Cortical Connections of the Macaque Anterior Intraparietal (AIP) Area

We traced the cortical connections of the anterior intraparietal (AIP) area, which is known to play a crucial role in visuomotor transformations for grasping. AIP displayed major connections with 1) areas of the inferior parietal lobule convexity, the rostral part of the lateral intraparietal area and the SII region; 2) ventral visual stream areas of the lower bank of the superior temporal sulcus and the middle temporal gyrus; and 3) the premotor area F5 and prefrontal areas 46 and 12. Additional connections were observed with the caudal intraparietal area and the ventral part of the frontal eye field. This study suggests that visuomotor transformations for object-oriented actions, processed in AIP, rely not only on dorsal visual stream information related to the object’s physical properties but also on ventral visual stream information related to object identity. The identification of direct anatomical connections with the inferotemporal cortex suggests that AIP also has a unique role in linking the parietofrontal network of areas involved in sensorimotor transformations for grasping with areas involved in object recognition. Thus, AIP could represent a crucial node in a cortical circuit in which hand-related sensory and motor signals gain access to representations of object identity for tactile object recognition.

Keywords: dorsal visual stream, grasping, parietal cortex, ventral visual stream, visuomotor transformations, 3D object coding

Introduction

The macaque anterior intraparietal (AIP) area, located rostrally in the lateral bank of the intraparietal sulcus (IIPS), is known to play a crucial role in visuomotor transformations for grasping. AIP is functionally distinct from the caudally adjacent oculomotor lateral intraparietal (LIP) area (Jeannerod et al. 1995; Sakata et al. 1997; Colby 1998; Andersen and Buneo 2002).

AIP neurons have been extensively studied by (Sakata et al. 1995; Taira et al. 1990; Murata et al. 1996, 2000) using a behavioral paradigm in which monkeys grasped or fixedated on different types of objects. Three classes of neurons were defined: “motor dominant,” discharging when grasping in both light and dark, “visual and motor,” discharging stronger when grasping in light than in dark, and “visual dominant,” discharging only when grasping in light. The visually responsive neurons were then grouped into 2 categories: “object type” and “nonobject type.” “Object-type” neurons also discharged during object fixation, showing selectivity for object intrinsic properties and suggesting coding of object’s 3-dimensional (3D) properties, such as shape, size, and orientation. In contrast, “nonobject-type” neurons were not active during object fixation but showed selective visual activity during grasping, which suggests coding of hand preshaping or hand-object interactions.

AIP is connected with the premotor area F5 (Luppino et al. 1999), where neurons with similar functional properties have been recorded (Raos et al. 2006). Furthermore, reversible lesion experiments have shown that AIP (Gallese et al. 1994) or F5 (Fogassi et al. 2001) inactivation produces grasping impairment, which results in a mismatch between the actual object shape and the appropriate hand preshaping. It has been proposed that AIP and F5 belong to a parietofrontal circuit dedicated to the extraction of object properties necessary for the generation of appropriate object-oriented hand actions (Jeannerod et al. 1995; Fagg and Arbib 1998).

Currently, there is very limited evidence for the possible sources of sensory information for AIP. Previous reports have shown that AIP is a target of projections from a caudal and ventral LIP sector (Nakamura et al. 2001), referred to as the caudal intraparietal (CIP) area, in which there are neurons coding 3D object’s visual features (Taira et al. 2000; Tsutsui et al. 2001, 2002, 2003). However, the functional properties of CIP cannot explain some of the AIP functional features, such as the “nonobject-type” visual responses. Moreover, human studies indicate that 1) appropriate grip selection for grasping familiar objects also relies on information on object identity, which is coded in the ventral visual stream (Jeannerod et al. 1994); 2) object-oriented actions also rely on anticipatory mechanisms concerning memory-based somatosensory expectations (Ehrsson et al. 2003); and 3) an intraparietal sector considered the human equivalent of the macaque area AIP (hAIP) is involved in integrating multimodal sensory information for object recognition and manipulation (Greffkes et al. 2002). All together, these observations suggest that AIP is a target also of visual information related to action coding and object identity and somatosensory information related to tactile expectations and haptic object coding. The possible pathways carrying these types of information to AIP still remain to be assessed.

The aim of the present study was to trace cortical AIP connections to identify the possible sources of sensory information for the AIP-F5 circuit. Preliminary data have been presented in abstract form (Luppino et al. 2004).

Methods

The experiments were carried out on 3 Macaca nemestrina (Cases 20, 14r, and 30) and 1 Macaca fascicularis (Case 17) in which neural tracers were injected in AIP. Additional data from 3 M. nemestrina (Cases 14l, 15, and 30r) and 1 M. fascicularis (Case 13), in which retrograde tracers were injected in the ventral premotor area F5, were used for a connectional definition of AIP. Data limited to the frontal AIP connections in Case 17 and the intraparietal connections of F5 in Case 17 and the intraparietal connections of F5 in Case 14r, and 30l) and 1...
Surgical and Electrophysiological Procedures
Each animal was anesthetized with either ketamine hydrochloride (15–20 mg/kg intramuscularly [i.m.]) or sodium thiopental (10–15 mg/kg intravenously [i.v.]) and placed in a stereotaxic apparatus. Under aseptic conditions, an incision was made in the scalp, the skull was trephined to remove the bone overlying the target region, and the dura was opened.

In all monkeys except one (Case 17, right and left hemispheres), the injection sites were chosen by using antero-posterior (AP) stereotaxic coordinates as a frame of reference and the intraparietal sulcus [IPS] as an anatomical landmark. In Case 17 (right and left hemispheres), which has been a subject in a previously published single-unit recording study (Murata et al. 2000), AIP was functionally identified before the tracing experiments (for a detailed description of behavioral, surgical, and recording procedures, see Murata et al. 2000). Briefly, for the purposes of the present study, in both hemispheres of this animal, the IIPS was explored through microelectrode penetrations spaced at approximately 1-mm intervals in the rostrocaudal direction. AIP was defined on the basis of the localization of single- and/or multiunit task-related activities during the execution of object grasping or object fixation tasks. AIP was located caudal to the hand representation of the primary somatosensory cortex in the rostral part of the IIPS, immediately rostral to area LIP. At the end of the experiments, small electrolytic cortical lesions (10 μA cathodal current for 10 s) were made at known stereotaxic coordinates, close to the studied region.

Tracer Injections and Histological Procedures
Once sites for the AIP injections were chosen, the fluorescent tracers fast blue (FB, 3% in distilled water, EMS-POLYLOY GmbH, Gross-Umstadt, Germany) and diamidino yellow (DY, 2% in 0.2-M phosphate buffer at pH 7.2; EMS-POLYLOY GmbH, wheat germ agglutinin, horseradish peroxidase conjugated (WGA-HRP, 4% in distilled water; SIGMA, St Louis, MO, USA), biotinilated dextran amine (BDA, 10% phosphate buffer 0.1 M, pH 7.4; Invitrogen-Molecular Probes, Eugene, OR, USA), and microruby (MR, 10% phosphate buffer 0.1 M, pH 7.4; Invitrogen-Molecular Probes), were slowly pressure injected at different depths in the IIPS, through a glass micropipette (tip diameter 50–100 μm) attached to a 1-μl or 5-μl Hamilton microsyringe (Reno, NV, USA). Table 1 summarizes the locations of the injections, the injected tracers, and their amounts. After the injections, the dural flap was sutured, the bone was replaced, and the superficial tissues were sutured in layers. During surgery, hydration was maintained with saline (about 10 ml/hr, i.v.) and temperature was maintained using a heating pad. Heart rate, blood pressure, respiratory depth, and body temperature were continuously monitored. Upon recovery from anesthesia, the animals were returned to their home cages and closely monitored.

All cases of F5 injections except Case 30 have been already used in previous concomitant studies (for details on surgical and tracer injection procedures, see Luppino et al. 1999, 2001; Gregoriou et al. 2006). In Case 13, DY (1 injection, 0.2 μl) was injected in the cortical convexity immediately adjacent to the inferior arcuate sulcus. In Cases 14 (FB, one injection, 0.2 μl) and 15 (DY, 2 injections, 0.2 μl each), tracers were injected in correspondence with the lip of the same sulcus. In Case 30, DY (1 injection, 0.2 μl) was placed deeply in the posterior bank of the inferior arcuate sulcus.

After appropriate survival periods (28 days for BDA, 12–14 days for FB, DY, and MR, and 2 days for WGA-HRP) following the injections, each animal was anesthetized with ketamine hydrochloride (15 mg/kg i.m.) followed by an i.v. injection of sodium thiopental (60 mg/Kg) and perfused through the left cardiac ventricle with saline, 3.5–4% paraformaldehyde, and 5% glycerol in this order. All solutions were prepared in phosphate buffer 0.1 M, pH 7.4. Each brain was then blocked coronally on a stereotaxic apparatus, removed from the skull, photographed, and placed in 10% buffered glycerol for 3 days and 20% buffered glycerol for 4 days. Finally, it was cut frozen in coronal sections 60-μm thick. The 2 hemispheres of Case 17 were cut separately.

Data Analysis
Injection Sites and Distribution of Retrogradely Labeled Neurons
The criteria used for the definition of the injection sites and the identification of retrograde and anterograde labeling have been fully described in previous studies (Luppino et al. 2001, 2003; Rozzi et al. 2006). The distribution of retrograde and anterograde (for WGA-HRP and BDA injections) labeling was analyzed in sections every 300 μm and plotted in sections every 600 μm, together with the outer and inner cortical borders, by using a computer-based charting system. Only anterogradely labeled terminals were plotted in Case 30l, given the very high sensitivity of BDA as an anterograde tracer and the relative paucity of retrograde labeling.

The distribution of the labeling in the IIPS, in the lateral fissure (LF), and in the superior temporal sulcus (STS) was then visualized in 2-dimensional (2D) reconstructions obtained by using the same software, according to the following procedure (for more details, see Matelli et al. 1998). In each plotted section, the cortex included in the region of interest was subdivided into columnar bins by lines perpendicular to the cortical surface and connecting the outer and inner cortical contours. To minimize the distortion caused by cortical curvature, the cortex was then unfolded at the level of a virtual line connecting the midpoints of all the perpendicular lines, approximately positioned at the border between layers III and IV. The unfolded sections were then aligned and the labeling distributed along the space between 2 consecutive plotted sections (600 μm). Sections through the IIPS were aligned to correspond with the lateral lip of the sulcus, those through the parietal operculum and the insula were aligned to correspond with the fundus of the upper bank of the LF, and those through the STS were aligned to correspond with the fundus and middle of the floor.

Data from individual sections were also imported into 3D reconstruction software (Bettio et al. 2001), which allowed us to obtain volumetric reconstructions of the monkey brain, including connectival data. The labeling distribution on the exposed cortical surfaces was visualized in dorsolateral and lateral views of the 3D reconstructions of the hemispheres. The labeling distribution within the arcuate sulcus was visualized in nonstandard views of the hemispheres in which the sulcal banks were exposed with appropriate dissections of the 3D reconstructions (see Rozzi et al. 2006).

The criteria used for the attribution of the labeling to parietal, frontal, and temporal areas were very similar to those previously described in detail by Rozzi et al. (2006). Briefly, areas of the cortical convexity of the inferior parietal lobule (IPL) were defined according to architectonic criteria (Gregoriou et al. 2006), whereas for the parietal operculum, we matched our data with the functional maps of the SII region of Krubitzer et al. (1995) and Fitzgerald et al. (2004). For the

### Table 1

<table>
<thead>
<tr>
<th>Monkey Species</th>
<th>Hemisphere</th>
<th>AP level</th>
<th>Tracer</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaca nemestrina</td>
<td>L 2.5 to 4.5</td>
<td>BDA</td>
<td>10%</td>
<td>2 x 1 μl</td>
</tr>
<tr>
<td>Macaca fuscata</td>
<td>L 0.5 to 1.5</td>
<td>DY</td>
<td>10%</td>
<td>2 x 1 μl</td>
</tr>
<tr>
<td>L 4 to 5.5</td>
<td>FB</td>
<td>3%</td>
<td>3 x 0.2 μl</td>
<td></td>
</tr>
<tr>
<td>Case 20</td>
<td>M. nemestrina</td>
<td>L 0.5 to 1.5</td>
<td>DY</td>
<td>3 x 0.2 μl</td>
</tr>
<tr>
<td>Case 30</td>
<td>M. nemestrina</td>
<td>L 2.5 to 4.5</td>
<td>BDA</td>
<td>10%</td>
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In Cases 14r, 17l, 20, and in all cases of F5 injections, 1 section out of each 5 sections was mounted, air-dried, and quickly coverslipped for fluorescence microscopy. In Case 17r, 1 section out of each 5 sections was processed for WGA-HRP histochemistry with tetramethylbenzidine as the chromogen (Mesulam 1982). In Case 30l, one series of each fifth section was processed for the visualization of BDA, using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) and 3,3'-diaminobenzidine as a chromogen. The reaction product was intensified with cobalt chloride and nickel ammonium sulfate. In all cases, one series of each fifth section was stained using the Nissl method (thionin, 0.1% in 0.1-M acetate buffer pH 3.7).

15 have been presented in a previous short report (Luppino et al. 1999). All experimental procedures were approved by the Veterinarian Animal Care and Use Committee of the University of Parma and complied with European law on the care and use of laboratory animals.
temporal cortex, we adopted the nomenclatures of Saleem and Tanaka (1996) and Boussaoud et al. (1990), and for the lower bank of the STS (STS), we also adopted the subdivision of Seltzer and Pandya (1978). In the frontal lobe, the agranular frontal areas were defined according to (Matelli et al. 1985; see also Geyer et al. 2000). The prefrontal cortex was subdivided according to Carmichael and Price (1994) and the prearcuate cortex according to Stanton et al. (1989).

**Laminar Distribution of the Labeling and Quantitative Analysis**
To obtain information on possible hierarchical relationships of the observed cortical connections, labeling attributed to a given area and reliably observed across different sections and cases was analyzed in each section every 300 µm in terms of the following: 1) the laminar distribution of the anterogradely labeled terminals and 2) percent of labeled neurons located in the superficial (I-III) versus deep (V-VI) layers. These data were then interpreted according to the criteria defined by Felleman and Van Essen (1991; see also Dum and Strick 2005) and fully described by Rozzi et al. (2006). Briefly, projections were interpreted, in general, as “feedforward,” “feedback,” and “lateral,” based on the laminar distribution of the labeled cells and terminals. However, as already noted by Felleman and Van Essen (1991), bilaminar retrograde labeling, that is, the relatively equal distribution of labeled neurons in the superficial versus deep layers, can be associated with different types of patterns of anterograde labeling. In these cases, possible hierarchical relationships were inferred on the basis of the laminar distribution of the labeled terminals observed in Cases 17r and 30l. In the agranular frontal cortex, connections characterized by anterograde terminals mostly in lower layer III were considered feedforward projections.

Furthermore, to have more objective information on the relative strength of the connections observed in the present study, in all cases of F5 injections and in Cases 20, 17r, and 14r, we counted the number of labeled neurons plotted in the ipsilateral hemisphere in sections every 600-µm intervals, beyond the limits of the injected area. Parietal afferents to F5 were expressed in terms of the percent of labeled neurons found in a given parietal subdivision, with respect to the overall labeling. Cortical afferents to AIP were expressed in terms of the percent of labeled neurons found in a given cortical region, with respect either to the overall labeling or to the extraparietal labeling.

**Photomicrographic Presentation**
The photomicrographs shown in this study were obtained by capturing images directly from the sections using a digital camera attached to the microscope. Individual images were then imported into Adobe Photoshop so that they could be processed, eventually assembled into digital montages, and reduced to the final enlargement. In most cases, image processing required lighting, brightness, and contrast adjustments.

**Results**

**Definition of AIP and Location of the Injection Sites**
AIP is considered to be the anterior portion of the IPS where motor, visuomotor and visual neurons are recorded during the execution of grasping movements (hand-related AIP neurons; Taira et al. 1990; Sakata et al. 1995; Murata et al. 1996; Murata et al. 2000). In Case 17r, the extent of AIP was determined physiologically (see Murata et al. 2000). The IPS was explored at 1-mm intervals with microelectrodes that penetrated to the fundus of the sulcus. Reconstructed electrode tracks were displayed on an unfolded map of the IPS that was aligned on the lateral lip of the sulcus (Fig. 1, upper right). Single- or multiunit activity (filled triangles) that was characteristic of AIP hand-related neurons was recorded within a limited region of the IPS. AIP hand-related neuronal recordings were located at levels about 5-13 mm caudal to the rostral tip of the IPS and extended from about 1.5 mm below the lip of the sulcus to within 2–3 mm of the fundus. The physiologically defined AIP in this case (thick dashed line) as well as the region identified as AIP in previous studies (e.g., Sakata et al. 1995; Murata et al. 2000) were used as a frame of reference to delineate AIP in the remaining cases of the present study. In the remainder of our figures, the rostral and caudal borders of AIP were based on stereotaxic coordinates and the dorsal and ventral borders were placed in relation to the lip and fundus of the IPS. Accordingly, the rostral and caudal borders of AIP were set at AP 6 and AP –2, respectively, in stereotaxic coordinates (Winters et al. 1969), the dorsal border at 1.5 mm below the lip of the sulcus, and the ventral border at 2–3 mm from the fundus.

In a previous short report (Luppino et al. 1999), we showed that AIP is robustly connected with the rostral ventral premotor (PMv) area F5 of the agranular frontal cortex. To see whether this aspect of AIP connectivity provides additional criteria for its definition, we analyzed the distribution of the retrograde labeling within the IPL following tracer injections in F5. In 4 monkeys, tracer injections were placed in different parts of F5, except its ventralmost one. Other electrophysiological studies have shown that in the F5 sectors involved in these injections, including both the posterior bank of the inferior arcuate sulcus and the adjacent cortical convexity, there is a representation of grasping movements (e.g., Rizzolatti et al. 1988; Cerri et al. 2003; Shimazu et al. 2004; Raos et al. 2006; Umiltà et al. 2007). Nearly all parietal projections to F5 originated from several IPL areas, except in Case 14l, where relatively dense labeling was located in the rostral part of the medial bank of the IPS (area PElp; Matelli et al. 1998). In the IPL, most of the labeling was observed in AIP, PF, PFG, and in the parietal operculum (SII region and retrosinular cortex), with some variability across cases. In particular, Case 30r displayed relatively stronger labeling in the parietal operculum and relatively weaker labeling in PF.
distribution of the labeling observed in Cases 13 and 14l on the IPL convexity cortex has been shown in a previous study (Gregoriou et al. 2006). In the IIPS, all cases exhibited dense retrograde labeling in AIP, PF, and the most medial part of PFG (Fig. 1, lower part). In contrast, LIP and VIP were virtually devoid of labeling. In the lowest right part of the figure, the labeled zones observed in each case were delineated and superimposed onto a single unfolded view of the IIPS. This composite view shows the following: 1) virtually the entire AIP projects to F5 and 2) the connectivity pattern with F5 clearly distinguishes AIP from the caudal area LIP and the ventral area VIP, confirming the validity of the estimated location of the caudal and ventral borders of AIP. Quantitative analysis showed that, considering the 4 cases all together, the mean percent value of labeling observed in AIP was 6.5% (range: 4.8--9%). This value was slightly higher than that observed in areas PF (mean: 5.2%; range: 2.7--6.7%) and PFG (mean: 5.3%; range: 4.8--9%). In the parietal operculum, the mean percent value of the observed labeling was much more variable, ranging from 2.6% in Case 13% to 24.7% in Case 30r (mean: 10.7%). These data indicated that, in spite of some variability, possibly due to a topographic organization of the parietal connectivity of F5, in all cases AIP represented 1 of the 4 main sources of parietal afferents to this PMv area.

The locations of the injection sites in AIP are shown in Figure 2. Across the cases, the injection sites varied in size, from relatively large (Case 17r) to relatively small (Case 20), and in location, from relatively caudal (Case 14r) to relatively rostral (e.g., Case 30l). All together, the injection sites involved almost the entire AIP except the ventral caudalmost part. In all cases, because of the pipette trajectories, the injection sites unavoidably involved the medialmost part of PFG or PG. This involvement was, however, minimal in Cases 20 and 30l. In Cases 17r, 20, and 30l, the injection core involved at least the middle cortical layers and the halo did not extend into the white matter. All together, the observed distributions and patterns of labeling were quite consistent, the main differences lying in the overall number of labeled neurons, which varied depending on the size of the injections and the injected tracer. In Case 14r, the injection site ran for several millimeters in the IIPS mostly through the lowest part of layer III. In spite of a quite low overall number of retrogradely labeled cells, the distribution of the labeling was consistent with that observed in Cases 17r, 20, and 30l. In Case 17l, the cores of the DY and FB injection sites (especially the DY injection site) were rather limited to the superficial layers. Moreover, a significant proportion of the FB injection site involved PFG. Data from these 2 injections were not considered in the description of the results.
**AIP Connections**

The AIP ipsilateral cortical connections will be described in this section on the basis of the distribution of the retrograde labeling observed in Cases 20, 17r, and 14r, and of the anterograde labeling observed in Case 30l. In Case 17r, the areal distribution of the anterograde labeling exactly matched that of the retrograde labeling.

**Connections with Parietal and Insular Areas**

Strong connections were found with areas PF, PFG, and PG of the IPL cortical convexity (Fig. 3, upper part), in full agreement with data from injections in these IPL convexity areas (Rozzi et al. 2006). In Cases 20 (Figs 3 and 4, j–o) and 14r (Fig. 3), the labeling tended to be confined to the dorsal part of the gyral cortex, whereas in Case 30l the anterograde labeling extended throughout the entire dorsolateral extent (Figs 3 and 5, g–k). In Case 17r, cortical damage that corresponded to the recording chamber implant (the unlabeled IPL convexity zone close to the injection site in the 3D reconstruction in Fig. 3) prevented accurate plotting of the labeling because of the presence of artifacts and nonspecific precipitation of the chromogen. Rich labeling was, however, observed outside the damaged zone in PF, PFG, and PG. Relatively weak labeling was observed in all cases in area MST (Figs 4, m–o and 5, l and m).

In the lIPS (Fig. 3, lower part), very dense intrinsic labeling extended throughout almost the entire AIP in all cases. The labeling also extended caudally for approximately 4 mm and involved the rostral portion of dorsal LIP (LPd; Figs 3, 4, m and 5, k).

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**Figure 3.** Distribution of the retrograde labeling, observed in Cases 20, 17r, and 14r, and of the anterograde labeling, observed in Case 30l, on the dorsolateral cortical convexity (upper part, 3D reconstructions of the injected hemispheres) and in the lIPS (lower part, 2D reconstructions of the bank). For Cases 20, 17r, and 14r each dot corresponds to one labeled neuron, whereas for Case 30l, the dot density is proportional to the density of observed labeled terminals. The unlabeled zone on the IPL convexity, close to the injection site of Case 17r, corresponds to a damaged cortical sector. Calibration bar shown for the hemisphere and for the 2D reconstruction of Case 20 applies to all cases. Ag, annectant gyrus; IO, inferior occipital sulcus; PMT, posterior middle temporal sulcus. Other abbreviations and conventions as in Figure 1.
This location appears to correspond to the rostral part of LIP in Blatt et al. (1990), where there is a central visual field representation and fixation-related and/or saccadic-related neuronal activity predominates (Blatt et al. 1990; Ben Hamed and Duhamel 2002). In addition, clusters of labeled cells or terminals were consistently observed more caudally and deeply in the bank, at about the level of the rostral end of the annectant gyrus (Figs 3, 4, n, and o, and 5, m). This labeled zone, which appears to be mostly located within the ventral subdivision of LIP (LIPv, Blatt et al. 1990), may correspond to area CIP, identified by Sakata et al. (see, e.g., Tsutsui et al. 2001). Some weak labeling was also observed ventral to AIP in VIP (Figs 3 and 5, i–k).

In the parietal opercular cortex (Fig. 6), labeling was confined to the SII region except for some weak labeling in the retroinsular cortex of Case 17r. The retroinsular labeling is likely due to the involvement of PFG in the injection site (Rozzi et al. 2006). In Cases 20, 30l, and 17r, rich labeling was mostly concentrated in 2 zones, one more rostral and medial (Figs 4, f and g, 5, e, and 6) the other more caudal and lateral, close to the lip of the upper bank of the LF (Figs 4, h, 5, g and h, and 6).

Comparison with functional studies of the SII region suggests that the rostral zone very likely corresponds to the hand representation of area PV (Krubitzer et al. 1995) and the anterior field of the SII digit representation as defined by Fitzgerald et al. (2004). The caudal zone appears to correspond...
with the posterior field of the SII digit representation as defined by Fitzgerald et al. (2004). In Case 30\textsubscript{l}, weaker anterograde labeling was located between the 2 densely labeled zones (Figs 5, f and 6). In Case 14\textsubscript{r}, the labeling was almost entirely confined to the caudal zone (Fig. 6) and was relatively weaker, even considering that the overall number of MR-labeled neurons in the ipsilateral hemisphere was considerably lower than that observed following the injections of DY and WGA-HRP. The relatively weaker labeling in the SII region observed in this case (caudalmost AIP injection) may reflect a possible topographical organization of AIP, in which visual dominant neurons, though largely intermingled with motor dominant neurons, tend to be more numerous in the caudal part of AIP (Luppino G, Murata A, unpublished data). Some labeling was also observed in the granular insula at about the same AP level of the rostral SII labeling, especially following larger injections (Figs 5, e and 6).

In all cases, relatively weak labeling was observed in 2 sectors of the superior parietal lobule. One sector was located caudally in the medial bank of the IPS (Fig. 5, l and m) in the location of area MIP (Colby et al. 1988). The other was located dorsally in the anterior wall of the parieto-occipital sulcus (Figs 4, p and 5, n), in the dorsal part of area V6A (Galletti et al. 1999), that is, in architectonic area V6Ad (Luppino et al. 2005). No labeling was observed in other parietal sectors or the occipital extrastriate areas.

Figure 5. Distribution of anterograde labeling observed in Case 30\textsubscript{l}, shown in drawings of representative coronal sections. Sections are shown in a rostral to caudal order (a-n). The levels at which the sections were taken are indicated in the dorsolateral view of the injected hemisphere shown in the upper right part of the figure. Conventions and abbreviations as in Figures 1, 3, and 4.
Connections with Frontal Areas

In all cases, dense labeling was observed in area F5. Furthermore, in agreement with previous observations (Luppino et al. 1999), the AIP connections with the agranular frontal cortex were virtually all confined to F5, except for the minor involvement of the caudal PMv area F4 in Case 17r (Fig. 7). The labeling in F4 may reflect the involvement of PFG by the injection site in this case (Rozzi et al. 2006). Within F5, dense clusters of labeled cells were observed at different rostrocaudal levels of the posterior bank of the inferior arcuate sulcus and in the immediately adjacent cortical convexity (Figs 4, c and d, 5, c and d, and 7). Accordingly, AIP appears to be connected with a large part of F5, as also expected by the observation of retrograde labeling in AIP following injections in different parts of this premotor area. In Case 30l, very likely because of the high sensitivity of BDA for tracing anterograde projections, some very sparse terminals were also observed in the hand representation of F1 (area 4) and in the rostroventral part of F2 (Figs 3, 5, d and 7). This label extended laterally to the junction with the orbital surface (Figs 3, 4, a and b, and 5, a). Weak retrograde and/or anterograde labeling was also consistently observed, even following injections in the rostral part of AIP, in the ventral part of the FEF (Figs 4, c, 5, c, and 7), as architectonically defined (Stanton et al. 1989). According to Bruce et al. (1985), this FEF sector hosts a representation of small-amplitude saccades. Weak connections, with variability across cases, were observed with the frontal opercular cortex (Figs 5, c and 6) in the location of area PrCO (Roberts and Akert 1963).

Connections with Temporal Areas

There were robust AIP connections with sectors of the lSTS and the ventrally adjacent gyral cortex (Fig. 8). In the lSTS, most of the labeling was located in areas TEa and TEM (Seltzer and Pandya 1978; area TEa/TEm in Boussaoud et al. 1990). Two densely labeled zones were located relatively rostrally in this sector (Figs 4, e--g, 5, f--i, and 8). The first one was centered at an AP level of about 12--13 and located in the inner part of the lSTS, in apparent continuity with the labeling observed in the gyral cortex. A further, more caudal zone extended in the mediolateral direction from the lower part of the fundus to the lip of the lSTS (Figs 4, i and j, 5, j and k, and 8). Here, the
labeling was relatively weaker and variable across cases. This zone appears to correspond to areas FST and TEO.

Additional temporal AIP connections were found with the gyral convexity cortex of the middle temporal gyrus (Fig. 8). These connections were quantitatively different across cases and particularly evident in Case 17r. The relatively rich labeling observed in Case 17r is unlikely to reflect the involvement of PFG by the injection site because this IPL convexity area does not display connections with the middle temporal gyrus (Rozzi et al. 2006). Thus, this labeling may depend on the size of the injection site (largest AIP injection). Nevertheless, in all cases 2 zones were more consistently labeled, a more rostral and a more caudal one, in areas TEpd and TEO, respectively. Further labeling was observed in Case 17r in area TEpv and in Cases 17r and 30l in area TEad (Fig. 5, e).

Finally, in all cases except Case 20, some weak labeling was observed in the upper bank of the STS, mostly corresponding to the rostral part of area STP (STPa; Figs 5, g and 8). This weak labeling may be due to the partial involvement of area PFG by the injection sites. However, PFG is a target of projections from STPa but also even more from the posterior (STPp) part of this area, close to MST (Rozzi et al. 2006). Thus, it is also possible that the variability of this weak labeling reflects the size of the injection site.

Figure 7. Cortical connections of AIP with postarcuate (upper part) and prearcuate (lower part) areas. The distribution of labeling is shown in nonstandard views of 3D reconstructions of the postarcuate and prearcuate cortex. Nonstandard views of a nearly intact right hemisphere show in darker gray the brain sector removed to expose the postarcuate and the prearcuate banks. In each dissected view of the hemispheres, the exposed bank is shown in darker gray, Conventions and abbreviations as in Figures 1 and 3.
Laminar Distribution of the Labeling and Quantitative Data

The patterns of the laminar distribution of the retrograde and anterograde labeling of the major AIP connections are summarized in Table 2, defined according to the criteria reviewed by Felleman and Van Essen (1991; see also Dum and Strick 2005). In the parietal cortex, AIP displayed lateral connections with IPL convexity areas and LIP and feedback connections with CIP. In the SII region, the retrograde labeling showed a bilaminar distribution (Fig. 9e), but anterogradely...
labeled terminals were by far densest in layer IV (Fig. 9c). Therefore, this connection can be considered feedforward. In the prefrontal cortex, retrograde labeling was by far predominant in the superficial layers, except for the area 12 labeling observed in Case 17r. The anterograde labeling showed a columnar pattern in area 46 (Fig. 9f) and a multilaminar pattern in area 12 (Fig. 9f) and in the FEF. In F5, anterograde (Fig. 9b) and retrograde labeling showed an unusual combination of laminar distribution: they were both by far more concentrated in the lower part of layer III. The temporal AIP connections appeared to be of the feedback type (Fig. 9c) in all areas except for area TEa (feedforward type; Fig. 9d, f, and b).

To estimate the relative strength of the connections observed in the present study, a quantitative analysis of the retrograde labeling distribution was performed in Cases 20 and 14r. The data were then transformed in terms of percentage of labeled neurons in the parietal, agranular frontal, prefrontal, and temporal cortex with respect to the total number of labeled neurons, excluding the injected area. Case 30l was excluded from this analysis because of the paucity of retrogradely labeled neurons. In Case 17r, cortical damage in the IPL convexity prevented a complete quantitative analysis of the retrograde labeling. Because of the very limited number of cases, these data should be considered with caution, but can be useful in giving more objective general information about the relative strength of the AIP connections traced in the present study. In Cases 20 and 14r, the labeled neurons in the parietal cortex were 71% and 73%, respectively. This value is in the same range of that observed following injections in IPL convexity areas (Rozzi et al. 2006). In Case 20, the contribution of the temporal, agranular frontal, and prefrontal projections was virtually identical (9–10%), whereas in Case 14r (caudal-most AIP injection), the contribution of the temporal projections was higher (17%) with respect to the agranular frontal (4%) and prefrontal projections (5%).

Table 2
Patterns of laminar distribution of retrograde and anterograde labeling and percent distribution of labeled neurons in the superficial versus deep layers (s/d ratio) observed for the major connections of AIP

<table>
<thead>
<tr>
<th>Retrograde</th>
<th>Insula</th>
<th>Frontal</th>
<th>Temporal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parietal</td>
<td>Parietal</td>
<td>Insula</td>
<td>Frontal</td>
</tr>
<tr>
<td>Case 20</td>
<td>Pattern</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>s/d ratio</td>
<td>56/44</td>
<td>42/58</td>
<td>80/20</td>
</tr>
<tr>
<td>Case 14r</td>
<td>Pattern</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>s/d ratio</td>
<td>60/40</td>
<td>51/49</td>
<td>70/30</td>
</tr>
<tr>
<td>Case 17r</td>
<td>Pattern</td>
<td>NE</td>
<td>B</td>
</tr>
<tr>
<td>s/d ratio</td>
<td>55/45</td>
<td>78/22</td>
<td>51/49</td>
</tr>
<tr>
<td>Anterograde</td>
<td>Pattern</td>
<td>NE</td>
<td>C</td>
</tr>
<tr>
<td>Case 17r</td>
<td>Pattern</td>
<td>NE</td>
<td>C</td>
</tr>
<tr>
<td>Case 30l</td>
<td>Pattern</td>
<td>NE</td>
<td>C</td>
</tr>
</tbody>
</table>

Note: B, bilaminar; I, infragranular; NE, not evaluated; S, supragranular; C, columnar; F, feedforward; M, multilaminar.

*PG, PFG, and PF.

Discussion
The present study provides the first complete description of the cortical connections of AIP, a parietal area that plays a crucial role in visuomotor transformations for grasping. Our results show that AIP displays major connections with parietal areas (PFG, PG, LIP, PF, and the SII region), frontal lobe motor areas (premotor area F5), prefrontal areas (46 and 12), and several sectors of the inferotemporal cortex (TEO, TEpD, TEM, and TEa). Thus, in addition to being a functionally distinct region (Murata et al. 2000), AIP has a unique set of anatomical connections. These connections appear to be useful for clarifying some controversial aspects of the models used to describe the neural mechanisms underlying visuomotor transformations for grasping (Milner and Goodale 1993, 1995; Jeannerod et al. 1995; Sakata et al. 1997; Fagg and Arbib 1998). These connections also suggest that AIP has a unique role in linking components of the hand motor system with components of the ventral visual stream involved in object recognition.

Connectional Distinctiveness of AIP
In the present study, the location and extent of AIP were estimated primarily on the basis of functional criteria (Sakata et al. 1995; Murata et al. 2000). Indeed, the caudal and ventral borders of AIP correspond well to the rostral border of LIP and the dorsal border of VIP, respectively, as defined in other studies (Blatt et al. 1990; Andersen, Asanuma, et al. 1990; Colby et al. 1993). The present data indicate that AIP can also be distinguished connectionally from LIP and VIP not only for the connectivity pattern with F5 but also for several other connectional features. First, LIP lacks connections with the agranular frontal cortex, 34% in the agranular frontal cortex, and 32% in the prefrontal cortex. These data indicate that, in spite of some variability across cases, AIP is a target of consistent projections from both the temporal and the prefrontal cortex. Furthermore, quantitative analysis of the retrograde labeling distribution in the agranular frontal cortex showed that in F5 the labeling was 97%, 98%, and 80% in Cases 20, 14r, and 17r, respectively, of the total amount of labeling observed in this cortical region. Thus, projections from the agranular frontal cortex to AIP originate almost exclusively from the premotor area F5.
SII region, the IPL convexity areas PF, PFG, and PG, the PMv area F5 and the prefrontal areas 46v (rostrally) and 12 (Cavada and Goldman-Rakic 1989a, 1989b; Andersen, Asanuma, et al. 1990; Blatt et al. 1990; Lewis and Van Essen 2000). Furthermore, AIP lacks connections with the visual extrastriate areas V3, V3A, V4, and MT, described for LIP. AIP, however, appears to share with LIP a connection with the ventral part of the FEF. Indeed, Schall et al. (1995) have shown that the FEF (especially its ventral part) is a target of a very large IPS territory that, rostrally, likely includes AIP. Finally, AIP, as LIP, is a target of areas TE and TEO, but is connected with ISTS sectors located more rostrally than those connected with LIP (Cavada and Goldman-Rakic 1989a; Andersen, Asanuma, et al. 1990; Blatt et al. 1990).

Second, VIPl, a fundal IPS region that is likely to correspond to the functionally identified VIP (Colby et al. 1993), is distinguished from AIP by a wide variety of cortical connections (Lewis and Van Essen 2000). Although both VIPl and AIP receive connections from PFG, PF, and the SII region, VIPl has connections with visual extrastriate areas, SPL areas, and area 2. These latter cortical sectors have minimal or no connections to AIP. In the frontal lobe, the VIPl connections are much more extensive than AIP connections, including agranular frontal sectors corresponding to F1 and different dorsal and ventral
premotor areas, the entire extent of the prearcuate gyrus, and (weakly) the caudalmost part of area 46. Finally, V1P lacks consistent connections with the inferotemporal cortex, but is robustly connected with the auditory-related area Toc and with STP.

Our data also show that the connectivity pattern of F5 with the IPL cannot distinguish AIP from the rostrally adjacent area PF and the laterally adjacent area PFG; both, similar to AIP, are main sources of projections to F5. Area PF (rostral part of area 7b) is mostly a face/mouth-related field where somatosensory responses predominate compared with the visual ones (Leinonen and Nyman 1979; Hyvärinen 1981). These data suggest that this area is mostly involved in somatomotor transformations for face and mouth movements that in F5 are represented in partial overlap with hand movements (see, e.g., Rizzolatti et al. 1988; Ferrari, Gallese, et al. 2003). Connectional evidence (Pandya and Seltzer 1982, Petrides and Pandya 1984; Rozzi et al. 2006) indicates that the connectivity pattern of this area differs markedly from that of AIP. In particular, the major parietal connections with somatosensory areas, including area 2, the premotor connections that consistently also involve F4 and the lack of projections from the temporal cortex, clearly distinguish area PF from AIP.

Area PFG (caudal part of area 7b), like AIP, appears to be involved in the control of hand actions (Mountcastle et al. 1975; Hyvärinen 1981). However, a large proportion of PFG neurons displays somatosensory responses, often associated with visual responses limited to the peripersonal space (Hyvärinen 1981; Gallese et al. 2002; Ferrari, Gregoriou, et al. 2003). Furthermore, PFG contains neurons active during the execution of combined face/hand movements (Ferrari, Gregoriou, et al. 2003; Yokochi et al. 2003) or coding the final goal of a complex action (Fogassi et al. 2005). Finally, PFG contains visually responsive neurons active during the observation of actions made by others (parietal mirror neurons, Gallese et al. 2002), suggesting a role for this area in action recognition. A class of these mirror neurons appears to be also engaged in understanding others' intentions (Fogassi et al. 2005). Thus, PFG and AIP appear to differentially contribute to the control of arm and hand movements. The present data, compared with a previous connectional study of PFG (Rozzi et al. 2006), indicate that the functional differences between AIP and PFG are parallel to striking differences in their cortical connectivity. Indeed, PFG, though sharing with AIP dense connections with several areas (PF, PG, SII region, F5, and 46), also displays relatively dense connections with areas (PEip, VIP, the retroinsular cortex, F4, and STP) weakly or not connected at all with AIP. In contrast, several areas consistently labeled following injections in AIP (e.g., LIP, TEa, TEm, TE/TEO, FEF, and 12) are very poorly or not connected at all with PFG. The present data, which show the existence of areas densely labeled following injections in AIP, but not in PFG or vice versa, represent a strong argument against a possible significant involvement of PFG in our injections.

**Functional Considerations**

The present study provides strong anatomical support to the notion of the joint involvement of AIP and F5 in a parietofrontal circuit dedicated to visuomotor transformations for grasping (see, e.g., Jeannerod et al. 1995). Furthermore, we found evidence for the direct connection of AIP with CIP, as already described by Nakamura et al. (2001). This connections, however, appears to be quite weak. Other projections, identified in the present study, may contribute to the functional properties of AIP.

First, AIP is a potential target of visuomotor information related to the control of arm movements in space or to the organization of hand action's sequences and their final goal, originating from PG and PFG, respectively (Mountcastle et al. 1975; Hyvärinen 1981; Fogassi et al. 2005; Rozzi et al. 2006). This information may contribute to the organization of grasping movements. Furthermore, the rostral part of LIP as well as the ventral part of the FEF could be the sources of information concerning fixation and/or saccadic movements (Andersen, Asanuma, et al. 1990; Andersen, Bracewell, et al. 1990; Blatt et al. 1990; Ben Hamed and Duhamel 2002), which are necessary for object exploration and accurate object-oriented actions.

Second, in agreement with Disbrow et al. (2003), we observed a strong anatomical link between AIP and SII. Accordingly, SII may contribute to the control of grasping movements not only, as theorized by Fagg and Arbib (1998), through the connections with F5 (e.g., Matelli et al. 1986; Ghosh and Gattera 1995; Tanne Gariepy et al. 2002; Disbrow et al. 2003) but also through connections with AIP. These connections appear to involve sectors of the SII region in which neurons have mostly proprioceptive responses and are active during object manipulation (Krubitzer et al. 1995; Fitzgerald et al. 2004). This finding may appear surprising, given that AIP neurons do not have somatosensory responses (Murata et al. 2000). Furthermore, AIP inactivation (Gallese et al. 1994) impairs the preshaping phase of the grasping movement much more than the actual object manipulation, which suggests impairment mostly in visuomotor transformations. The primate SII, however, is a higher order somatosensory area, involved, for example, in tactile object recognition (Reed et al. 2004) and in coding tactile expectancies about the contact with objects (Carlsson et al. 2000). Thus, it is possible that the following occurs: 1) AIP forwards to SII motor signals related to hand actions, contributing to haptic coding of objects and 2) SII is a source of information about tactile feedback or memory-based somatosensory expectations, contributing in AIP to the selection or update of grasping motor programs (Gentilucci et al. 1995) or to the control of fingertip forces for grasping stability (Ehrsson et al. 2003).

Third, AIP is robustly connected with ventral area 46, a well-known major target of IPL projections (Cavada and Goldman-Rakic 1989b; Blatt et al. 1990; Rozzi et al. 2006), and also to area 12, which appears to be almost selectively connected with AIP in the parietal cortex. Area 12 is part of a ventrolateral prefrontal domain that is a well-known target of the inferotemporal areas (e.g., Webster et al. 1994). This prefrontal domain is considered to be involved in higher order processing of nonspatial information (see, e.g., Wilson et al. 1993; Passingham et al. 2000; Romanski 2004), where, according to Wilson et al. (1993), objects are encoded in working memory. Thus, projections from area 12 may contribute to the activity of AIP neurons related to the visual memory of object features (Murata et al. 1996).

Finally, the present data suggest that information from ventral visual stream areas involved in object discrimination and recognition (see, e.g., Tanaka 1996) can be directly conveyed to AIP. Other studies have described a direct link between inferotemporal areas and LIP (Cavada and...
Connections of the Macaque Area AIP

Goldman-Rakic 1989a; Andersen, Asanuma, et al. 1990; Blatt et al. 1990; Morel and Bullier 1990; Baizer et al. 1991). However, Webster et al. (1994), by injecting tracers in the middle temporal gyrus, observed dense labeling in a large lIPS territory, which, although entirely attributed to LIP, likely involves AIP. The present data clearly indicate that these inferotemporal-parietal connections involve not only the eye-related area LIP but also the hand-related area AIP. In this respect, it is interesting to note that AIP and LIP appear to be the only parietal areas consistently connected with the inferotemporal areas of the STS and the middle temporal gyrus. Indeed, the IPL convexity areas PFG, PG, and Opt also have some inferotemporal connections, which, however, appear to mostly involve the inferior temporal gyrus (Zhong and Rockland 2003; Roazzi et al. 2006).

Although the inferotemporal areas of the middle temporal gyrus are involved in object recognition and may convey to AIP information about object identity, the rostral ISTS appears to be also involved in visual coding of object-oriented actions (Perrett et al. 1989) and in 3D shapes perception (Janssen et al. 2001). Accordingly, the ISTS projections to AIP could be responsible for the "nonobject-type" visual responses of the hand-related AIP neurons, as suggested by Murata et al. (2000) and may contribute to the visual properties of the "object-type" neurons. In turn, actual or memory-based visual and/or motor signals from AIP could contribute to the coding of 3D objects and surfaces in the inferotemporal cortex (Uka et al. 2000; Janssen et al. 2001). In this respect, our data provide the anatomical substrate for the contribution, suggested by Tanaka (1996), of the dorsal visual stream representations of 3D object shapes to the object coding of the ventral visual pathway.

According to one widely accepted view, first advanced by (Goodale and Milner 1992; see also Milner and Goodale 1993, 1995), the primate dorsal visual stream, including area AIP, is crucial for visuomotor transformations, that is, an automatic conversion of visual information into motor commands ("vision for action" system). This process occurs independently from perceptual processes, which are considered a province exclusively of the ventral visual stream ("vision for perception" system). The existence of anatomical connections between the temporal lobe and the IPL, however, challenges this proposed dichotomy between the dorsal and the ventral visual stream. Indeed, alternative models have been proposed, concerning mostly the functional role of the IPL convexity, a target of higher order multisensory areas of the STS (e.g., Roazzi et al. 2006). In this context, it has been hypothesized that the primate IPL convexity is part of a separate visual pathway, where information from the dorsal visual stream and higher order multisensory areas of the STS is integrated with information about motor programs. The result of this integration would be crucial for the control of perception-based motor programs (Rizzolatti and Matelli 2003; see also Fogassi and Luppino 2005). Similarly, Jeannerod and Jacob (2005) have proposed that, in humans, the IPL is involved in high-level visuomotor representations, contributing, in the right hemisphere, to the perception of spatial relationships or, in the left hemisphere, to the storage of complex representations of actions. However, Husain and Nachev (2007) have proposed that part of human IPL convexity is involved not in motor control, but in nonspatial functions that are unique to humans.

Our data suggest that perceptual processes also influence the visuomotor transformation processing elaborated in the macaque lIPS, for which there are close homologies to the corresponding region of the human brain (see, e.g., Grefkes and Fink 2005). Specifically, the functional properties of AIP can reflect object and action coding taking place in the inferotemporal cortex. Accordingly, visuomotor transformations for grasping would rely on information related not only to "how" an object is made but also to "what" the object target of the action is. This last information could be crucial for the selection of the most appropriate grasping configurations as a function of the specific target object. The present study, therefore, provides a strong anatomical basis for the theoretical model of Fagg and Arbib (1998), in which this type of information has been predicted.

The present data may also provide insights in understanding specific deficits following cortical lesions in humans. In particular, Jeannerod et al. (1994) have described the behavior of a patient with a bilateral posterior parietal lesion, which likely affected areas of the dorsal visual stream. This patient was severely impaired in grasping neutral geometrical objects but performed much better in grasping familiar objects. These observations indicate that in humans, semantic cues are used for guiding object-oriented actions. Our data would predict that in this patient the access to AIP from the dorsal visual stream was disrupted, but access to AIP from the ventral visual stream was intact.

As far as we know, our study is the first to provide evidence for a direct link between components of the hand motor system and areas responsible for object recognition. This finding appears to be useful for clarifying the neural mechanisms underlying tactile object recognition in primates. In humans, tactile object recognition involves both the SII region (Caselli 1993; Reed et al. 2004), where object shape is coded haptically, and an occipitotemporal sector (lateral occipital complex; Anmedi et al. 2001; James et al. 2002; Reed et al. 2004), the putative homolog of the monkey inferotemporal cortex, where object identity is potentially coded. In the monkey, SII does not appear to be connected with the inferotemporal cortex but, as shown in our study, is robustly connected with AIP. Indeed, in humans, an anterior IPS sector, considered as the homologue of the monkey AIP (hAIP), is involved in haptic and visual object encoding and recognition (Grefkes et al. 2002). Therefore, it seems plausible to suggest that AIP, through its connections with inferotemporal areas, is crucial for the access of hand motor and haptic representations to the representations of object identity. This connection would be crucial for our ability to find with our hands a specific object among others, for example, in the dark or in our pockets. It may also provide the neural substrate responsible for preserving tactile object recognition in patients with visual agnosia, who display damage in the ventral visual stream (Farah 1990).

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Notes
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References


