections were then aligned and the labelling distributed along the space between two consecutive plotted sections (600 μ m).

Several criteria were used to locate specific cortical areas and to assign labelled cells to these areas. In the occipito-parietal cortex, we recognized areas V1, V2, V3, V3A, V6 and V6A on the basis of their cytoarchitectonic organization as well as the functional data collected during recording sessions. As far as the identification of the other cortical areas is concerned, the decision was essentially based on their brain location as described in the literature (see Results). In some cases, differences in laminar distribution of anterograde and/or retrograde labelling were used to trace the border between neighbouring labelled areas.

Results

Identification of injection sites

The microelectrode penetrations carried out in two animals for several months before injecting neuronal tracers allowed us to study the functional properties of neurons in the occipito-parietal cortex. Areas V1, V2 and V3 were recognized on the basis of the coherence of RF size and location with respect to those predicted by a number of previous studies (Daniel & Whitteridge, 1961; Gattass *et al.*, 1981, 1988). Area V6 was easily recognized in behaving animals among the other visual areas hidden in the depth of POS and POM (see Galletti *et al.*, 1999b). Cells of area V6 were very sensitive to the visual stimulation and their visual RFs were easy to map. In contrast, several cells of area V6A, located just dorsal to V6, were activated only by complex visual stimuli, or were completely insensitive to visual stimulation (Galletti *et al.*, 1999a). Also, the activity of many V6A cells were modulated by arm movements in darkness (Galletti *et al.*, 1997; Fattori *et al.*, 1999b), a behaviour never seen in V6 (Galletti, unpublished data). Finally, the RF size of V6 neurons was typically smaller than that of V6A cells, and consistently larger than that of cells in V3, an area that borders ventrally and posteriorly on area V6 (see Galletti *et al.*, 1999b).

At the end of recording sessions, penetrations were carried out with recording syringes in three hemispheres of two animals (cases 16L, 17L, 17R) in order to inject neuronal tracers into different parts of area V6. The following are descriptions of the type of neurons encountered along those penetrations and of the injection site identification.

Case 16L

As shown in Fig. 2, the recording needle passed first through area V1 in the occipital operculum. The RFs of recorded cells were very small, located in the lower visual field, near the vertical meridian, at about 7° of eccentricity. The needle then passed through area V1 in the calcarine fissure (RFs at about 10° of eccentricity), and area V2 in the posterior bank of POS (RFs larger than those in V1; 13° of eccentricity, located nearer to the horizontal meridian). The recording needle was then advanced into V3 (RFs larger than those in V2; 24° of eccentricity, located in proximity of the vertical meridian) and finally into V6.

Two regions of V6 were traversed, in sequence; in both, the RFs were larger than in V3. In the first one (Fig. 2, RFs 1–5), RFs were located within the lower visual field, with RF centres at an eccentricity of about 40° and the borders, on average, between 20° and 60° of eccentricity. In the second region of V6 (Fig. 2, RFs 6–9), RFs were located in the upper visual field, with RF centres at an

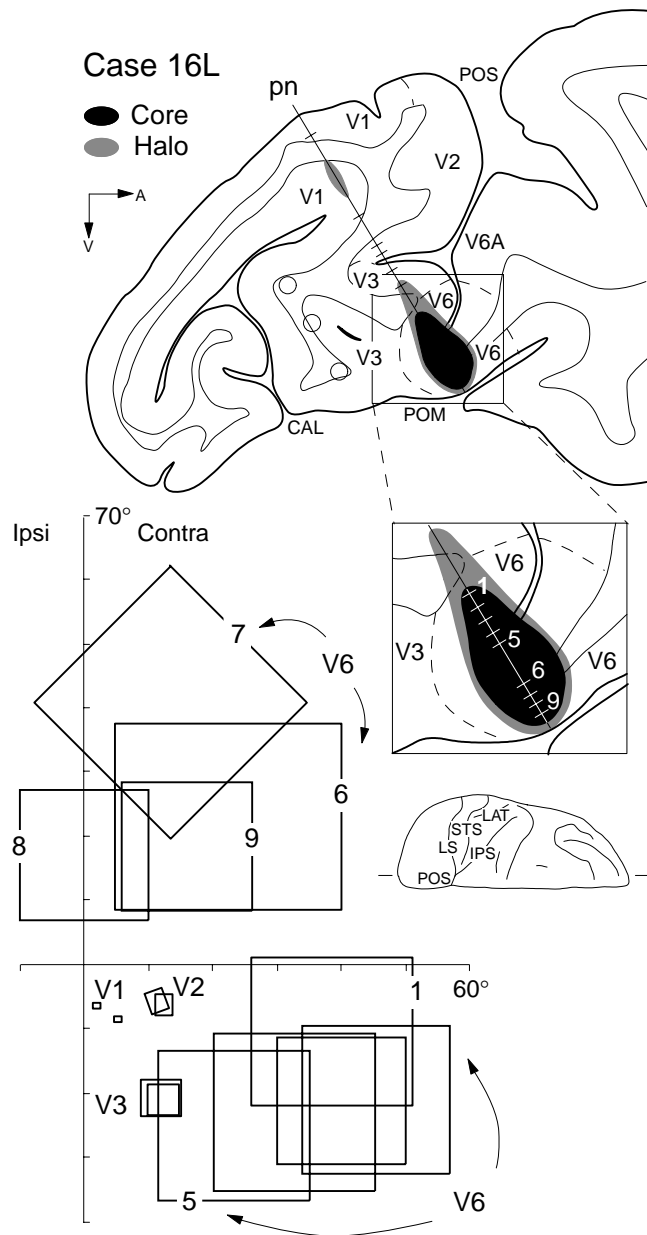


FIG. 2. Reconstruction of the penetration carried out with the recording syringe in case 16L. Top: Parasagittal section of the brain containing the reconstruction of the penetration carried out with the recording syringe (pn). The section was taken at the level indicated on the brain silhouette below. The assignment of borders between cortical areas on the section (dashed lines) is the result of the reconstruction of a series of microelectrode penetrations carried out in that cortical region (see Galletti *et al.*, 1999b). Three small circles in the occipital pole indicate the locations of small electrolytic lesions made along one of these penetrations. A picture of the original section is shown in Fig. 5. Dashes along the penetration track indicate locations of recording sites. The recording sites in area V6 are indicated in the enlargement of POS fundus shown in the inset at the centre of the figure; sites are numbered from 1 to 9. Core and halo zones of injection site are reported in the section and in the inset as black and grey areas, respectively. Bottom: RFs mapped at different recording sites. A, anterior; V, ventral; Ipsi, ipsilateral visual field; Contra, contralateral visual field.

eccentricity of about 30° and the borders between 10° and 60° of eccentricity. This type of retinotopic organization was typical of this part of V6, as described in Galletti *et al.* (1999b). WGA-HRP

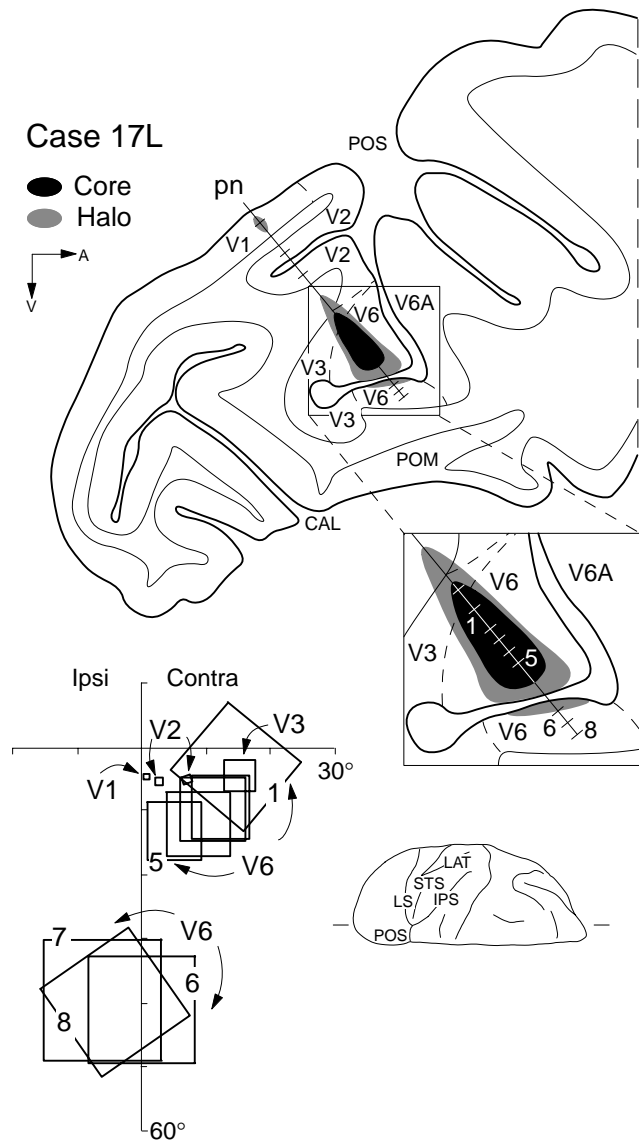


FIG. 3. Reconstruction of the penetration carried out with the recording syringe in case 17L. Details and abbreviations as in Fig. 2.

was slowly injected in both the lower and upper field representations of V6 (see Materials and methods for a detailed description of injection).

Case 17L

As shown in Fig. 3, the recording needle first passed through area V1 in the occipital operculum (RFs at 4.5° of eccentricity, located near the lower vertical meridian), then twice through area V2 in the posterior bank of POS (6° and 8.5° of eccentricity, respectively, with RFs moving from the vertical to the horizontal meridian). After about $3000\ \mu\text{m}$ of neural silence (white matter), the recording syringe entered into area V3 (RFs larger than those in V2; 15° of eccentricity, located near the horizontal meridian) and then into V6. The passage from V3 to V6 was clearly indicated by the sudden increase in RF size. While the electrode advanced into the grey matter of V6, the RFs moved from the horizontal to the vertical meridian; they were located within the lower visual field, with RFs centres at an eccentricity of about 15° and RFs borders, on average, between 5°

and 25° of eccentricity (Fig. 3, RFs 1–5). These data are in agreement with our recent studies showing that in the most lateral part of the posterior bank of POS (e.g. in the most medial part of the annectant gyrus) the central visual field representation of area V6 merges with the cortex of area V3 (see Galletti *et al.*, 1999b).

After a brief period of neural silence, due to the passage through the cleft of POS, the electrode entered into the grey matter of the fundus of POS, into another part of V6. As also noted in previous studies, the RFs in the fundus of POS were more eccentric than in the posterior bank of POS (Fig. 3, RFs 6–8; see also Galletti *et al.*, 1999b). In order to inject the tracer into the central representation of V6, the recording syringe was withdrawn as far as to re-enter the posterior bank of POS, where the WGA-HRP was injected (see Materials and methods for a detailed description of injection).

Case 17R

As shown in Fig. 4, the recording needle passed through area V1, both in the occipital operculum (RFs at 5° of eccentricity) and in the calcarine fissure (RFs at 7° of eccentricity). Then, it passed through area V2 in the posterior bank of POS (RFs at 10° of eccentricity) and reached area V6 in the anterior bank of POS. The RFs in V6 were large, as usual, and very eccentric (Fig. 4, RFs 1–3). They were located within the lower visual field, with the RF centres more eccentric than 50° and RF borders between 35° and more than 70° of eccentricity. RFs moved from the vertical to the horizontal meridian, and from periphery to more central field representations, according to previous descriptions of Galletti and coworkers in other cases (Galletti *et al.*, 1999b). FB was slowly injected in this part of area V6 (see Materials and methods for a detailed description of injection).

Case P11L

In one case, WGA-HRP was injected in the dorsal part of the anterior bank of POS in an anaesthetized animal (see Materials and methods for a detailed description of injection). As shown in Fig. 1, the injection site in this case was restricted to area V6A, located dorsally to area V6. Figure 5 shows, together with the injection site, the strong projections to the dorsal premotor area F2 (Matelli *et al.*, 1991) arising from V6A. Although a detailed description of V6A connections is out of the scope of the present work, the frontal projections of V6A are here reported to highlight the absence of frontal connections after V6 injection (see Discussion).

Extent of injection sites

Our estimate of the effective zone of uptake of neuronal tracer was made on the basis of the extent of the injection 'core' plus the surrounding 'halo' of intense diffuse labelling, where labelled cells were visible against the uniformly stained background.

The section shown in Fig. 6 is a low power microphotograph taken at the injection site level in case 16L and corresponds to that presented in Fig. 2. As one can see from Fig. 2, the core of tracer injection was inside the limits of area V6, whereas the halo zone involved the infragranular layers of V3.

Figure 3 shows that the core of tracer injection in case 17L was inside the limits of area V6, whereas the halo zone involved dorsally a very narrow strip of V3.

In case 17R, a significant leakage of tracer was observed along the needle track in the posterior bank of POS, at a level of area V2 representing a small central part of the visual field (see Fig. 4). Area V3 was not involved by the leakage because the needle did not cross this area. It was located more posteriorly, in the fundus of POS (see Fig. 4) as recognized by previous series of microelectrode penetrations carried out in this same hemisphere. Despite the leakage, the

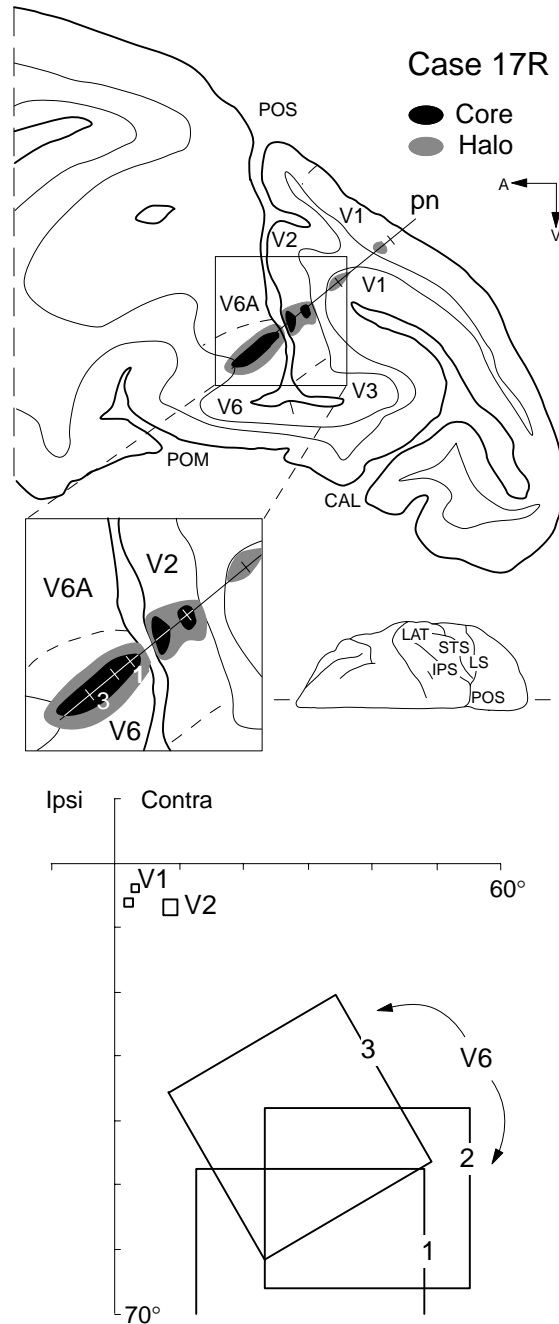


FIG. 4. Reconstruction of the penetration carried out with the recording syringe in case 17R. Details and abbreviations as in Fig. 2.

bulk of tracer was injected in area V6, and Fig. 4 shows that both core and halo zones of injection site in the anterior bank of POS were well inside the limits of this area.

Electrophysiological data collected with the recording syringe allowed us to check whether substantial quantities of tracer were picked up by the cortical areas traversed by the recording syringe (areas V1, V2, V3; see Figs 2–4). As it is well known that areas V1 and V2 are retinotopically interconnected, we looked for labelled cells and/or terminals in regions of V1 and V2 corresponding retinotopically to those passed through by the recording needle. In case 16L, neither cells nor terminal labels were found in those parts of areas V1 and V2. In case 17L, only a few labelled cells (22 cells in

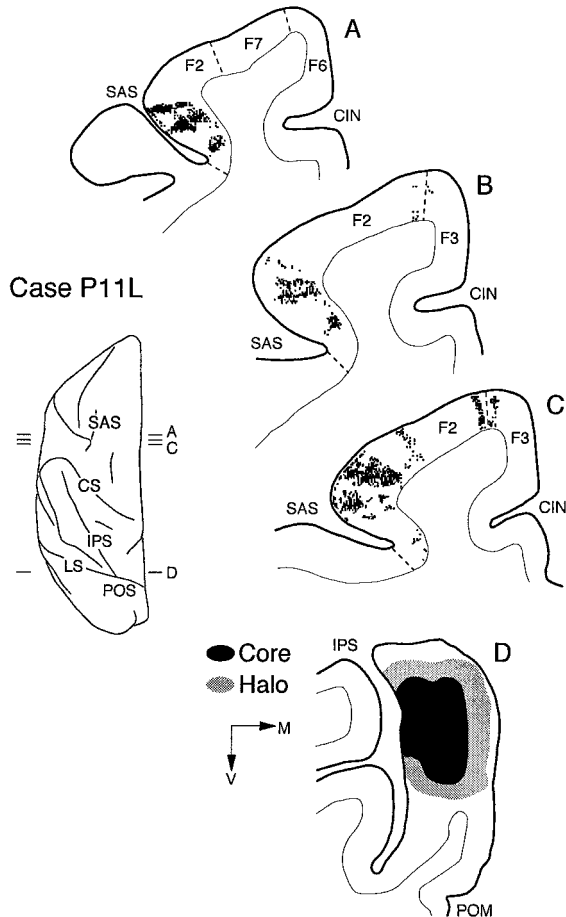


FIG. 5. Frontal projections after V6A injection. (A–D) Coronal sections of case P11L, taken at the level indicated on the brain silhouette shown on the left. Sections A–C contain anterograde labels (dots), after WGA-HRP injection in area V6A, in the dorsal premotor area F2, defined according to Matelli *et al.* (1991). Section D shows the core and halo zones of injection site. CIN, cingulate sulcus; CS, central sulcus; SAS, superior arcuate sulcus; V, ventral; M, medial. Details and abbreviations as in Fig. 2.

total, distributed over six sections) were found in regions of V2 and V1 retinotopically consistent with V1/V2 regions traversed by the recording syringe (see Fig. 3 and encircled dots in sections A and B of Fig. 9). In case 17R, where a significant leakage of tracer in V2 was observed, labelling was found in the retinotopically corresponding region of area V1 (see encircled dots in Fig. 11).

In cases 16L and 17L the recording syringe traversed V3 before entering V6, and the halo zone of injection site partially involved V3 itself (see Figs 2 and 3). As it is well known that V3 is retinotopically interconnected with areas V1 and V2, the possibility exists that labelling observed in these two areas was due to the involvement of V3 rather than to V6 injection. However, two main considerations led us to the conclusion that tracer uptake in area V3 in cases 16L and 17L, if any, was not primarily responsible for the labelling observed in V1 and V2. First, labelling involved large extents of V1 and V2 (see Figs 8–11), that corresponded better to the large visual field representation present at the injected V6 rather than to the very small field representation of V3 involved at injection site (see Figs 2 and 3). Second, in the case 17R, where the injection site did not involve V3, labelled cells were found in regions of V1 and V2 retinotopically

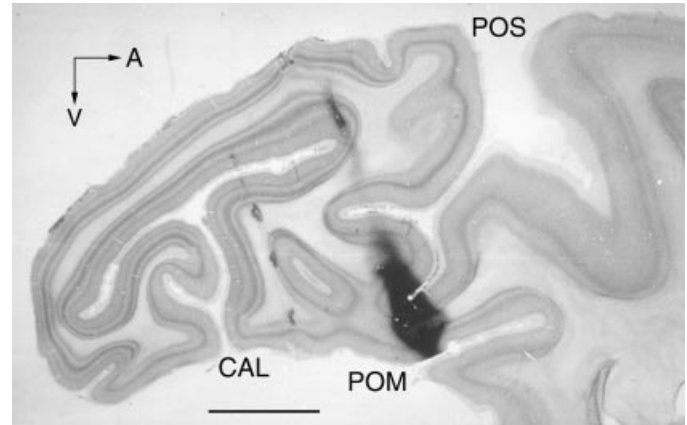


FIG. 6. Injection site in case 16L. Nissl-stained parasagittal section passing through the injection site in case 16L. The section is the same reported in Fig. 2. A, anterior; V, ventral. Scale bar, 5 mm.

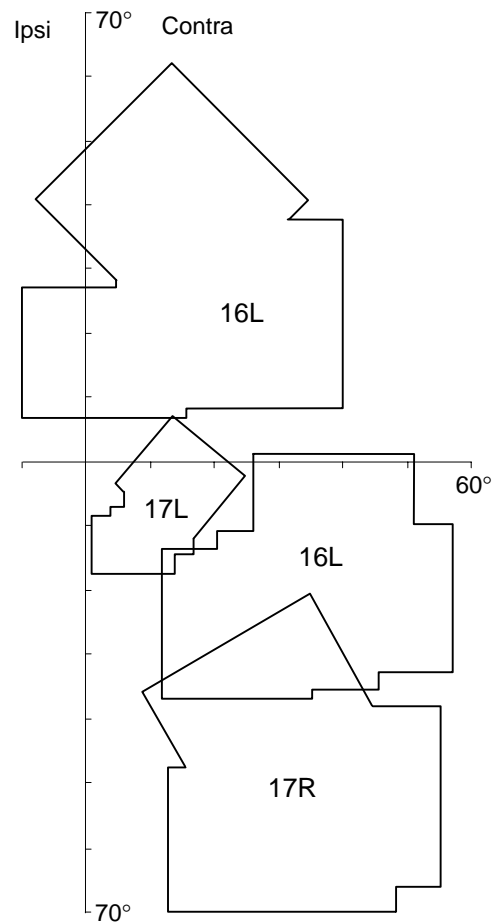


FIG. 7. Visual field representation in V6 at injection site level. Outlines of the most external RF borders mapped at the injection sites in cases 16L, 17L and 17R. The single RFs encountered in V6 at the injection site level are shown in Figs 2–4. Ipsi, ipsilateral visual field; Contra, contralateral visual field.

corresponding to the field representation of V6 at the injection site (see Fig. 11).

Figure 7 summarizes the RF locations of visual neurons recorded in the parts of V6 injected in cases 16L, 17L and 17R. Note that the

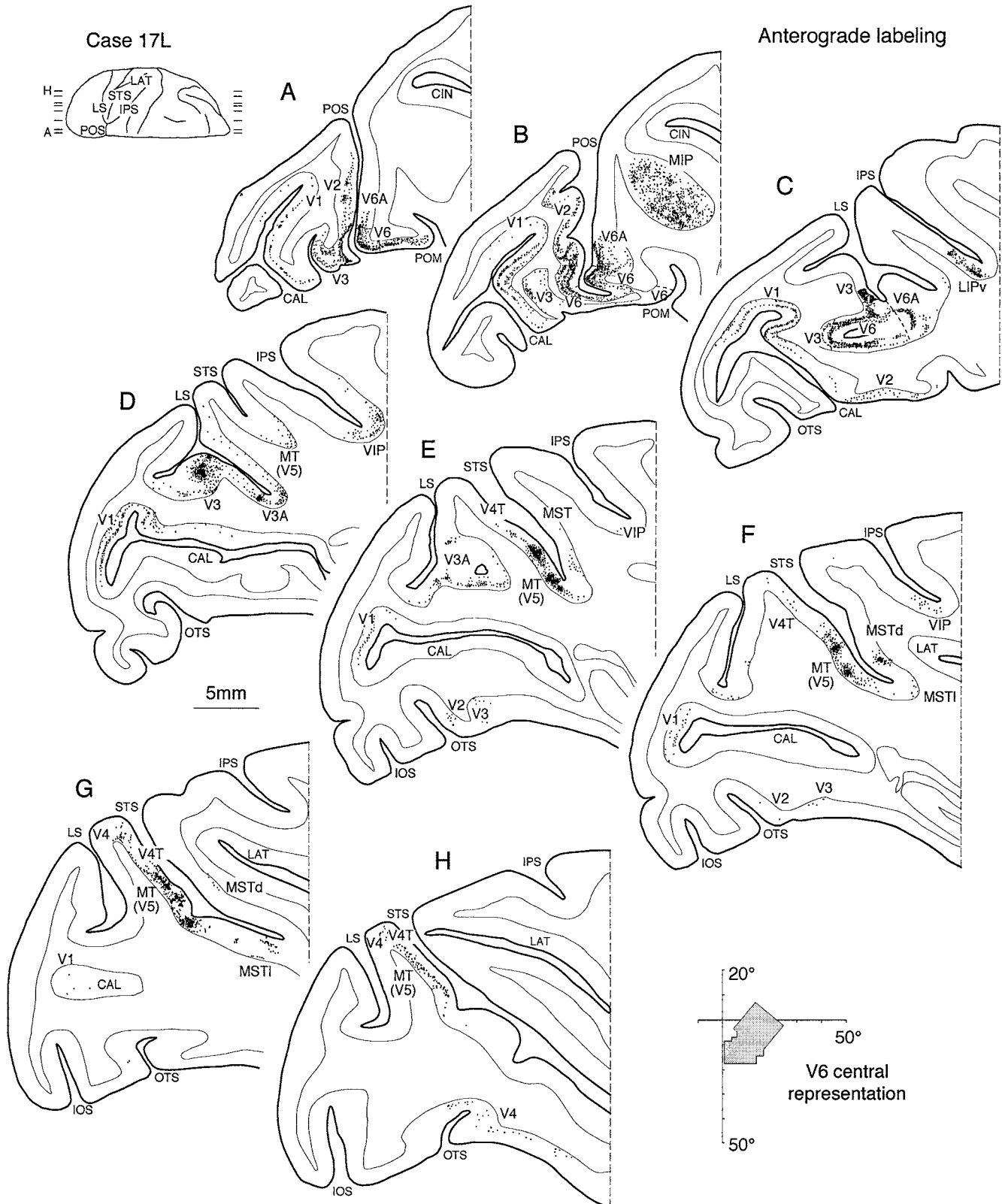


FIG. 10. Cortical distribution of anterograde labeling after central V6 injection of WGA-HRP (case 17L). (A–H) Parasagittal sections taken at the levels shown on the brain silhouette reported on the bottom right of the figure. Dot densities on sections are proportional to anterograde labeling. Other details as in Fig. 8.

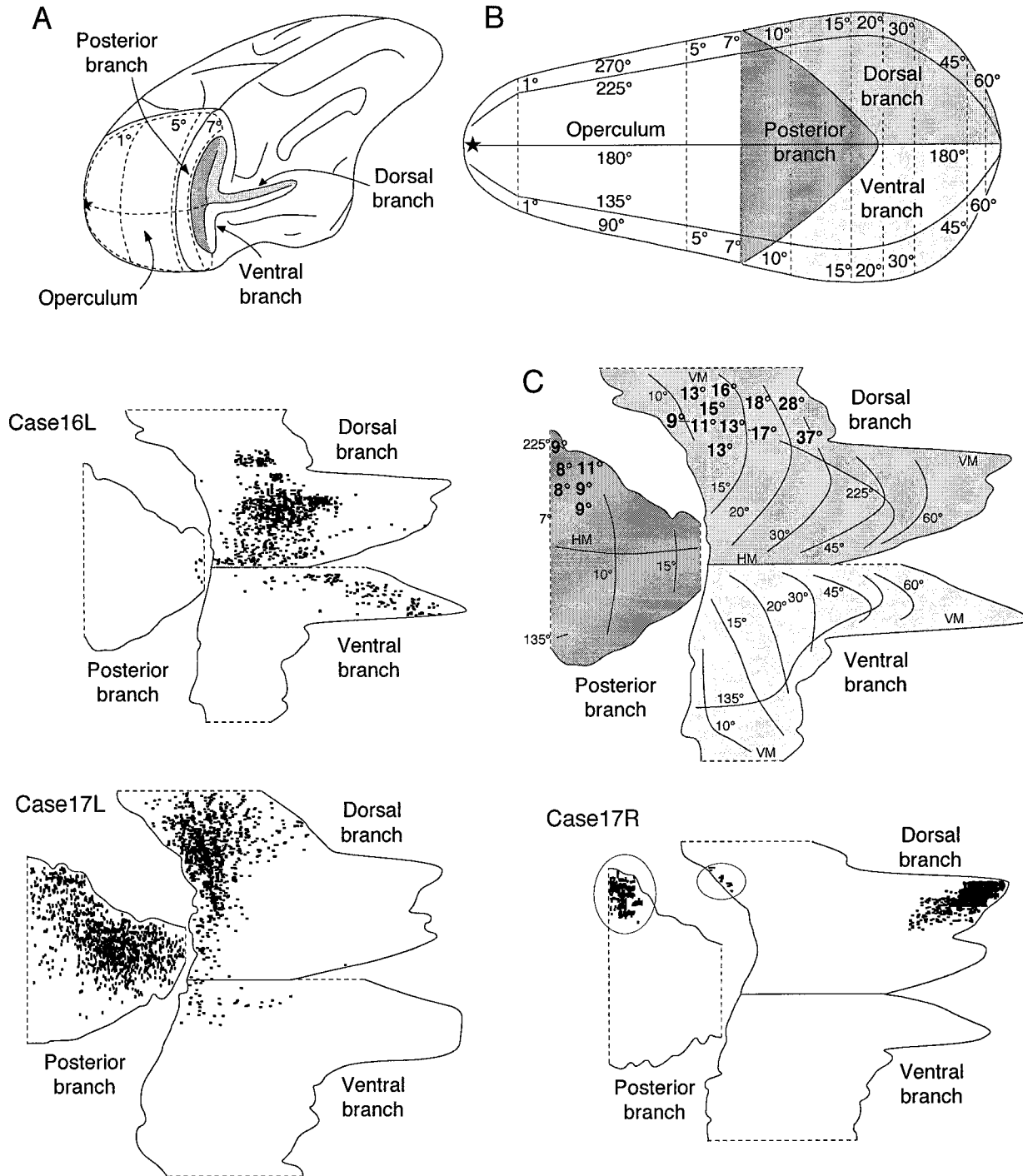


FIG. 11. Cortical distribution of V1 cells projecting to V6. (A) Postero-medial view of a left hemisphere to show the occipital operculum, and the posterior, dorsal and ventral branches of CAL (shown with different scales of grey), where area V1 is located in the macaque brain. (B) Flattened map of the occipital operculum and CAL. The bidimensional map shows the visual field representation in V1 as reported by Daniel & Whitteridge (1961). (C) The topographic data of Daniel & Whitteridge (1961) were superimposed on the flattened map of CAL of case 16L. Numbers in bold indicate the eccentricity of V1 RFs we encountered when recording from that part of V1 in case 16L. HM, horizontal meridian; VM, vertical meridian. In the remaining part of the figure, the flattened maps of cases 16L, 17L and 17R are reported. Each single dot on the maps represents a retrogradely labelled cell in V1 after V6 injection. Encircled dots in the flattened map of case 17R represent labelled cells due to the tracer leakage along the penetration track (see Results).

central part of the visual field representation was injected in case 17L whereas the visual periphery was injected in cases 16L and 17R. Both lower and upper visual field representations were injected in case 16L; only the lower one in case 17R.

Cortical connections of V6

In this section we will report in detail the cortical connections of cases 16L and 17L, the first injected in the peripheral field

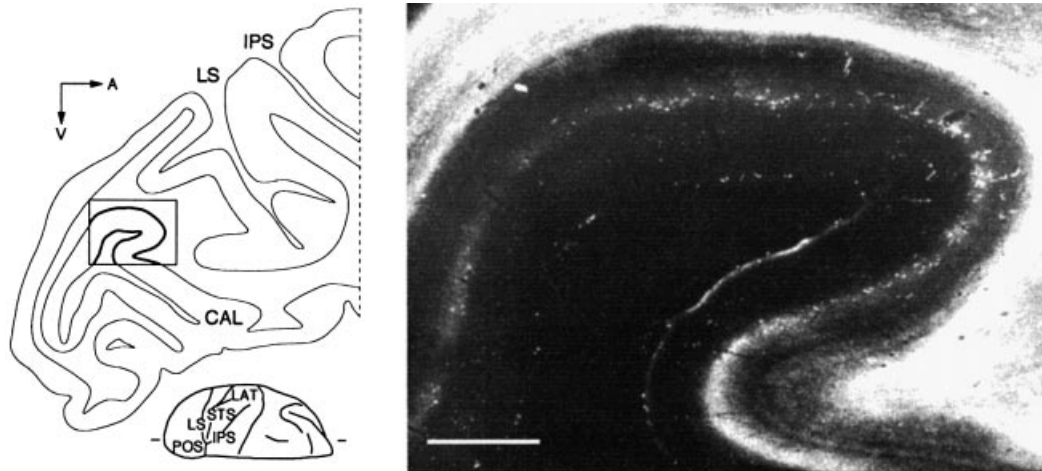


FIG. 12. V1 labelling after V6 injection. Left: Parasagittal section of case 17L taken at the level indicated on the brain silhouette shown below. This section was taken at about the same level of section D in Fig. 9. Right: Dark-field photomicrograph of the squared region of CAL cortex shown on the left, containing transported labelled material after V6 injection. A, anterior; V, ventral. Scale bar, 1 mm.

representation of V6 and the second in the central field representation.

Figures 8 and 9 show the locations of retrogradely labelled cells in a series of parasagittal sections in cases 16L and 17L, respectively. Figure 10 shows the locations of anterograde labelling in the case 17L. By comparing the labelling reported in Figs 9 and 10, both referring to the case 17L, it is evident that all the cortical connections of area V6 are reciprocal. The same pattern of connection was observed in case 16L.

After tracer injection into V6, regardless of central or peripheral field representation injected, the same cortical areas in the occipital and posterior parietal cortex, and in the caudal part of superior temporal sulcus (STS) were labelled constantly. Strong intrinsic connections, spreading over large part of V6, were also observed in both cases (Figs 8C and D, 9A–C and 10A–C). No labelled cells or terminals were found in the inferior temporal, mesial and frontal cortices.

Area V1

Area V1 was extensively labelled after V6 injections. The labelling was found in different regions of V1, whose visual field representation was congruent with that of the injected V6 (see Figs 2–4). In Fig. 11, labelling was reported on the flattened maps of the calcarine sulcus (CAL). The maps clearly show that V1 regions projecting to V6 occupy complementary parts of the striate cortex in the three cases. In case 16L, labelled cells were found in the dorsal and ventral branches of the CAL (see also Fig. 8C–G). In case 17L the posterior branch of CAL and the posterior part of the dorsal branch were labelled (see also Fig. 9A–G). Finally, in case 17R a cluster of labelled neurons was located in the most rostral part of the dorsal branch of CAL. According to the Daniel and Whitteridge's map of V1 (Daniel and Whitteridge, 1961), (reported in Fig. 11B and C), labelled regions would contain the representation of the peripheral parts ($> 20^\circ$ of eccentricity) of the lower and upper visual fields in case 16L, the central part ($5\text{--}20^\circ$) of the lower visual field in case 17L and the far periphery of the lower visual field in case 17R. All these data are in good agreement with the field representations in the injected V6 (see Figs 2–4). Note that the occipital operculum, that contains the central 5° representation of the visual field, was devoid

of labelling. As this visual field representation was never injected in our cases, this observation too supports the view that V1–V6 reciprocal connections are strictly retinotopically organized.

Retrograde and anterograde labelling in V1 occupied more or less the same cortical regions but showed different lamination patterns (compare Figs 9 and 10). The darkfield picture of a section reported in Fig. 12 and the reconstruction of an adjacent section in Fig. 13 show that retrogradely labelled cells were mainly confined within layer 4B, with some labelled cells in supragranular layers 2 and 3, whereas anterograde labelling were found into layers 4B, 5 and 6.

Areas V2 and V3

Extensive labelling was observed in areas V2 and V3. The distribution of labelled cells and terminals involved different parts of these areas in the two cases. In case 16L, labelling was found in the cuneate cortex (Fig. 8B), in the cortex hidden in the POM (Fig. 8C and D) and in the cortex of the ventral part of the brain, both dorsally (Fig. 8C) and ventrally to the CAL (Fig. 8E). In case 17L, labelled cells and terminals in areas V2 and V3 were found in the posterior bank of POS, in the cuneate cortex (Figs 9A and B, and 10A and B), in the upper branch of CAL (Figs 9C and 10C) and in the posterior bank and fundus of the lunate sulcus (LS, Figs 9C and D, and 10C and D). Sparse labelling was found in the ventral surface of the brain, around the occipito-temporal sulcus (Figs 9E and F, and 10E and F). According to the retinotopic maps of areas V2 and V3 reported by Gattass and coworkers (Gattass *et al.*, 1981, 1988), there was a good correspondence between the visual field representation at the level of labelled regions and the field representation at injection sites. Note, for instance, that V2 and V3 labels in the ventral part of the hemisphere were grouped together in case 17L (Fig. 9E and F). According to Gattass *et al.* (1981, 1988), ventral V2 and V3 represent the upper visual field, and the border between V2 and V3 represents the horizontal meridian. Accordingly, in case 17L the horizontal meridian and a small part of the upper visual field were involved at the injection site (see Fig. 7). Furthermore, the labelling found only in case 16L (periphery V6 injection) in the cortex hidden in POM (Fig. 8C and D) is consistent with the recent demonstration that the far periphery of V3 is represented in this cortical region (Galletti *et al.*, 1999b).

Area V3A

Very strong labelling was observed in area V3A in both cases 16L and 17L. Labelled cells and terminals were found in the fundus and anterior bank of LS (Figs 8E, 9D and E, and 10D,E). Medially, the labelled region extended from the fundus as far as the two thirds of the anterior bank of LS in case 16L (Fig. 8E), whereas it remained confined to the fundus of LS in case 17L (Figs 9D and 10D). Laterally, in case 16L labelling was virtually absent in the region where the annectant gyrus merges with the anterior bank of LS (Fig. 8F), whereas strong labelling was observed in the same cortical region in case 17L (Figs 9E and 10E). These results are consistent with previous mapping studies (Van Essen & Zeki, 1978; Zeki, 1978a) showing that V3A contains: (i) lower field representation posteriorly, in the fundus of LS, and an upper field representation anteriorly, in the anterior bank of LS and (ii) a central representation laterally and a more peripheral one medially.

Area V4 and dorsal prelunate area

The exposed surface of the prelunate cortex contains two adjacent areas: the dorsal prelunate area (DP), medially, and the dorsal component of area V4, laterally (Van Essen & Zeki, 1978; Maguire & Baizer, 1984; Van Essen, 1985; Gattass *et al.*, 1988). Area DP represents the upper visual field (Van Essen, 1985), whereas the dorsal V4 represents part of the lower visual field (Van Essen & Zeki, 1978; Maguire & Baizer, 1984; Gattass *et al.*, 1988). The ventral component of V4 is located on the ventral surface of the hemisphere and represents the upper visual field and a part of the lower one (Gattass *et al.*, 1988).

We found few labelled cells and terminals in both DP and V4. In DP, labelled cells were observed only in case 16L (Fig. 8D), in accordance with the fact that a substantial part of the upper visual field representation in V6 was injected in this case. In area V4, very weak labelling was observed in both cases. A few labelled cells were found in the dorsal component of V4 (Figs. 8E, 9G and H, and 10F–H), whereas sparse labelling was observed in the ventral component of this area (Figs 8F, 9H and 10H). This slight quantitative difference between dorsal and ventral components seems to be congruent with the observation that part of the lower visual field is represented in the ventral component of V4 (Gattass *et al.*, 1988). However, the weakness of the overall V4 labelling, together with the absence of V4 labelling in the case 17R (where area V3 was certainly not involved in tracer uptake), casts serious doubt on the attribution of this connection to V6 injection and suggests that the tracer diffusion in area V3 may be responsible for this labelling.

Area V4T

In both cases, extensive labelling was observed in a strip of cortex located in the dorsal part of the posterior bank of STS, just above middle temporal area/area V5 (MT/V5). According to the electrophysiological mapping studies of this cortical region, this labelled area is likely to correspond to area V4T (Desimone & Ungerleider, 1986; Gattass *et al.*, 1988). Labelling in V4T was stronger medially in case 16L (Fig. 8F) and laterally in case 17L (Fig. 9H), in good agreement with the visuotopic organization of V4T (peripheral representation medially and central representation laterally, Desimone & Ungerleider, 1986; Gattass *et al.*, 1988). Mapping studies agree that V4T contains only a representation of the lower visual field, but disagree on its extent: according to Gattass *et al.* (1988) this representation is limited to the central 20°, whereas according to Desimone & Ungerleider (1986) it extends as far as

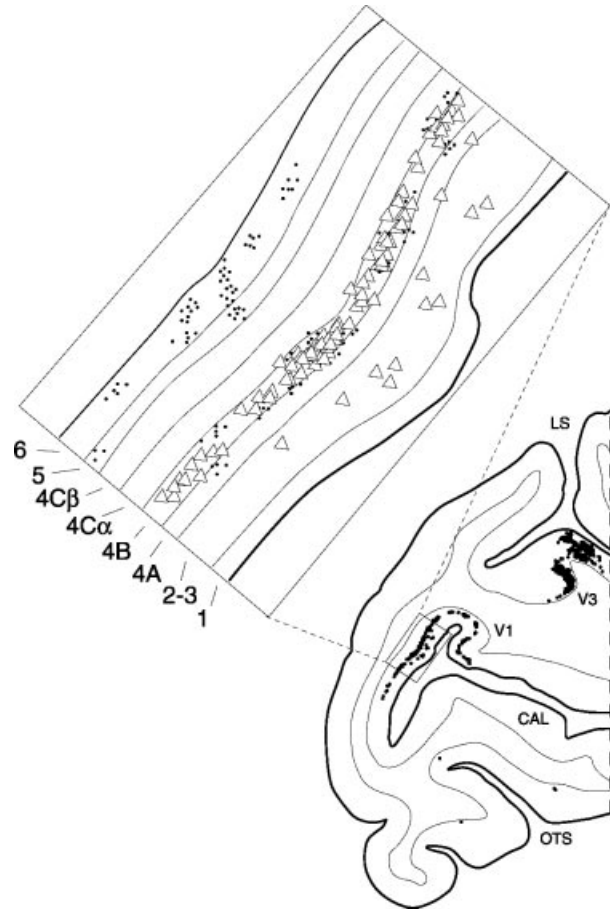


FIG. 13. Laminar pattern of labelling in V1 after V6 injection. The figure shows a parasagittal section of case 17L taken at about the same level of section D in Fig. 9 and the section shown in Fig. 12. Each single dot represents a retrogradely labelled cell. The inset on the top of the figure shows an enlargement of a part of the posterior branch of CAL (squared area on the section). Triangles are single retrogradely labelled cells; small dots, anterograde label. Numbers and letters indicate the cortical layers.

about 60°. Our data agree with this latter view, as in case 16L (20–60° of the visual field at injection site) V4T was labelled strongly.

Area MT/V5

V6 injections produced strong labelling in the posterior bank of STS, ventrally to V4T labelling, and in the fundus of this same sulcus (Figs 8E and F, 9D–H and 10D–H). According to mapping studies of this region, this labelling is likely within the limits of area MT/V5 (Gattass & Gross, 1981; Van Essen *et al.*, 1981; Desimone & Ungerleider, 1986). Mapping studies reported that MT/V5 represents the central 30°–40° of the visual field, with the foveal representation located antero-laterally and the periphery medio-dorsally within the STS. Our data show that the central 20° representation of V6 (case 17L) is more heavily connected with MT/V5 than the peripheral V6 representation (case 16L). In particular, labelling in the most lateral part of area MT/V5 was stronger in the centrally injected case 17L (Figs 9H and 10H) than in the peripherally injected case 16L (Fig. 8H). In case 17R, where injection was in the far periphery representation of V6, MT labelling was virtually absent. These results suggest that connections between V6 and MT/V5 are retinotopically arranged.

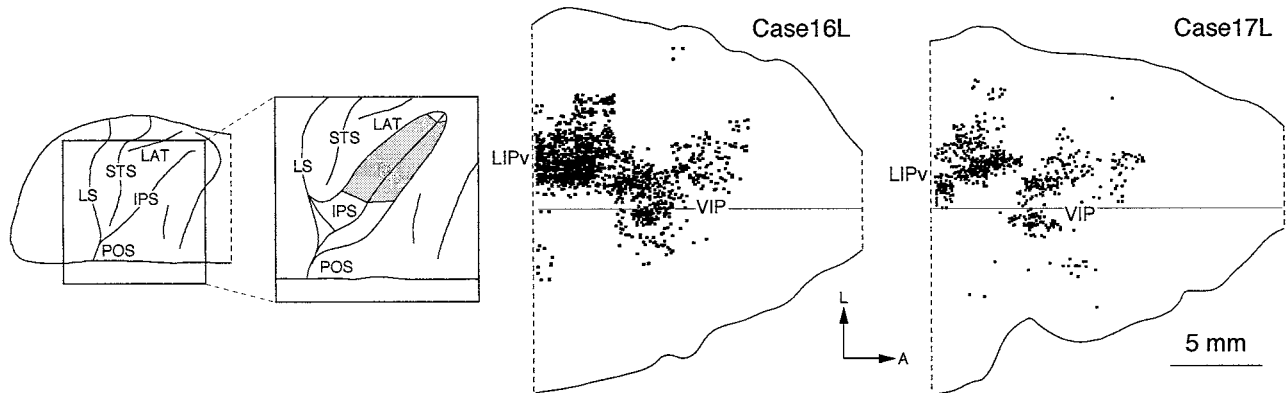


FIG. 14. Cortical distribution of LIPv and VIP cells projecting to V6. On the left, the dorsal view of the posterior half of the left hemisphere and an enlargement of the IPS region (inset) are shown. In the inset, the IPS is open to show (in grey) the cortical region reported to the right as flattened maps. The maps were obtained by aligning the unfolded sections at the fundus of IPS. Each single dot on the maps represents a retrogradely labelled cell. Based on their locations, the two labelled regions in each map likely correspond to areas LIPv and VIP as indicated. A, anterior; L, lateral.

Medial superior temporal area

In both cases, moderate labelling was observed in the anterior bank of STS (Figs 8F–H, 9E–G and 10E–G), in a region that, according to mapping studies, is likely to correspond to the medial superior temporal area (MST; Maunsell & Van Essen, 1983a; Desimone & Ungerleider, 1986). The distribution of labelled cells and terminals was quite different in the two cases. Whereas in case 16L there was a single labelled region in the anterior bank of STS (Fig. 8F–H), in case 17L two regions were labelled: a dorsal one, approximately corresponding to the dorsal part of the region labelled in case 16L (Fig. 9E and F), and a ventral one, in the fundal region of STS (Figs 9F and G, and 10F and G). The different locations of labelling in the two cases agree with the view of a coarse retinotopic organization in MST, with the central representation located dorsally and the peripheral one ventrally. The second, ventralmost spot of projection observed in the case of central V6 injection could be in area FST (according to Desimone & Ungerleider, 1986) or the lateral component of MST (MSTl; according to Komatsu & Wurtz, 1988), both regions being located below the antero-ventral border of MST (or MSTd according to Komatsu & Wurtz, 1988), and both containing central representations of the visual field.

Area V6A

Area V6A has been defined recently as a 'horseshoe-shaped' strip of cortex located dorsally to V6 in the anterior bank of POS and anteriorly to V6 in the mesial surface of the hemisphere (Galletti *et al.*, 1999a). Following V6 injection, area V6A was labelled strongly in all cases, but not in its whole dorso-ventral extent; labelled cells and terminals were always confined to the ventral part of V6A, that is to the part of V6A bordering area V6 (Figs 8A–D, 9B and C, and 10B and C).

Recent physiological data have shown that V6A is not retinotopically organized (Galletti *et al.*, 1999a). Upper and lower visual field, as well as central and peripheral parts, can be represented in any part of V6A. This is particularly true for the ventral part of V6A, where adjacent cells can show RFs in either the central part of the visual field or in the far periphery. Therefore, the segregation of labelling observed in area V6A can not be explained as the result of a visuotopic organization of connections. Rather, it likely reflects a possible functional dishomogeneity within area V6A (see Discussion).

Medial intraparietal area

Labelled cells and terminals, though differentially distributed, were present in both cases in the posterior part of the medial wall of IPS (Figs 8B and C, 9B and 10B). In case 16L, labelled cells and terminals were confined to the ventral part of the medial wall of IPS, whereas in case 17L they involved almost the whole dorsoventral extent of the wall. All these labels can be attributed to the medial intraparietal area (MIP; Colby *et al.*, 1988; Colby & Duhamel, 1991), a cortical region whose visuotopic organization is, at present, completely unknown.

Ventral part of the lateral intraparietal area

In both cases, labelled cells and terminals were found in the ventral part of the middle third of the lateral bank of IPS (Figs 8C and D, and 10C). The flattened maps of IPS reported in Fig. 14 show that the extent of labelled region in the lateral bank of IPS was similar in cases 16L and 17L, though it was stronger in the former. This labelled region is likely to be coextensive with area LIPv, described as the ventral component of the lateral intraparietal area by Blatt *et al.* (1990). According to these authors area LIP on the whole would be roughly retinotopically organized; the lower visual field would be located caudally and the upper one rostrally. LIPv would contain a representation of the periphery of the visual field, whereas the dorsal part of area LIP would represent the central part of it. Our results partially disagree with this view. The fact that in case 16L (peripheral V6 injection in both upper and lower visual field representations) labelling in LIPv were more or less coextensive with those of case 17L (central V6 injection in lower field representation) suggests that LIPv contains a nonretinotopic representation of the whole visual field, with a large representation of the periphery as suggested by Blatt *et al.* (1990).

Ventral intraparietal area

Labelled cells and terminals were found in the fundus of the middle third of the IPS (Figs 8E and F, 9D–F and 10D–F), a region likely corresponding to area VIP (Maunsell & Van Essen, 1983a; Blatt *et al.*, 1990; Colby & Duhamel, 1991; Colby *et al.*, 1993; Duhamel *et al.*, 1998). Figure 14 shows that the extent of this labelled region was similar in cases 16L and 17L, occupying both banks of the IPS fundus posteriorly, and the lateral bank only anteriorly.

TABLE 1. Numbers and percentages of labelled cells after V6 injection

Cortical area	Case 16L Labelled cells		Case 17L Labelled cells		Proportion of total labelled cells in the infragranular layers* (%)
	(n)	(%)	(n)	(%)	
V1	1420	8.1	3147	19.4	0
V2	1097	6.3	1129	7	17
V3	2130	12.2	2608	16.1	55
V3A	3543	20.3	913	5.6	68
DP	64	0.4	0	0	–
V4	86	0.5	117	0.7	–
V4T	594	3.4	324	2	71
MT/V5	2236	12.8	4046	24.9	64
MST	530	3	667	4.1	97
V6A	3538	20.2	693	4.3	47
MIP	556	3.2	1763	10.8	62
LIPv	1193	6.8	501	3.1	49
VIP	487	2.8	328	2	85
Total cells	17474	100	16236	100	–

*Percentage of labelled cells in the infragranular layers with respect to the total labelled cells in the same cortical area.

The ventral intraparietal area (VIP) has been described in physiological studies as a visual area lacking in visuotopic organization (Colby & Duhamel, 1991; Colby *et al.*, 1993). In good agreement with this view, our data indicate that the extent of labelled region in VIP after V6 injection is similar in cases 16L and 17L. The fact that V6 connections with area VIP were stronger in case 16L could be due to the presence in VIP of both the upper and lower field representations (Duhamel *et al.*, 1991, 1998), and/or to a wider peripheral representation in area VIP with respect to the central one, a fact that is not explicitly reported in literature.

Strength of V6 connections

A quantitative study was undertaken to evaluate the strength of V6 connections and to make a comparison between cases. In this study, data from case 17R were discarded because, as discussed above, tracer uptake in V6 in that case was poorer than in the other two cases, and also involved the cortical areas traversed by the recording syringe. In cases 16L and 17L, only the retrogradely labelled material was taken into account.

The number of labelled cells was calculated in both cases in each section every 600 μm . Labelled cells in the halo zone and intrinsic connections of area V6 were excluded from this count. The total number of labelled cells found in a given cortical area, and its percentage with respect to the total number of labelled cells were taken as indicative of the strength of that particular V6 connection.

Table 1 shows the numbers and percentages of labelled cells in the cases 16L and 17L. In case 16L, 26 adjacent sections covered the entire labelled region, spanning from the midline to about 17 mm laterally to it. A total of 17 474 labelled cells were found in 13 different cortical areas. In case 17L, 33 sections covered the labelled region, spanning from the midline to about 20 mm laterally to it. The labelled cortical areas were the same found in the case 16L, except for area DP. The total number of labelled cells was 16 236.

The last column of Table 1 reports the percentage of infragranular labelled cells with respect to the total labelled cells in that area. Note that in V1 no labelled cells were found in the infragranular layers, whereas in high-order visual areas such as MST and VIP almost all the labelled cells were located in the infragranular layers, in agreement with the view that the number of projecting cells increases

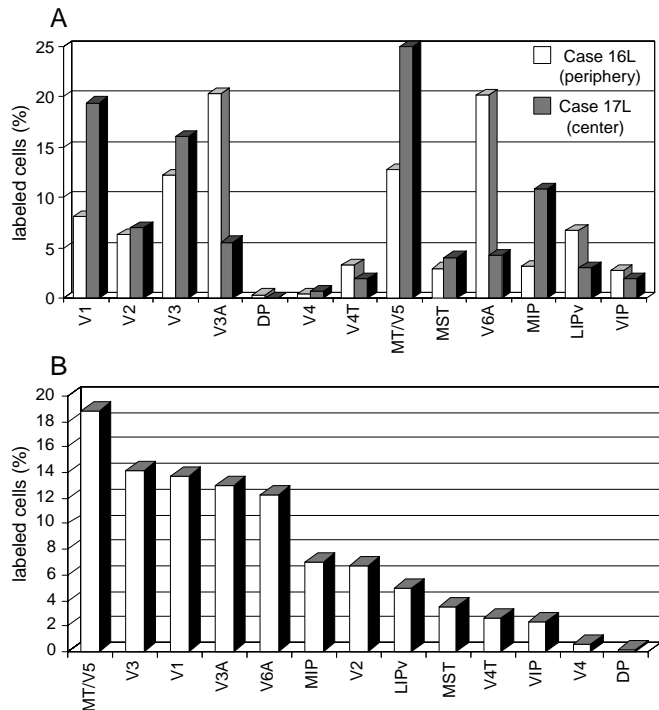


FIG. 15. Strength of V6 cortical connections. (A) Percentages of labelled cells (columns) present in different cortical areas after V6 injection. For each area, the percentages of labelled cells in the two cases 16L and 17L are reported. (B) Average percentages of labelled cells (columns) present in different cortical areas after V6 injection. The value of each column is the mean value of the two cases 16L and 17L as reported in A.

in the infragranular layers according to the hierarchic level of the area.

Figure 15A reports the percentage distribution of labelled cells in the different areas in the two cases (first two columns of Table 1). It is evident that for some cortical areas the connection with the central field representation of V6 was the strongest (e.g. areas V1, MT/V5, MIP), whereas for others the strongest was the connection with the peripheral field representation of V6 (e.g. areas V3A, V6A, LIPv). For the remaining cortical areas, the differences between cases were negligible.

Data plotted in Fig. 15B are averaged from the two cases and show the mean strength of V6 connections apart from differences between field representations. Areas V1, V3, V3A, MT/V5 and V6A are strongly connected with V6; labelled cells in these areas ranging from about 12% of the total in V6A to about 19% in MT/V5. Areas V2, MIP and LIPv are moderately connected with V6 (about 5–6%), whereas the connections of V6 are weaker with areas V4T, MST and VIP (about 2–3%). Very few labelled cells were found in V4 and DP.

Discussion

In this study, we injected WGA-HRP into different parts of V6, representing different parts of the visual field, to study the cortico-cortical connections of this area as well as the visuotopic organization of connections. To achieve this goal, we used a specially devised recording syringe that allowed us to record single unit activity in behaving animals and to map visual receptive fields at the injection site. Although we are aware that the intracortical spread of tracer

certainly exceeded the volume of cortex sampled by the micro-electrode of the recording syringe, electrophysiological data directly showed that at least those particular parts of the visual field representation were injected. The visual field representation involved by tracer injection was also inferred from the distribution of labelled material within the topographically organized area V1 after V6 injections. According to this analysis (see Fig. 11), the central 20° of visual-field representation was injected in one case (17L), whereas the peripheral (> 20°) field representation was injected in other two cases (16L, 17R).

Irrespective of whether WGA-HRP was injected into central or peripheral field representations in V6, strong-to-moderate labelling (cells and terminals) was found in areas V1, V2, V3, V3A, MT/V5, V6A, MIP and LIPv, whereas weaker but consistent labelling was observed in areas V4T, MST and VIP.

Visuotopic organization of V6 cortical connections

Six out of 11 areas connected with V6 (i.e. V1, V2, V3, V3A, V4T, MT/V5) are retinotopically organized visual areas (Daniel & Whitteridge, 1961; Van Essen & Zeki, 1978; Zeki, 1978a; Gattass & Gross, 1981; Gattass *et al.*, 1981, 1988; Van Essen *et al.*, 1981; Desimone & Ungerleider, 1986). Present results show that V6 connections were retinotopically in register with the topographic maps of these areas. According to this view, cortical areas where the central field representation is particularly magnified, such as V1 and MT/V5, were more strongly labelled after central than peripheral V6 injection. Oddly, area V3A, which is supposed to represent the central 20–25° of both lower and upper quadrants of the contralateral hemifield (see Van Essen & Zeki, 1978; Gattass *et al.*, 1988), contained many more labelled cells after peripheral than central V6 injection. This could be due to the fact that both lower and upper quadrants of V6 were injected in that case (16L), and/or that in V3A the periphery of the visual field (outside 20–25° of eccentricity) is more represented than previously supposed.

The remaining five cortical areas connected with V6 (i.e. MST, V6A, MIP, LIPv and VIP) are high-order visual areas. The V6 connections with area MST seem to be consistent with the coarse retinotopic organization described in this area (Desimone & Ungerleider, 1986; Komatsu & Wurtz, 1988). Present data suggest the existence of a coarse retinotopic organization also in area MIP, with the central representation located dorsally and the peripheral one ventrally in the medial bank of IPS. In areas LIPv, VIP and V6A the location of labelling was about the same following either central or peripheral V6 injections. This finding disagrees with the view that LIPv is at least partially retinotopically organized (Blatt *et al.*, 1990), whereas is consistent with the evidence that VIP and V6A are nonretinotopically organized (Colby *et al.*, 1993; Duhamel *et al.*, 1998; Galletti *et al.*, 1999a).

Labelling in VIP and V6A did not involve the whole extent of these areas. In VIP, it involved both the medial and lateral banks of IPS caudally, but only the lateral bank rostrally. The rostral part of the medial bank of IPS, near the fundus, which is believed to be part of area VIP by many authors (Maunsell & Van Essen, 1983a; Blatt *et al.*, 1990; Colby & Duhamel, 1991; Colby *et al.*, 1993; Duhamel *et al.*, 1998), was free of labels in our cases. In V6A, labelling involved only the ventral part of the area. These data suggest the existence of functional subdivisions within the nonretinotopically organized areas VIP and V6A. In the case of V6A, the internal subdivision is also supported by a number functional data. For instance, the ventral part of this area contains a larger visual field representation than the dorsal one (Galletti *et al.*, 1999a), in good agreement with the stronger connections observed in case 16L, where peripheral V6 was injected.

Also, the ventral part of V6A shows a higher sensitivity to the visual stimulation than the dorsal one (Fattori *et al.*, 1999a). Finally, the ventral part of V6A, but not the dorsal one, contains cells that are able to directly encode the visual space (Galletti *et al.*, 1993, 1996, 1999a). All these functional data support the view, suggested by present data, that area V6A contains two functionally different areas: the dorsal V6A (V6Ad), not receiving a direct input from area V6, and the ventral V6A (V6Av), receiving a direct input from V6.

Comparison with other studies

Two previous studies on macaque monkey are particularly related to the present work. In the first (Colby *et al.*, 1988), area PO was injected with retrograde tracers. In the second (Shipp *et al.*, 1998), the V6 complex was injected with WGA-HRP.

Colby *et al.* (1988) found that PO receives retinotopically organized inputs from areas V1, V2, V3, V3A, V4, V4T and MT, and additional projections from several divisions of the parietal cortex [MST, PP (7a), PIP, MDP (7m), LIP, MIP, VIP]. In some cases, they also observed weak projections from the frontal and prefrontal cortices. The relationship between the anatomically defined area PO and the functionally defined area V6 was treated at length by Galletti *et al.* (1999b). To briefly summarize the conclusions, there are reasons to believe that the territory of area PO includes large part of V6 plus the ventral part of V6A. According to this view, the injection sites of Colby *et al.* (1988) would involve V6 and, partly, V6A. This could explain the differences observed in this work, i.e. the fact that we did not find projections from the parietal areas 7a and 7m, or from the frontal and prefrontal cortices. As area V6A is the recipient of projections from these same cortical areas (Shipp *et al.*, 1998), it seems likely that an involvement of V6A in the cases reported by Colby and coworkers could explain the additional parietal, frontal and prefrontal inputs they observed.

A further difference between our data and those of Colby and coworkers regards the description of a posterior intraparietal (PIP) area input to PO, not found after V6 injection. Actually, this difference is only apparent, because we consider area PIP as part of the central representation of area V6 (see Galletti *et al.*, 1999b). Therefore, in the present study, labelling in the PIP region was considered as an intrinsic connection of area V6.

The major differences between present results and Shipp's report (Shipp *et al.*, 1998) regard the absence, in the latter, of V1 and V4T inputs to V6, as well as the presence, in the same, of V6 afferents from the parietal areas 7a and 7m and from some subdivisions of the dorsal premotor frontal cortex. We believe that these differences are due to the differences in the injection sites. Shipp and coworkers injected WGA-HRP mainly in the dorsal half of the anterior bank of POS. They reached the region to be injected passing through the dorsal, exposed surface of the caudal part of superior parietal lobule. Thus, area V6A turned out to be involved in all the cases they studied, whereas area V6 was partly involved only in the cases with deepest injections (e.g. case SP19; see Fig. 1 of Shipp *et al.*, 1998). The lack of labelling in areas V1 and V4T in the Shipp's study was probably due to a marginal involvement of area V6, whereas parietal and frontal labelling to an involvement of area V6A even in their ventralmost injections. This interpretation is supported by data on parieto-frontal connections of the superior parietal lobule, showing that injections in dorsal premotor cortex produce labelling in area V6A but not in V6 (Matelli *et al.*, 1998). Further support to this view comes from our results of WGA-HRP injection in area V6A (case P11L). As shown in Fig. 5, V6A sends strong projections to the frontal lobe, in particular to the dorsal part of area 6 in the periaruate

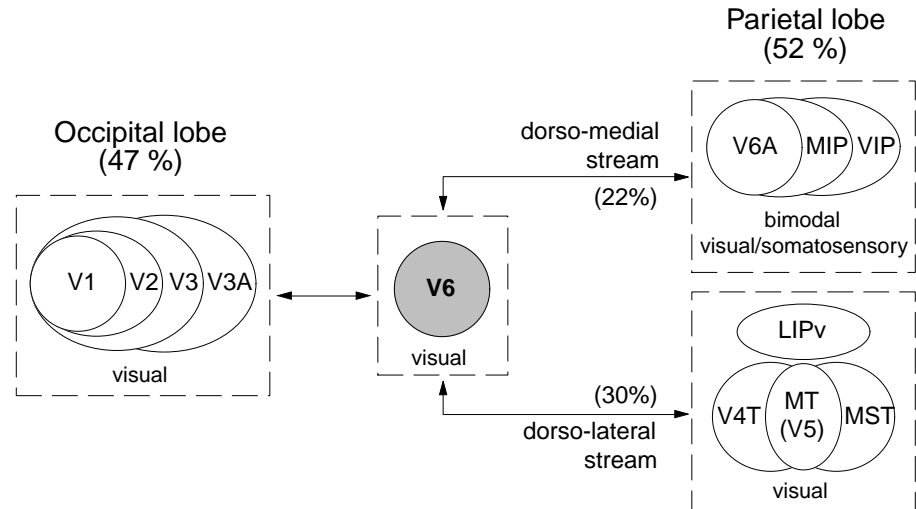


FIG. 16. The occipito-parietal network processing visual information. Connectivity diagram based on the V6 cortical connections described in this work.

cortex. By contrast, no labelling was found in the frontal cortex after V6 injection.

Functional considerations

As summarized in Fig. 16, about half of the cortical connections of V6 is with the visual areas of the occipital lobe (V1, V2, V3, V3A), and the other half with the areas of the 'dorsal visual stream': 30% with visual areas of the dorso-lateral visual stream (V4T, MT/V5, MST, LIPv) and 22% with bimodal sensory (visual and somatosensory) areas of the dorso-medial visual stream (V6Av, MIP, VIP). Area V6 is not directly connected with the 'ventral visual stream', nor with the mesial and frontal cortices.

The type of visual information exchanged between interconnected areas could be inferred by the functional properties of neurons of these areas. Cells in layer 4B of V1 receive a magnocellular input from the lateral geniculate nucleus and project to V6 (present results), to the thick stripes of V2, to V3 and to area MT/V5 (Zeki, 1969; Lund *et al.*, 1975; Maunsell & Van Essen, 1983a; Felleman & Van Essen, 1984; Livingstone & Hubel, 1984; Ungerleider & Desimone, 1986; Zeki & Shipp, 1988). Many cells in layer 4B of V1, thick stripes of V2, areas V3, MT/V5 and V6, as well as most cells of the cortical regions of the dorsal visual stream connected with these areas (V3A, V6A, MST) are tuned for orientation and/or motion direction (Dow, 1974; Zeki, 1974, 1978b; Van Essen *et al.*, 1981; Maunsell & Van Essen, 1983b; Albright, 1984; Livingstone & Hubel, 1984; Desimone & Ungerleider, 1986; Hubel & Livingstone, 1987; Galletti *et al.*, 1991, 1996). Some of these areas are particularly rich of cells sensitive to orientation of visual stimuli (V3, V3A; Zeki, 1978a, 1978b; Felleman & Van Essen, 1987), others to the direction of movement (MT/V5, MST; Zeki, 1974; Van Essen *et al.*, 1981; Maunsell & Van Essen, 1983b; Desimone & Ungerleider, 1986). Areas V6 and V6A are rich of cells sensitive to both orientation and direction of movement (Galletti *et al.*, 1996, 1999a, 1999b).

In summary, the visual areas connected with V6 form an occipito-parietal network that could perform the fast form and motion analyses needed for the visual guiding of action, as originally suggested by Milner & Goodale (1995) for the dorsal visual stream. Area V6 in particular seems to be well equipped to do this task. It analyses both form and movement in the visual field, and is directly connected with bimodal areas containing representations of the arm (MIP and V6A, Colby & Duhamel, 1991; Johnson *et al.*, 1996; Galletti *et al.*, 1997), the head (VIP, Duhamel *et al.*, 1991, 1998), of oculomotion (V6A,

Galletti *et al.*, 1991; Nakamura *et al.*, 1999) and of spatial locations of objects to be grasped (V6A and VIP, Galletti *et al.*, 1993, 1995; Duhamel *et al.*, 1997). All these bimodal areas are, in turn, directly connected with the premotor area 6 (Johnson *et al.*, 1993, 1996; Tanne *et al.*, 1995; Matelli *et al.*, 1998; Shipp *et al.*, 1998). We advance the hypothesis that the occipito-parietal network above described, which includes V6 together with many areas of the dorsal visual stream, controls visually guided arm movements as well as their coordination with the eyes and the head.

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Abbreviations

CAL, calcarine fissure; DP, dorsal prelunate area; FB, fast blue; FST, fundal superior temporal area; IPS, intraparietal sulcus; LIP, lateral intraparietal area; LIPv, ventral part of area LIP; LS, lunate sulcus; MIP, medial intraparietal area; MT/V5, middle temporal area/area V5; MST, medial superior temporal area; MSTd, dorsal component of area MST; MSTl, lateral component of area MST; PIP, posterior intraparietal area; PO, parieto-occipital area; POM, medial parieto-occipital sulcus; POS, parieto-occipital sulcus; RF, receptive field; STS, superior temporal sulcus; V6Av, ventral part of area V6A; VIP, ventral intraparietal area; WGA-HRP, wheatgerm agglutinin conjugated with horseradish peroxidase.

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