We present a comprehensive analysis of the cortical connections of the insular and adjacent cortical areas in the domestic cat by using microinjections of wheat-germ agglutinin conjugated to horseradish peroxidase. We examined the identity and extent of the cortical fields connected to each area, the relative anatomical weights of the various connections, their laminar origin, and their paths across the cerebral commissures. Our main finding is that despite their relatively small size and close apposition, the connections of the insular and adjacent areas are far more widespread and more specific to each area than previously realized, suggesting that each area is involved in disparate aspects of cortical integration. The granular insular area is linked to a constellation of somatosensory, motor, premotor and prefrontal districts. The dysgranular insular area is chiefly linked to lateral prefrontal and premotor, lateral somatosensory and perirhinal cortices. The dorsal agranular insular area is connected with limbic neocortical fields, while the ventral agranular insular area is associated with an array of olfactory allocortical fields. The anterior sylvian area is associated with visual, auditory and multimodal areas, with the dorsolateral prefrontal cortex, and with perirhinal area 36. The parainsular area is linked to non-tonotopic auditory and ventromedial frontal areas. Trajectories followed by the callosal axons of each of the investigated areas are extremely divergent. As a whole, the picture of the insular region that emerges from this and a parallel study (Clascá et al., J Comp Neurol 384:456–482, 1997) is that of an extreme heterogeneity, both in terms of histological architecture and neural connections. Comparison with earlier published reports on primates suggests that most, but not all, of the areas we investigated in cats may have an direct counterpart within the insula of Old World monkeys.

Introduction

Extensive arrays of long cortico-cortical connections are a key anatomical feature of higher association cortices in primates and carnivores. These long pathways, which include intra-hemispheric as well as commissural connections, are regarded as a pivotal substrate for the complex integration capabilities of the association cortices (Reinoaso-Suárez, 1984). Precise anatomical mapping of cortical pathways, therefore, is crucial for understanding the functional properties of the these cortices. However, data on the cortical connections of many association cortices remain scant and fragmentary even in model species as well-studied as the domestic cat.

A loosely defined 'orbito-insular' region situated in the anterior sylvian and orbital gyri and adjacent sulci of cats has long been regarded a major association cortex in this species (Avanzini et al., 1969; Avanzini and Kopf, 1984; Guldin and Markowitsch, 1984; Guldin et al., 1986; Yasui et al., 1987, Norita et al., 1991), large portions of the orbito-insular region remain unexplored with modern tracing methods. Moreover, these previous studies have not, in general, mapped their findings with cytoarchitectonic or stereotaxic references, making it difficult to compare their data. In addition, since their lesions or tracer injections often extend to the claustrum, and this nucleus itself has widespread cortical connections (Clascá et al., 1992), the significance of the reported findings is unclear. Although additional data is available in different studies focused on other cortical zones (Reale and Imig, 1980; Craig et al., 1982; Burton and Kopf, 1984; Reinoaso-Suárez, 1984; Cavada and Reinoaso-Suárez, 1985; Reinoaso-Suárez and Roda, 1985; Room et al., 1985; Witter and Groenewegen, 1986; Room and Groenewegen, 1986; Bowman and Olson, 1988; Avendano et al., 1988; Clarey and Irvine, 1990; Bowman and Olson 1988; Ghosh, 1997a,c), it is not possible to infer from the published data data either the precise extent of the cortical territories connected to the insular region, or the relative anatomical weight of the various connections. Overall, data on the laminar origin of cortical input to the insular region, or on the comissural connections of this region, are almost nonexistent.

We set out to analyze systematically the cortical connections of the orbito-insular region with the following specific goals: (i) to elucidate the areas connected to the various areas of this region; (ii) to determine the relative anatomical weight of the various connections, their laminar origin and their paths across cerebral comissures; (iii) to gain insight into the functional realm of each field through a comparison of its cortical connections to data from previously published physiological studies; and (iv) to explore the possibility that some of these pathways resemble cortical connections described in the insular areas of Old World primates. For this study, we took advantage of data from a parallel study of thalamic connections in the cat’s orbito-insular region (Clascá et al., 1997). Results show that the cortical connections of the various areas in the orbito-insular region are far more widespread and more specific to each area than previously realized, and suggest that each area may be involved in disparate aspects of cortical integration. Preliminary results have been reported previously in abstract form (Clascá et al., 1996).

Materials and Methods

The brains of 31 adult domestic cats of either sex were used in the present study. All procedures involving live animals were carried out in accordance with European Communities Council Directive 86/609/EEC guidelines.

Surgery

Animals were anesthetized with sodium pentobarbital (30 mg/kg), and additional doses (10 mg/kg) were administered as required throughout the surgical procedure to keep the animal arreflectic while preserving...
spontaneous ventilation. We exposed the target zone through a small craniotomy. To address the possibility that some cortical connections are not homogeneously distributed within the areas investigated, we performed either small injections involving limited parts of an area, or larger ones, covering most or all of the area. Under direct visual guidance, we made unilateral microinjections in the cortex of a mixture of 30% horseradish peroxidase (HRP) + 2% wheat germ agglutinin conjugated to HRP (WGA–HRP). In two animals (nos 201 and 365), we injected a 50% solution of HRP in distilled water. In most cases, we made a single injection of 40–60 nl. In four experiments, we made two or three contiguous deposits (60 nl each) to impregnate a more extensive zone. We adjusted the depth and angle of injection to impregnate all cortical layers as evenly as possible. In most cases, we used a 1 μl Hamilton syringe with a beveled and gauged tip; however, in experiments aimed at the deep sulcal cortex, we air-pressure injected the tracer through a glass micropipette (15–25 μm external diameter at the tip) using a Picospritzer II (General Valve, Fairfield, NJ). After injection was complete, we covered the exposed cortex with a film of hemostatic gelfoam, sealed the bone with dental cement, and sutured muscle and skin. Amoxycillin (3 mg/kg/day) was administered preoperatively and throughout the postoperative period.

**Histology**

Between 46 and 54 h after the injection, the animals were overdosed with sodium pentobarbital (80 mg/kg), and transcardially perfused with saline (5 min). 1% paraformaldehyde + 1.25% glutaraldehyde in phosphate buffer (pH 7.4, 4°C, 45 min), and 10% sucrose in the same buffer for 20 min. We then split the brains along a coronal plane, and subsequently cryoprotected the tissue by soaking in phosphate-buffered 30% sucrose solution for 24 h. Using a freezing microtome, we cut the whole brain into 50 μm thick serial coronal sections, collecting six parallel series of sections. Two series of sections were used for histochemically revealing HRP using tetramethylbenzidine (TMB) (Mesulam, 1978). These sections were then mounted, air dried, lightly counterstained with thionin and gauged tip; however, in experiments aimed at the deep sulcal cortex, we air-pressure injected the tracer through a glass micropipette (15–25 μm external diameter at the tip) using a Picospritzer II (General Valve, Fairfield, NJ). After injection was complete, we covered the exposed cortex with a film of hemostatic gelfoam, sealed the bone with dental cement, and sutured muscle and skin. Amoxycillin (3 mg/kg/day) was administered preoperatively and throughout the postoperative period.

**Microscope Examination of the Sections**

For each brain, we analyzed and drew an entire series of TMB-reacted sections throughout the rostrocaudal extent of both cerebral hemispheres (one section every 250 μm). Using either a camera lucida mounted on a stereomicroscope, or an inverted projector, we traced section contours, heavier labeling and tissue landmarks (vessels, the inner borders of cortical layers I and VI, and the outer limit of layer V) as revealed by the thionin counterstain at 6×. Subcortical fibers, anterogradely labeled axon terminals and faintly labeled cell somata were subsequently recorded, re-examining the sections under brightfield and/or darkfield optics and polarized light at 50–300× in a Zeiss microscope. This was done by hand on the camera lucida drawings, using...
Large number of studies from this and other laboratories. For clarity, the Reinoso-Suárez map (Reinoso-Suárez, 1984) with data collated from a stereotaxic coronal planes. To this end, we revised and updated the required comprehensive delineation of the cat's cerebral areas adjusted to Correct identification of the areas labeled by the axonal transport accurately positioning the labeling.

The spread of labeling across the cerebral hemispheres and the large number of sections tended to obscure the relative weight of the various projections. We therefore decided to complement the examination of single sections with a numerical analysis of labeled cells, area by area, across an entire series of sections. It must be emphasized that these counts were never intended as a quantitative estimate of the actual total population of labeled cells, but rather as an aid in perceiving the overall amount of the various sets of labeled neurons. Cell counts were made by hand on the section drawings. The resulting numbers fluctuated widely between the various experimental cases (range 577–5969 cells; mean ± SD 2344 ± 1341). To normalize for comparison between experiments, cell numbers were converted to percentages against the sum of all the labeled cortical cells counted in the same experiment.

The previously drawn labeling and tissue landmarks as references for accurately positioning the labeling.

Reconstruction of the Injection Sites
We first analyzed and reconstructed the injection sites. We considered valid for subsequent analysis only the injection experiments which had impregnated all cortical layers in a roughly proportionate manner, and had no significant spread of tracer to the subcortical white matter or the claustrum. A total of 13 injection experiments that did not meet these criteria were discarded. The results reported here, therefore, are based on the analysis of a total of 18 valid cases (Figure 2).

In TMB-stained material, the core of the tracer deposits contains a dense black precipitate, which glows purple under dark-field optics. A translucent halo of precipitate, which has a golden glow in dark-field, extends for few hundred microns around the core. As a conservative estimate, we considered 'injection site' to be both core and halo. For comparison of the position of the injections in the various brains, we reconstructed the tracer deposits, section by section, on a single 'unfolded' cortical map of the orbitosylvian region (Figure 2; for details on the procedure to generate this map see Clascá et al. (Clascá et al., 1997)).

Abbriviations given in Table 1. Figure 1 summarizes the resulting cortical map over 'flat' medial and lateral views of the cat's cerebral hemisphere. In this type of diagram, the anteroposterior extent of the areas is accurately matched to stereotaxic coronal planes, but the relative extent of sulcal cortex becomes substantially underrepresented. The map is not based on the analysis of any single brain but, rather, it represents an idealized 'average' shape and extent of cortical areas. As a 'working diagram', this map may need revision as new experimental data become available.

Analysis of the Cortical Labeling
In our material, the cytoarchitecture revealed by the thionin counterstain of the TMB-reacted sections made it possible to delineate most of the cortical fields. When thionin staining was incomplete, adjacent cresyl violet or acetylcholinesterase stained sections were used to elucidate the boundary of a cortical field. However, a number of fields, such as the striate visual areas in the suprasylvian sulcus (Tusa et al., 1978; Tusa and Palmer, 1980; Updyke, 1986; Grant and Shipp, 1991), or the auditory fields in the anterior ectosylvian and posterior ectosylvian gyri (Reale and Imig 1980; Clarey and Irvine, 1990; Winer, 1992), are largely defined by physiological mappings, and in these regions we relied on gyral patterns and stereotaxic references reported in the original studies.

The spread of labeling across the cerebral hemispheres and the large number of sections tended to obscure the relative weight of the various projections. We therefore decided to complement the examination of single sections with a numerical analysis of labeled cells, area by area, across an entire series of sections. It must be emphasized that these counts were never intended as a quantitative estimate of the actual total population of labeled cells, but rather as an aid in perceiving the overall amount of the various sets of labeled neurons. Cell counts were made by hand on the section drawings. The resulting numbers fluctuated widely between the various experimental cases (range 577–5969 cells; mean ± SD 2344 ± 1341). To normalize for comparison between experiments, cell numbers were converted to percentages against the sum of all the labeled cortical cells counted in the same experiment.

To visualize the spatial distribution of the labeled connections across the cortical mantle, we generated reconstructions of the labeling onto lateral and medial views of the individual cerebral hemispheres. In these reconstructions, the individual labeled cells in each serial section were represented as dots along parallel lines matched to the anteroposterior level of the section.

In addition to labeling in the cortical gray matter, examination of TMB-stained material under dark-field and polarized light revealed the entire course of the axons through the white matter, including the interhemispheric commissures. We recorded these fibers on the drawings of the serial coronal sections. To facilitate comparison between cases, we reconstructed, section by section, the position of the labeled abbreviations given in Table 1. Figure 1 summarizes the resulting cortical map over 'flat' medial and lateral views of the cat's cerebral hemisphere.
interhemispheric axons on a standard midsagittal section of the cat's cerebral commissures.

To determine the cortical layers of origin for the labeled projections more precisely than by an inspection of single sections, we decided to count, for each area in both cerebral hemispheres, the neuronal somata labeled either in the superficial (IV–II) or deep (V–VI) cortical layers. We then calculated the ratio between both groups of layers, and subsequently compared the ratios of the various areas.

Figure 2. Location and extent of the tracer deposits in the 18 valid experiments analyzed in this study. Injection sites are represented on an 'unfolded' map of the cortex of the insular region (Clascá et al., 1997). For orientation, the straight solid line indicated by two open arrows indicates the fundus of the anterior rhinal and pseudosylvian sulci (which are continuous, cf. the inset in the lower right corner). Scale indicates stereotaxic coronal levels (in millimeters).
Results

Microinjections of WGA-HRP in the cortex of the orbito-insular region produced widespread anterograde and retrograde labeling in both cortical hemispheres. A detailed account of the percentage distribution of retrograde labeling in every case is provided in graphic format in Figure 3, which also gives a global overview of our findings. When this information is compared with the locations of injections (Fig. 2, and black squares in Fig. 3), it can be seen that the patterns of labeling in experiments injected in the same area are remarkably consistent. Additionally, this analysis reveals a fine-grained variability in the relative weight of the various connections that, to some extent, can be correlated with small differences in the location of the injections.

The following account reports the observations for some ‘representative’ cases, usually one for each area investigated. Only features departing from the patterns seen in the representative case are described for the remaining cases. The spatial

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**Figure 3.** Percentage analysis of the retrograde labeling in the injected cerebral hemisphere (upper half of the figure) and contralateral side (lower half) in each of our 18 valid injection cases. Cortical areas are listed in the left column. For brevity, some areas are grouped according to proximity and functional affiliations. The experiment number is indicated in the row at the center of the table (bold italics); compare with the position of the injection sites shown in Figure 2. For each experimental case, the percentage of neurons labeled in a given area over the total number of labeled cortical cells is represented following the color code at the bottom. See Table 1 for abbreviations.
distribution of the labeling is documented by the drawings and photomicrographs in Figures 4–15. Note that although axonal tracing with WGA–HRP produced both anterograde and retrograde labeling, for clarity only retrogradely-labeled somata are represented in the line drawings.

**Injections in the Granular Insular Area (GI)**

Area GI encompasses the posterodorsal orbital gyrus and ventral lip of the orbital sulcus between anteroposterior planes (AP) +20 and +17 (Clascá et al., 1997). Four experimental cases (nos 983, 935, 818 and 847) have injections restricted to, or primarily located in, area GI (Fig. 2).

The representative GI case is no. 983, illustrated in Figures 4 and 5A–D. Labeled cells are spread over a broad zone of the injected hemisphere. Heavy labeling is located in (i) the dorsal bank of the anterior ectosylvian sulcus (fourth somatosensory area, SIV), as well as nearby zones of the anterior ectosylvian gyrus (second somatosensory area, SII); (ii) sectors of the lateral bank and bottom of the presylvian sulcus that include area 6aγ (Avendaño et al., 1992), and the dorsal border of the dorsolateral prefrontal sector (DIP) (Cavada and Reinoso-Suárez, 1985); (iii) areas 3a, 3b and 4 in the dorsal lip of the coronal sulcus and adjacent portions of the sgymodium; (iv) a ventral zone of area 2 in the dorsal bank of the orbital sulcus; and (v) the fifth somatosensory area (S-V) (Mori et al., 1991) in the dorsal bank of the suprasylvian sulcus. There is additional labeling in both lips of the cruciate sulci (areas 6αα and 6γγ, and medial sectors of areas 3a and 4), in the dysgranular insular area, as well as in caudal and ventral portions of the anterior ectosylvian sulcal cortex1 (fields PAE and VAE). In the medial aspect of the hemisphere, numerous cells are labeled in area 7m (Avendaño and Verdú, 1992) and adjacent zones of the posterior cingulate area (CgP). A further collection of cells is labeled in the medial bank of the posterior rhinal sulcus (area 35 and the dorsolateral entorhinal area, DIE). In the contralateral hemisphere, areas GI, DI, S-IV, S-II and 3a contain the largest numbers of labeled neurons; however, in contrast to the injected hemisphere, no cells are labeled in 7m and SV (Figs 4 and 5B).

In most areas, the distribution of anterogradely labeled fibers closely matches that of the labeled somata (Fig. 5A,B), although there appear to be some differences in the overall amount of anterograde labeling in the various areas. The heaviest anterograde labeling is present in areas SIV, 3a and 6aγ, where it is arranged in a columnar fashion that largely matches the distribution of the retrogradely labeled somata. On the other hand, areas 3b, S-II and the motor fields of the cruciate sulci show faint anterograde labeling (Fig. 5C,D). In most areas, layers I, III and VI contain the densest aggregates of anterogradely labeled fibers and terminals.

The remaining three GI injection experiments (Fig. 6) largely concur with the findings in case no. 983. On the other hand, each case shows some particular features that, at least in part, may reflect the specific location of the tracer deposits within GI. For example, the injection in no. 935 partially overlaps that in no. 983, but it also spreads to a more rostral and dorsal portion of GI (Fig. 2). Compared to case no. 983, labeling in no. 935 is almost absent in areas 5, 7m and Cg; even scarcer in 3b; but fairly heavier in S-II and PAE (Figs 3 and 6). Likewise, in case no. 818, which involved a caudodorsal portion of GI as well as a small border zone of the ventral anterior ectosylvian field (VAE), labeling of the somatosensory cortex is less extensive, and mainly restricted to S-IV, 3a, S-II and S-V. Moreover, areas weakly labeled in case no. 983, such as area 36 and the anterolateral lateral suprasylvian area (AILS), contain significant labeling in no. 818. Case no. 847 received a large tracer deposit that encompasses most of GI and two small bordering zones of areas DI and Ald. Despite the fact that the zone impregnated nearly doubles in extent that of no. 983 (Fig. 2), this injection basically yielded the same labeling pattern, with some additional cells and fibers labeled in area 36 of the perirhinal cortex, as well as in the infra-limbic (IL), prelimbic (PL) and anterior cingulate (CgA) areas.

**Injections in the Dysgranular Insular Area (DI)**

Area DI extends over the anteroventral aspect of the orbital gyrus and the lateral lip of the presylvian sulcus between AP +22 and +18 (Clascá et al., 1997). Two valid experimental cases (nos 851 and 907) have injections centered in DI (Fig. 2).

Case no. 851 is described as the representative, and illustrated in Figures 7 and 8A,C. The tracer deposit in this case covers a large extent of DI, along with a small border zone of Ald (Fig. 2). Despite the relatively large size of the injection, however, labeling spreads over a smaller zone than after similar, or even smaller, injections in adjacent area GI. Labeling is heavy in 6aγ, DIP and 2 in the dorsal lip of the orbital sulcus, as well as in areas A5. However, unlike in any of the GI-injected cases, the remaining somatosensory and motor fields are not labeled. Moreover, also unlike injections limited to GI (case nos 983, 935 and 818), there are labeled neurons in IL, and in a rostral zone of the posterior rhinal sulcus that is transitional between 35 and DIE. In addition, labeling in the gustatory area (G) is heavier than following injections in GI. In the opposite hemisphere, the densest labeling involves DI and GI. The tangential distribution of anterograde labeling basically matches that of the labeled somata. The densest anterograde labeling is present in the presylvian sulcus and area 35, where it mainly involves layers V1 and I (Fig. 8A,C).

Being limited to a rostral and dorsal portion of DI and a border zone of GI (Fig. 2), the tracer deposit in no. 907 is substantially smaller than that in no. 851. As would be expected from the smaller size of the deposit, fewer neurons are labeled in the cortex; nevertheless, their distribution is a virtual replica of case no. 851 except for the absence of labeling in the rostral perirhinal cortex and S-IV, and a few labeled cells in 3a and SV (Figs 5 and 6).

The minute tracer deposit in case no. 363 is placed in a region that, according to our map, corresponds to a ‘junction’ zone between areas DI, GI, Ald and AS (Fig. 2). Accordingly, labeling in this case (Fig. 3) involves some of the areas labeled by injections in DI or GI (areas 4, 6, 5 and SIV); others labeled by injections in Ald (PL and AL); as well as some further areas typically labeled by injections in AS (posterior suprasylvian area, PS; temporal auditory field, Te – see below).

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**Figure 4.** Retrograde cortical labeling following a representative tracer injection in the granular insular area (case no. 983). (A–G) Distribution of labeled neurons on selected coronal sections. Each black dot represents a single cell. Thin lines perpendicular to the cortical surface indicate approximate area boundaries, while a dashed line parallel to the surface marks the outer limit of layer V. For abbreviations, see Table 1. In (D), the dark gray area represents the tracer deposit. The level of sections in (A–C) is indicated in (A). For orientation, an open arrow points to either the anterior or posterior rhinal sulci in (A–G). Bar = 2 mm. (H) Reconstruction of retrograde labeling on medial (left) and lateral (right) views of the injected hemisphere. (I) Reconstruction of labeling in the contralateral hemisphere. For clarity, caudal portions of the hemisphere that did not contain labeling are cut off. Compare with Figure 1 for the approximate location of the cortical areas. Although dots are intended to represent single labeled cells, they coalesce into solid lines in areas of high labeling density.
Injections in the Agranular Insular Areas (Areas AId and AIv)

The agranular insular cortex comprises the dorsal bank and fundus of the anterior rhinal sulcus between AP +19 and +13 (Fig. 2). An isocortical agranular dorsal field (AId) extends along the dorsal bank. The ventral agranular subfield (AIv) is cyto-architectonically transitional with the olfactory allocortex, and makes up the bottom and deep part of the ventral bank of the sulcus. Since AId and AIv are ‘folded’ within the anterior rhinal sulcus, it is not easy to reach them as selectively as would be desirable, and none of our three valid injections in the anterior rhinal sulcus involved a single area independently. Moreover,

Figure 5. Photomicrographs of anterogradely labeled axons and retrogradely labeled neuronal somata after injections in the granular insular area (A–D, case no. 893) or agranular insular area (E, case no. 788). Dark-field illumination under polarized light. Dorsal is at the top. Bar = 1 mm. (A) Labeling in the dorsal lip of the anterior ectosylvian sulcus (area S-IV) of the hemisphere injected in no. 983. Note the clustering of labeled terminals and somata in column-like aggregates (arrows) separated by similar patches of non-labeled tissue. (B) Labeling in contralateral S-IV. Note that retrogradely labeled somata are mostly situated in the supragranular layers. The arrowhead points to the lumen of the anterior ectosylvian sulcus. (C) Labeling in the anterior ectosylvian gyrus (area S-III). The arrowhead indicates the anterior ectosylvian sulcus while a star marks the suprasylvian sulcus. Observe the clusters of neurons labeled across the dorsoventral extent of the area, and the scarcity of anterograde labeling. (D) Labeling in the cruciate sulcus (arrowhead) corresponding to areas 6aα and 6aβ. (E) Labeling in the ventromedial prefrontal cortex produced by an injection in the agranular insular areas (no. 788). The most ventral aspect of both left and right frontal cortices is shown. An arrow indicates the midline. Note the absence of labeling in the right frontal cortex.

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Figure 6. Fine-grained variability in the spatial distribution of the retrograde labeling after different injections in the granular insular area. Labeling in the injected hemisphere in case nos 935 (A), 818 (B) and 847 (C) is illustrated. The relative position of these three injections, and of that in case no. 983 (Fig. 4) can be directly compared in Figure 2. Other conventions, as in Figure 4.

Figure 7. Retrograde labeling produced by injections in the dysgranular insular area. (A–H) Case no. 851. Conventions as in Figure 4. (A–G) Labeled neuronal somata on representative coronal sections; (H) Reconstruction of the labeling in the injected hemisphere. Compare with Figure 1 for an approximate location of the cortical areas. (I) Case no. 907. Reconstruction of the labeling in the injected hemisphere. The location of this injection can be directly compared to that of no. 851 in Figure 2. For abbreviations, see Table 1.
the claustrum is wrapped around AId and AIv, and narrowly separated from them by a thin extreme capsule. Thus, it was technically difficult to avoid some tracer spill over the claustrum, and it was decided to include two injections with some tracer spread to the claustrum (nos 788 and 711) among the valid cases. Overall, even if none of the three valid injections in AId and AIv is per se an ideal experiment, one can draw a consistent picture of the cortical connections from the comparison of the labeling patterns in the three experiments.

The injection in no. 788 involves a sector of AId and an

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**Figure 8.** Sub-area and laminar specificity of the cortical connections. Dark-field photomicrographs under polarized light. Dorsal is at the top and medial on the right. Bar = 1 mm. 
(A) Labeling in the banks of the presylvian sulcus following a dysgranular insular area injection (case no. 851). The lumen of the sulcus is indicated by a black arrowhead. Clusters of labeled terminals and somata are located in the most lateral zone of the dorsolateral prefrontal sector (single white arrow), and in area 6A (double white arrow). Note the numerous small neurons labeled in layer II of the gustatory area (white arrow in the lower left). (B) Labeling in a matching coronal level from a brain injected in the anterior sylvian area (no. 677). Note the conspicuous column-like clusterings of labeled cells and terminals (arrows) in the medial bank of the sulcus, which corresponds to the dorsolateral prefrontal sector. Comparison with (A) shows that the dysgranular insular and anterior sylvian areas are linked to non-overlapping domains of the dorsolateral prefrontal sector. (C) Labeling in the posterior rhinal sulcus after the injection in no. 851. Labeling is present in the medial bank of the sulcus corresponding to areas the dorsolateral entorhinal cortex and medial part of 35. A black arrowhead indicates the lumen of the sulcus. (D) An equivalent coronal level of the posterior rhinal sulcus in case no. 677. Observe that, in contrast to (C), labeling here involves only the lateral bank and lip of the sulcus (areas 36 and lateral half of 35). Note in addition, that, unlike in the presylvian sulcus (A,B), labeling in the posterior rhinal sulcus (C,D) is largely confined to the deep cortical layers.

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**Figure 9.** Retrograde cortical labeling produced by injections in the agranular insular fields. Conventions as in Figure 4. For abbreviations, see Table 1. (A–G) Case no. 788, injected in the dorsal and ventral agranular insular areas. Distribution of labeled neuronal somata is illustrated on representative coronal sections in (A–G). (H) Labeling on medial and lateral views of the contralateral hemispheres; (I) reconstruction of the injected hemisphere. (J) Labeling in the injected hemisphere in case no. 711, which received an injection in the ventral agranular area and the prepyriform cortex (Fig. 2).
adjacent zone of Alv at about AP +16.5. The tracer spilled over a small ventral portion of the dorsal claustrum (Fig. 2). Cortical labeling (Figs 9 and 5E) is confined to ventral isocortical areas and allocortical olfactory fields. The largest set of HRP-positive cells is situated in the ventral and medial frontal region [ventral prefrontal sector (VPf), IL and PL]. Area DI and portions of AS adjacent to the injection also contain numerous labeled cells. The perirhinal cortex, particularly area 35, is labeled across an extensive anteroposterior range. In the allocortex, the densest labeling involves the prepyriform cortex (Ppc), while some few neurons are labeled in area te (TT). In the contralateral hemisphere, Alv, VPf and 35 show the heaviest retrograde labeling. Interestingly, there are virtually no HRP-positive cells in contralateral Alv, and allocortical fields are not labeled.

Anterograde labeling is heavy in all the sectors of the prefrontal cortex, but particularly in VPf (Fig. 5E). Additional anterograde labeling is present in the medial part of 35, as well as in DIe, where it is situated deep to the lamina dissecans.

The tracer deposit in case no. 811 involves Alv, Alv and AS between AP +14.5 and +16 (Fig. 2), and completely spares the claustrum. Retrograde labeling in this case (Fig. 3) shows features similar to no. 788, plus others typical of AS injections, such as labeling of auditory fields A-2 and Te, visual fields of the suprasylvian sulcus and CgP (see below). Anterogradely labeled fibers in DI and VPI show a density and laminar distribution similar to case no. 788; however, unlike no. 788, anterograde labeling in the prefrontal cortex is mainly restricted to VPf.

The tracer deposit in case no. 711 involves Alv and the adjacent Ppc. Labeling is restricted to allocortical and ventral frontal isocortical regions (Fig. 9). While labeling in the isocortical and transitional areas is circumscribed to VPf, IL, and the agranular orbital area (AO), labeling in allocortical fields such as TT and Pp is heavier and more widespread than the previous two cases.

Injections in the Parainsular Area (Pi)

Area Pi covers the ventral bank and bottom of the suprasylvian sulcus, except for its caudal end. Our series includes two valid Pi injection experiments (nos 709 and 778), which are partially overlapping (Fig. 2).

Case no. 709 (Fig. 10) received an injection in the rostral tip of the ventral bank of the suprasylvian sulcus, a zone that corresponds to the rostral third of Pi. In the injected hemisphere, the largest collections of HRP-positive cells are situated in VPf and Te. Other labeled areas include IL or PL, Te, 35 and 36. In the opposite hemisphere, aside from homotopic labeling in Pi, the heaviest labeling is situated in neighboring area Te, with some additional cells labeled in VPf, IL and PL. Anterogradely labeled fibers overlap the regions containing retrogradely labeled somata. Most abundant in VPf, Te and 36, they are mainly distributed in layers 1, III and VI.

The deposit in case no. 778 is situated roughly at the center of area Pi (Fig. 2). As in the previous case, numerous neurons are labeled in VPf, Te, 35 and 36 (Fig. 10). Additional cells are labeled in AO, in caudal portions of AS and in PL. On the other hand, unlike no. 709, large collections of cells and terminals are labeled in EP and A-2. In the opposite hemisphere, the heaviest anterograde and retrograde labeling involves areas Pi, Te, AS and 36.

Injections in the Anterior Sylvian Area (AS)

Area AS covers the rostral two-thirds of the anterior sylvian gyrus and dorsal lip of the suprasylvian sulcus (Clasca et al., 1997), and there were six valid injection cases in this area (Fig. 2). The representative case is no. 677 and consisted of two contiguous injections that impregnated a relatively large zone in the crown of the anterior sylvian gyrus (Fig. 2). Several major arrays of labeled cells and terminals are present in the injected hemisphere (Figs 8B, D, 11 and 12). One array is spread along the lateral bank and lip of the suprasylvian sulcus and adjacent portions of the posterior ectosylvian and fusiform gyri. Most of the labeling is situated in retinotopic areas PLS, DLS, PS and an adjacent zone referred to as EPP (Fig. 12B, C), while other labeling probably belongs to areas VLS, 21b and AIIS. A second array of labeled cells and terminals involves a zone of DIP (Fig. 11, 8D), with some additional cells scattered in DmP. A third array is spread on the anterior and posterior sylvian gyri (A-2, Te; Fig. 12A), and the ventral bank of the anterior ectosylvian sulcus (field VAE). There is a smaller labeling focus in the ventral lip of the splenial sulcus, a zone that would correspond to a border between Cg and the cingulate visual area (CVA; Fig. 12D). Further collections of labeling are situated in area 36 and lateral parts of area 35 (Fig. 8D). In the contralateral cortex, the heaviest retrograde labeling is located in AS, A-2 and VAE, and there is additional labeling scattered in PLS, DLS, EPP and PS. However, in stark contrast to the injected hemisphere, the prefrontal and perirhinal cortices of the contralateral hemisphere are not labeled.

In general, anterogradely labeled terminals largely overlap the locations of labeled somata. Anterograde labeling is heaviest in DIP (Fig. 8B) and the suprasylvian visual areas (Fig. 12B, C), but area 36 contains few labeled terminals (Fig. 8D). In most areas, the densest anterograde labeling is seen in layers 1, III and VI.

The remaining five valid experiments with an injection in AS involved some sectors of this field not affected by the injection in no. 677. Despite small differences, the resulting labeling patterns (Figs 3 and 13) are basically like the one just described for no. 677. The deposit in case no. 201 is limited to a rostral zone of AS that was not involved by the injection in no. 677 (Fig. 2). In comparison to no. 677, labeling in no. 201 (Fig. 13A) is scarcer in caudal portions of the suprasylvian sulcus, as well as in areas Te and 36. On the other hand, no. 201 has HRP-positive cells in areas not noticeably labeled in no. 677, specifically 31V, PAE and 6aβ. In a further experiment (no. 705), the injection spread over an anteroverentral sector of AS within the lip of the anterior rhinal sulcus (Fig. 2). The scarcity of labeling in the posterior sylvian gyrus and suprasylvian sulcus, and the relatively large numbers of neurons labeled in the orbital gyrus (DI, GI, Ald), are salient features of this case (Fig. 13C). The injection in no. 570 involved the crown of the anterior sylvian gyrus between AP +15 and +13 (Fig. 2). Scant labeling of Ps and of the perirhinal cortex are the only significant departures from the pattern seen in no. 677 (Fig. 13B). A further case (no. 399)
Figure 12. Axon terminals and neuronal somata labeled by an injection in the anterior sylvian area (case no. 677). Dark-field photomicrographs under polarized light. Dorsal is at the top and medial on the right. Bar = 1 mm. (A) Labeling in the posterior ectosylvian gyrus (auditory field V). For orientation, an arrowhead points to the posterior ectosylvian sulcus. (B) Labeling in the ventral bank of the suprasylvian sulcus (arrowhead) at about AP +4, corresponding to visual field PILS. (C) Labeling in a more caudal portion of the suprasylvian sulcus and adjacent posterior ectosylvian gyrus (areas PILS and EPp). Abundant anterograde labeling amidst the retrogradely labeled somata indicates the strong reciprocal character of these connections. (D) Labeling in CgP in the ventral lip of the splenial sulcus (arrowhead) at about AP +9. E. Labeling in PS at about AP –0.5. For orientation, an arrowhead indicates the posterior rhinal sulcus.

Figure 11. Retrograde labeling after a representative tracer injection in the anterior sylvian area (case no. 677). Conventions as in Figure 4. See Table 1 for abbreviations. Compare with Figure 1 for an approximate location of the cortical areas. (A–G) Coronal sections illustrating the labeling. Note that the section in (C) corresponds to a dorsomedial portion of the hemisphere, near the corpus callosum (CC). (H) Reconstruction of the retrograde labeling in the injected hemisphere. (I) Labeling in the contralateral hemisphere. Compare with Figure 1 for an approximate location of the cortical areas.
injected in a caudal and ventral portion of AS, largely spared by
the injection in no. 677 (Fig. 2), yielded fairly heavier labeling in
Te, EP and VAE than in no. 677, and scant labeling of ALs, PLS,
VLS and Cg (Fig. 13D). The injection in case no. 760 impreg-
nated a cortical territory that, as far as can be said from our
reconstruction of the injection sites, is almost totally contained
within the zone injected in no. 677 (Fig. 2), and the labeling
resembles that of case no. 677. However, the labeled cells in
auditory area A-2 and the ventral auditory field (V) in no. 760 are
fairly more numerous and more dorsally located than those in no.
677 (compare Figs 11H and 13E).

Patchy Tangential Distribution of Labeled Neurons and
Terminals
On the individual coronal sections, HRP-positive neurons and

Figure 13. Fine-grained variability in the cortical input to the anterior sylvian area. (A) Case no. 201. (B) Case no. 570. (C) Case no. 705. (D) Case no. 399. (E) Case no. 760. Only the
lateral aspect of the injected hemisphere is shown in each. See Figure 1A for a map of the cortical areas. The position and extent of the injections in these five experiments, as well
as that in case no. 677 (Fig. 11), can be directly compared in Figure 2. Note the fluctuations between cases in the relative amount and distribution of the labeled somata.

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fibers are most often found gathered together, forming small clusters or column-like arrays of variable size, separated by zones of non-labeled or poorly labeled tissue. When serial reconstructions were made (Figs 4, 6, 7, 9–11 and 13), it became apparent that, in many areas, these aggregates of labeling corresponded to domains of variable size and shape that involve only limited portions of the labeled areas. In some areas, these domains are fairly small (∼300–800 µm in diameter; Fig. 5C–E). In other areas, the labeling spreads over wider zones; however, even here, there was a tendency to waxing and waning of the labeled cells and terminals (Figs 5A, 8A,B, 12C,E) that suggests a preferential labeling of smaller cortical domains. Although the distance between the sections sampled precludes a more fine-grained assessment, present observations show that the cortical connections of the insular fields do not involve the whole extent of the labeled areas, but rather a patchwork of restricted cortical domains within these areas. These domains are irregularly shaped, are ∼300–800 µm in diameter, and are separated by zones of either non-labeled or poorly labeled cortex. Together with similar findings on the connections of the somatic, auditory and visual cortices (Avendaño et al., 1988; Rouiller et al., 1991; Schwark et al., 1992, Morley et al., 1997), our observations in a
variety of sensory, association and limbic areas strongly suggest that this pattern may reflect a general underlying organization of the cortico-cortical connections.

Interhemispheric Pathways Across the Cerebral Commissures

In addition to labeling in the cortical gray matter, our experiments revealed the entire course of the axons through the white matter, including the interhemispheric commissures. Figure 14 summarizes these observations. Injections in each of the areas investigated labeled two sets of interhemispheric axons. One set followed a ventral route through the external capsule, before crossing the midline in the posterior limb of the anterior commissure, while the other set follows a dorsal route, crossing the midline in the corpus callosum. It is noteworthy, however, that the injection limited to AIv and PpC (case no. 711) labeled only ventrally directed commissural axons.

While the paths of ventrally directed axons labeled after injections in all areas seem to be largely overlapping, paths taken by dorsally directed axons diverge markedly with each area injected (Fig. 14). Injections in AId and AIv labeled axons in the rostroventral edge of the corpus callosum. After the DI injections, labeled fibers turn dorsally and then extend across the ventral portions of the genu of the callosum, with some few additional axons scattered up to AP +14.5. Following injections in AS, either in the crown of the anterior sylvian gyrus or in the dorsal bank of the pseudosylvian sulcus, the main bundle of labeled axons extends first dorsocaudally and then crosses the body of the corpus callosum between AP +12 and +10, although additional labeled axons are scattered over a broader zone (AP +13 to +7.5). Finally, following injections in Area Pi, labeled fibers form a conspicuous bundle that extends first caudally in the lateroventral wall of the temporal horn of the lateral ventricle, and then turns medially, around the occipital edge of the ventricle, to join the inferior branch of the forceps minor. This bundle crosses the midline through the dorsal hippocampal commissure and ventral splenium (AP +6.5 and +3.5).
**Laminar Origin of Afferent Cortical Projections**

The layers with the most abundant retrogradely labeled somata were, in decreasing order, layers III, II, V and VI. This pattern can be observed in Figures 4 and 5 and 7–12. To substantiate our impressions based on the observation of single sections, we counted, in each experiment, the somata labeled in the infragranular (V–I) or supragranular (IV–II) layers of the cortex in each cortical area in both cerebral hemispheres on all the drawn sections. The ratio of supragranular to infragranular cells was calculated for each experiment, and then averaged among the cases injected in the same area.

The charts in Figure 15 summarize the results of this analysis. Note that, in most cortical areas, 75–95% of the projections to the insular and adjacent areas originate in layers III–II. In fact, some projections like those of field G to DI and to GI are 97–99% supragranular (Fig. 8A). There is an interesting exception, however: ≈80% of the projections from the perirhinal cortex (areas 35 and 36) to areas GI, DI, AI and AS arise from neurons in layers V–VI (Fig. 15C,D).

**Discussion**

The main finding of this study is that, despite their relatively small size and close apposition, the insular region areas connect to largely non-overlapping sets of cortical areas, suggesting that they may be involved in rather different aspects of cortical integration. The following discussion explores: (i) the patterns of cortical connections characteristic to each field; (ii) the functional affiliations that can be inferred from the presence of such connections; and (iii) the similarities to connections described in the insular fields of Old World primates.

**Methodological Considerations**

Injections placed in the same area produced largely similar patterns of cortical labeling in different animals. However, our quantitative and topographical analysis of the labeling consistently reveals fluctuations between cases in the relative amount and spatial distribution of the connections labeled in particular areas. Differences in the efficiency of the axonal transport labeling method are an unlikely explanation for these fluctuations, since they would affect the global amount of labeling, rather than the amount in any particular area. In our view, these fluctuations probably reflect the combination of two factors. On one hand, the distribution of cortico-cortical connections within the injected areas may not be homogeneous, but patchy. Previous data showing that injections in distant areas selectively label small, patch-like domains of the orbital or anterior sylvian cortex (areas 35 and 36) to areas GI, DI, AI and AS arise from neurons in layers V–VI (Fig. 15C,D).

**Highly Convergent Inputs from Face, Neck and Upper Limb-related Sensorimotor Regions Characterize the Granular Insular Area**

The cortical connections of the dorsolateral portion of the orbital gyrus now identified as area GI had not previously been investigated with modern methods. Results show that this area is strongly associated with a wide array of somatic and motor districts in both cerebral hemispheres, with additional connections to dorsolateral prefrontal and perirhinal cortices (Fig. 16).

A heavy reciprocal pathway links GI with a zone of area 3a in the rostral tip and dorsal lip of the coronal sulcus of both cerebral hemispheres. This zone responds to cutaneous and proprioceptive stimulation of the face (Landgren and Olsson, 1980; Iwata et al., 1990b), and elicits simple face and perioral movements upon intracortical microstimulation (Woolsey, 1958; Iwata et al., 1985, 1990a). More caudal zones of area 3a in the anterior coronal gyrus, which are also connected to GI, receive proprioceptive information from the forelimb (Dykes et al., 1980), project to the cervical spinal cord, and elicit movements of shoulder muscles upon microstimulation (Woolsey, 1958; Ghosh, 1997a). Connections of GI with area 3b are weaker, and mostly concentrated in a zone that reportedly processes cutaneous input from forepaw digits, forelimb and trunk (Felleman et al., 1983). Our data indicate that GI lacks direct connections to more lateral portions of areas 3a and 3b, which process tongue, teeth and jaw inputs (Felleman et al., 1983; Iwata et al., 1986, 1987; Taira, 1987), although it does have dense interconnections with area 2 in the dorsal bank of the orbital sulcus and adjacent portions of the coronal gyrus, a zone...
associated with intraoral somatic input (Iwaya et al., 1990b; Nishikawa et al., 1993).

Connections with area SIV are heavy and involve the whole extent of this field in both hemispheres. Area SII connections are heavier in the anteroverentral zones of the area, which have been shown to respond to cutaneous stimulation of the face and digits (Burton et al., 1982). Following injections in SII and SIV, Burton and Kopf reported labeling in the orbital gyrus, and noted the poor topographic arrangement of these connections (Burton and Kopf, 1984). Our findings confirm these observations; in addition, they show that connections of SII and SIV are restricted to GI and have a markedly bilateral character. Together with previous reports (Burton and Kopf, 1984; Barbaresi et al., 1987), these data suggest a dense somatosensory representation of the posterior rhinal sulcus (Witter and Groenewegen, 1986; Yasui et al., 1987). This region of the parietal cortex is connected to other somatic fields such as SII, SIV and area 5, and it has been suggested that this zone may be a gateway for interactions between the hippocampal formation and neocortical somatosensory areas (Friedman et al., 1986; Witter and Groenewegen, 1986).

Thus, it may be concluded that, as a unifying theme, GI connections mainly involve cortical regions related to the sensorimotor control of the face, eyes, neck, upper limbs and trunk. The limited physiological data available on this cortex (Korn et al., 1969; Landgren and Olsson 1980) concur with this conclusion. Moreover, thalamic input to Area GI mainly arises from spinotegmental-recipient and motor nuclei (Fig. 17) (Claus et al., 1997). The available evidence is compatible with the notion that GI may be part of a complex network of cortical fields and thalamic nuclei that participate in the sensorimotor control of face, eyes, neck and upper limb. Nociceptive input may play a particularly important role in GI. Furthermore, widely converging projections from a variety of sensorimotor areas in both hemispheres suggest that GI neurons are likely to have large receptive fields, which may extend across the body midline.

The Dysgranular Insular Area May Participate in the Sensorimotor Integration of Inputs from the Mouth and Upper Digestive Tract

Previous data on the cortical connections of the anteroverentral portion of the orbital gyrus are scant. Our data show that, in comparison with adjacent area GI, the cortical connections of DI appear to be much more limited. The main connections involve cortical zones that are also connected to GI, such as the cortex of the presylvian sulcus, lateral area 2 and area 35 (Fig. 17). The relationship of these fields to orofacial sensorimotor control has been discussed above.

Other connections, such as those with G and IL, appear to be specific to DI. The primary gustatory cortex has not been clearly delineated in cats, although anatomical and physiological data from different laboratories (Burton and Earls, 1969; Nomura et al., 1980; Niimi et al., 1989) consistently indicate that a distinct portion of granular cortex extending from AP +22 to +26 and situated between DI, 3b and 6a has thalamic connections and physiological responses equivalent to those of the primary gustatory cortex in other mammals. Like other studies (Craig et al., 1982; Avendaño and Verdú, 1992) we refer to this zone as 'area G'. Our observation of reciprocal connections between DI and IL confirms earlier findings by other researchers (Room et al., 1985; Yasui et al., 1987).

In addition to these cortical connections, thalamic connections (Fig. 17) (Claus et al., 1997) indicate that DI may receive ascending input from the oral mucose and upper digestive tract. Stimulation of the vagus and splanchnic nerves was reported to evoke activity in the anteroverental orbital gyrus of carnivores (Kaada 1960; Korn, 1966). In addition, DI may have a direct 'efferent' character, since it projects directly to the nucleus of...
the solitary tract and adjacent reticular formation (Yasui et al., 1990), as well as parabrachial complex (Yasui et al., 1985b).

Cortical stimulation of the anteroventral part of the orbital gyrus was reported to elicit complex lip and tongue movements, salivation and changes in gastric motility (Hess et al. 1952; Kaada, 1960). Area DI of cats has yet to be explored with the modern functional techniques, but the existing evidence suggests that it may integrate non-lemniscal somatic, gustatory and visceral information from the oral mucose and upper digestive tract, and participate in the cortical modulation of the motor/secretory activity of these structures.

The Dorsal Agranular Insular Area is Linked to Limbic Neocortical Fields, While the Ventral Agranular Insular is Linked to Olfactory Areas

It is clear from the comparison of the labeling in cases no. 788, 711 and 811 (Figs 2 and 3) that the cortical connections of AId and AIv differ. Area AId is richly connected with ventral isocortical regions PIV, PL, IL, DI, 36 and 35. The finding of strong connections linking AId with VPf, IL and PL fits well with reports of labeling in the cortex of the anterior rhinal sulcus following injections in ventral portions of the gyrus proreus (Cavada and Reinoso Suárez, 1985; Room et al. 1985; Yasui et al., 1987). The large extent of medial frontal cortex labeled by relatively small tracer injections in AId suggests that these reciprocal pathways are both highly convergent and highly divergent. The AId connections, on the other hand, are basically limited to allocortical fields like Pp, TT and the olfactory tubercle. In addition, AId has some connections with VPf, AO and IL. It is interesting to note that AId is richly connected with thalamic nuclei associated with the prefrontal cortex (mediodorsal, midline, and parafascicular nuclei; Fig. 17), while AIv has only scant thalamic connections, and these mainly with midline nuclei (Clascá et al., 1997).

The specific functional significance of most of the fields to which AId and AIv are connected is poorly understood at present. In broad terms, however, the anatomical data support the notion that AId is linked to frontal and parahippocampal areas involved in the control of complex motivational and visceral behavior (Cavada and Reinoso-Suárez, 1985; Room et al., 1985; Witter and Groenewegen, 1986), whereas AIv may process highly elaborated olfactory information (Krettek and Price, 1977a; Cavada, 1984; Room et al., 1984).

The Parainsular Area is Involved in Auditory Processing, and Closely Associated with Medial Prefrontal Cortex

There is no previous direct study of the cortical connections of the ventral bank and bottom of the pseudosylvian sulcus. Results show PI is prominently linked with the medial prefrontal and anterior limbic cortices on the one hand, and with the perirhinal and ventral auditory cortices on the other (Fig. 17).

In the frontal cortex, the heaviest PI connections involve VPf, and adjacent parts of IL and PL. Although our series only includes two valid PI injections, the labeling data suggest that frontal connections are heaviest near the rostral edge of the sulcus, a result that concurs with reports of labeling in the pseudosylvian sulcus after tracer injections in VPf (Cavada and Reinoso-Suárez, 1985; Room et al., 1985; Musil and Olson, 1991). The finding of PI connections with the perirhinal cortex, particularly with area 36, as well as with adjacent portions of fields Te and EP accords with descriptions of labeling in the pseudosylvian sulcus following injections or lesions in these areas (Paula-Barbosa et al., 1975; Witter and Groenewegen, 1986; Bowman and Olson, 1988). Areas Pi, Te and rostral area 36 are reciprocally connected, and have similar connections with the medial geniculate thalamic complex (Fig. 16), and the lateral amygdaloid nucleus (Krettek and Price, 1977a; Room and Groenewegen 1986; Winer, 1992; Shimonaga et al., 1994; Clascá et al., 1997). It has been proposed that these ventral auditory areas are mainly involved in the cortical modulation of emotional responses to auditory stimuli (Romanski and Ledoux, 1993).

The Anterior Sylvian Area is a Complex Auditory–Visual Field, Closely Associated with the Dorsolateral Prefrontal Cortex

As a whole, the cortical connections of A5 shown in this study are in basic agreement with previous anterograde degeneration (Heath and Jones, 1971; Paula-Barbosa et al., 1975; Cranford et al., 1976) or axonal transport studies (Imig and Reale, 1980; Squartrito et al., 1981; Guldin and Markowitsch, 1984; Reinoso-Suárez, 1984; Reinoso-Suárez and Roda, 1985; Cavada and Reinoso-Suárez, 1985; Guldin et al., 1986; Witter and Groenewegen, 1986; Bowman and Olson, 1988b; Norita et al., 1991; Olson and Musil, 1992). In addition, our findings reveal the relative anatomical weight of the various inputs, and the extent of the cortical territories that originate and receive these connections. Area A5 connections mainly involve several anatomically and functionally separate regions of the cerebral cortex: (i) an array of parieto-temporal fields; (ii) the dorsolateral prefrontal cortex; (iii) perirhinal area 36; and (iv) a portion of the posterior cingulate cortex (Fig. 17).

Parieto-temporal areas connected to A5 tend to fall into three broad categories: (i) monomodal retinotopically organized suprasylvian visual fields; (ii) monomodal auditory fields; and (iii) multisensory cortices. The lateral bank of the suprasylvian sulcus contains several topographic retinal representations (Palmer et al., 1978; Tusa et al., 1979; Updyke, 1986; Grant and Shipp, 1991). According to Rosenquist’s parceling (Rosenquist, 1985), A5 connections involve fields PILS and DLS, while weaker connections reach fields AILS and VLS. While the specific role of the individual suprasylvian visual fields remains to be determined, there is evidence suggesting that PILS and DLS are prominently involved in visually guided orientation behaviors (Hardy and Stein, 1988; Payne et al., 1996). Among the monomodal auditory fields, the connections involve fields A2, Te, V and Ep (Reale and Imig, 1980). As a whole, these ventral auditory areas have been related to motivational or emotional aspects of auditory discrimination (Shimonaga et al., 1994; Campeau and Davis, 1995). Multisensory parieto-temporal fields connected to A5 would include PS, EPp and VAE. Fields EPp and PS are situated along the border between the visual and auditory cortical districts (Rosenquist, 1985; Updyke, 1986; Bowman and Olson, 1988a,b; Payne, 1993). Both fields share similar cortical and thalamic connections and contain cells responding to visual stimulation with no clear retinotopic arrangement, intermingled with others responding to auditory stimuli (Palmer et al., 1978; Updyke, 1986). Field VAE has similarities to EPP and PS, both in terms of cortical and thalamic connections and of response properties (Mucke et al., 1982; Roda and Reinoso-Suárez, 1983; Symmonds and Rosenquist, 1984; Reinoso-Suárez and Roda, 1985; Olson and Graybiel, 1987). However, field VAE also contains some cells responding to somatic stimulation (Clarey

Area AS is prominently linked to DIP. This pathway is heavy, reciprocal and basically ipsilateral. The DIP zone connected to AS is adjacent to the lateral frontal eye field of Guittot and Mandl (Guittot and Mandl, 1978a), and its connections suggest it could be similarly involved in visuomotor processing (Cavada and Reinoso-Suárez, 1985). Congruent with previous data (Room and Groenewegen, 1986; Witter and Groenewegen, 1986; Yasiu et al., 1987), the present results show that AS has heavy links to 36, but few connections to 35 (Fig. 8D). A smaller reciprocal connection links AS with a zone of CgP that has a markedly visuomotor character ( Olson and Musil, 1992; Vanduffel et al., 1995).

The present data also correlate well with available functional studies of this cortex. For example, most cells in dorsal parts of the anterior sylvian gyrus are responsive to visual stimuli (Bignall et al., 1966; Benevento and Loe, 1975; Hicks et al., 1988a,b), and this zone is richly connected with monomodal visual areas. In addition, AS has been reported to contain substantial numbers of neurons responsive to auditory stimuli, and a few of these cells are responsive to both auditory and visual stimuli (Hicks et al., 1988b). Such neurons are reportedly more frequent in the caudal and ventral aspects of AS, a fact that may correlate with our observation that these portions of AS have scant links to monomodal visual areas and abundant connections to monomodal auditory and multisensory fields (compare the labeling in nos 201 and 570 with that of nos 705 or 599; Fig. 13). Hicks and colleagues reported some neurons that were responsive to somatic stimuli in the anterior sylvian gyrus but they did not specify their location (Hicks et al., 1988b). An interpretation consistent with our findings is that these somatic-responsive cells would be near the rostral border of AS, a zone that receives some projections from S-IV and GI (case nos 201 and 705; Fig. 13).

It has been proposed that the cortex of the anterior sylvian gyrus is functionally organized as a mosaic of small neuronal clusters, each one related to the processing of a specific sensory modality (Hicks et al., 1988b). The ‘associative’ character of this cortex might thus occur mainly at the population level, while at the single-cell level, most cells would remain responsive to a single sensory modality. However, studies in the adjacent VAE field suggest that multimodal convergence can take subtle forms, e.g. through the spatial congruency of the receptive fields of cells that are responsive to different modalities (Wallace et al., 1992). Whatever the case, the available anatomical and functional data seem consistent with the notion that AS may integrate highly elaborated visual and auditory information with ongoing activity in the dorsolateral prefrontal cortex, as a part of a network of cortical areas engaged in the cortical modulation of orientation responses.

Connections within the Orbitosylvian Region

Connections between some of the areas under study are substantial, while connections between other areas are weak or absent. The connections mainly involve adjacent sectors of neighboring areas. For example DI has substantial connections with GI, but these connections appear to be restricted to the zone of GI that is adjacent to DI (note that dorsocaudal injections in GI in nos 818 and 935 produced almost no labeling in DI; Figs 3 and 5). Area GI is weakly connected to AId, and has no direct connections to Alv. On the other hand, areas DI and AId are richly interconnected. The connections of GI and DI with AS and PI are sparse, suggesting they are more or less functionally isolated.

Divergent Pathways Across the Cerebral Comissures Reveal a Basic Heterogeneity Between the Various Fields of the Insular Region

Our data show that interhemispheric connections of the insular and adjacent areas follow both a ‘ventral’ route, which crosses the midline in the anterior commissure, and a ‘dorsal’ route, which crosses the midline through the corpus callosum/dorsal hippocampal commissure. This finding is in consonance with earlier observations after massive tracer injections and commissure sectionings (Jouandet, 1982; Jouandet et al., 1986). In addition, our experiments reveal a rather unexpected spatial arrangement of these connections: despite the close apposition of the areas investigated, the paths followed by dorsally routed axons diverge widely (Fig. 14). This divergence is suggestive of basic differences in the pathfinding cues and/or timing followed by commissural axons from the various areas during development. In addition, it should be remembered that the anlagen of the anterior commissure, the corpus callosum and the dorsal hippocampal commissure are adjacent in the early embryo, but are then widely separated by the massive enlargement of the callosum that occurs during subsequent development (Sidman and Rakic, 1982). It is tempting to speculate that neighboring axons that selectively cross the midline through one or another commissure at relatively early developmental stages may occupy widely separated locations in the adult.

It is also striking that callosal axons from the investigated areas tend to converge at the midline with callosal axons from the cortical districts with which each area is preferentially associated. For example, connections of GI and DI extend across the genu of the callosum at the same levels occupied by motor and somatosensory callosal axons (Lomber et al., 1994; Matsunami et al., 1994). Likewise, callosal axons to and from area AS cross the midline in a central portion of the body of the callosum that is reported to contain the callosal connections of auditory, visual and cingulate cortices (Payne and Siwak, 1991; Lomber et al., 1994; Clarke et al., 1995). Area AId connections cross the rostrum, a region that contains interhemispheric fibers from the prefrontal cortex (Jouandet, 1985). Very much like the adjacent olfactory cortices (Jouandet et al., 1982, 1986; Payne, 1994), area Alv commissural connections are only established via the anterior commissure. In addition to a substantial pathway through the anterior commissure, large numbers of interhemispheric area PI connections cross at the dorsal hippocampal commissure and rostral part of the ventral splenium, a zone that is known to contain fibers from the entorhinal cortex (Jouandet et al., 1985, 1986).

Laminar Distribution of the Cortical Connections

Quantification of the laminar distribution of afferent projections substantiated our initial impression that, in most cortical areas, the somata of most of the cells projecting to the orbito-insular region were situated in layers III–II (Fig. 15). Areas 35 and 36 are the consistent exception, since they show preferential labeling of their deep layers in all cases, except in those injected in PI. This result is in consonance with reports that areas 35 and 36 project predominantly from deep cortical layers to a variety of frontal, parietal and temporal fields (Witter and Groenewegen, 1986; Bowman and Olson, 1988b).

Laminar distribution of labeled cortico-cortical somata and terminals has received much attention in recent years as a
morphological criterion for inferring the flow of information across the cortex (Maunsell and Van Essen 1983; Rouiller et al., 1991; Scannell et al., 1995). Criteria for establishing such hierarchical relationships include the laminar patterns of both afferent and efferent projections. Laminar distribution of the anterogradely labeled terminals could not be reliably resolved in our material because of the possibility of dendritic and local collateral staining with WGA-HRP. However, since the areas of the orbito-insular region mainly receive supragranular projections from most other areas, it is tempting to speculate that they may occupy ‘high’ hierarchical levels within their transcortical networks of sensory-motor integration. Furthermore, the mainly infragranular projection from the perirhinal cortex could be taken as evidence of a ‘feedback’ type connection with the insular areas. It is to be noted, however, that the general validity of these anatomical criteria regarding hierarchy is still unclear in carnivores (Reinoso-Suárez, 1984; Schwark et al., 1992; Turman et al., 1992; Kittes and Hollrigel, 1996).

Comparisons with Cortical Areas in the Insular Lobe of Old World Primates
Cytoarchitectonic, connective and electrophysiological studies over the past two decades have characterized a number of areas in the insular lobe of macaques. Comparison with these primate areas is relevant because the ‘insular’ areas of carnivores were originally named in explicit reference to the insular lobe of Old World monkeys and anthropoids (Brodmann, 1909; Gurewitsch and Chatschaturian, 1928; Ariens Kappers et al., 1936), and this label has persisted to the present day. However, these early comparative studies were based on criteria that would be regarded as thin, at best, by modern standards (Clasçá et al., 1997). Thus, the question as to which cortical areas in carnivores, if any, bear resemblance to contemporary definitions of areas in the insula of Old World monkeys is still open.

It is widely acknowledged that carnivores and primates have evolved along separate lines from a primitive common ancestor for ∼65 million years (Northcutt and Kaas, 1995). Therefore, while it is still possible to recognize a number of area homologs based on similarities in relative position, connectivity, biochemical markers and functional properties (Payne, 1993; Krubitzer, 1995; Payne et al., 1996), other cortical areas may have less obvious equivalents, and may be species specific (Preuss, 1995). Based on thalamic connectivity and cytoarchitecture, and a review of the literature, we have recently suggested a number of equivalences between GI, DI, Ald, Alv and Pi in cats and areas characterized in the macaque insula (Clasçá et al., 1997). Interestingly, however, we could not identify a field resembling the cat area AS in the macaque insula. Findings in the present study add further support to these comparative interpretations.

Cortical connections described here for the cat GI are remarkably similar to connections described in the granular insular area of macaques. As in cats, the macaque granular insular cortex is largely, if not exclusively, a somatosensory field, with particular relation to nociception (Sudakov et al., 1971; Robinson and Burton, 1980; Friedman et al., 1986; Schneider et al., 1995). The granular insular cortex of macaques is richly connected to a number of somatic and motor fields, including areas 4, SII, and lateral zones of areas 6 and S1 (Künzle, 1977; Mesulam and Mufson, 1985; Friedman et al., 1986; Barbás and Pandya, 1987; Burton et al., 1995). As in the cat’s GI, the granular insular cortex of macaques is connected with the rostral part of the perirhinal cortex (Suzuki and Amaral, 1994). Likewise, the granular insula of macaques is heavily linked to area 7b and the retrolinseus areas (Mesulam and Mufson, 1985; Friedman et al., 1986; Cavada and Goldman Rakic, 1989); S-V and S-IV of cats, both richly connected to GI, have been compared, respectively, to 7b (Avendaño et al., 1988) and the retrolinsular area (Burton and Kopf, 1984). The granular insula of macaques is heavily connected to area 12 of the prefrontal cortex (Preuss and Goldman-Rakic, 1989; Carmichael and Price, 1995), and the cat GI has connections to a zone in the caudolateral part of DIP; nevertheless, it is difficult to say whether the connections bear a direct relationship, given the highly specific development of the primate prefrontal cortex (Preuss, 1995).

In macaques, there is a dysgranular insular area that is adjacent to the primary gustatory cortex and involved in gustatory and visceral functions (Mesulam and Mufson, 1985; Pritchard et al., 1987; Ogawa, 1994; Zhang et al., 1998). Cortical connections of this area include lateral premotor cortices, the most lateral parts of areas 6 and S4 (including the precentral and gustatory opercular cortex), area 13 of the orbitofrontal cortex, and rostral portions of areas 35 and 36 (Mesulam and Mufson, 1985; Matelli et al., 1986; Barbás, 1988; Suzuki and Amaral, 1994; Carmichael and Price, 1995). Overall, the connections shown here for cat area DI seem to have a rather direct parallel in the above macaque connections. Likewise, a connection to area 13 of the orbitofrontal cortex in macaques (Carmichael and Price, 1995) might have a counterpart in the connections of DI to caudal DIP.

In macaques, an agranular insular cortex with an architecture that is transitional between that of the neocortex and the prepyriform cortex (Mesulam and Mufson, 1985) has been described and subdivided into a number of subareas (Carmichael and Price, 1994). As a whole, this macaque insula cortex is richly connected to the orbital and medial prefrontal cortex, the entorhinal and perirhinal cortex, the dysgranular insular area, and the parainsular cortex (Mesulam and Mufson, 1985; Insausti et al., 1987; Suzuki and Amaral, 1994; Carmichael and Price, 1995), a pattern that resembles that shown here for area Ald. Moreover, in macaques, the most medial (ventral) agranular subareas, but not the lateral ones, receive anatomically and electrophysiologically demonstrable olfactory input from the prepyriform cortex (Carmichael et al., 1994).

Topological relationships as well as cortical and thalamic connections (Clasçá et al., 1997) (see also present results) suggest a parallel between the cat area Pi, and the agranular and dysgranular isocortex of the macaque temporal operculum (Morán et al., 1987). In a way reminiscent of Pi, the connections of the temporal operculum involve medial frontal (areas 14, 25 and 24), perirhinal, and association auditory areas (Insausti et al., 1987; Morán et al., 1987; Suzuki and Amaral, 1994). A further parallel is the scarcity of connections with the insular cortex, which, as in cats, are restricted to the agranular insular area (Mesulam and Mufson, 1985; Morán et al., 1987).

In the literature, the cortex of the anterior sylvian gyrus has epitomized the cat’s insular cortex. However, based on the analysis of its thalamic connections, we have proposed avoiding the term ‘insular’ and, instead, named it AS, according to its gross anatomical location (Clasçá et al., 1997). The cortical connections reported here add further support to this view. Unlike the areas in the insular lobe of macaques, the main cortical connections of AS involve extrastriate visual, secondary auditory or multimodal (auditory–visual) cortical fields. As pointed out above, the connections of the macaque’s insular lobe are mainly with somatic, visceral, motor and limbic cortical fields. It is thus possible that area AS is specific to felines (Clasçá et al., 1997). An
alternative possibility, given the highly specific expansion and gyral pattern of the cerebral cortex in Old World primates, is that fields similar to AS may be located outside the insular lobe in macaques. In this regard, the parallel connectivity and functional affiliations between the cat AS and the multimodal cortex in the caudal dorsal bank of the macaque superior temporal sulcus (Jacobson and Trojanowski, 1977; Barbas and Mesulam, 1981; Barbas, 1988; Yeterian and Pandya, 1989; Huerta and Kaas, 1990) is intriguing.

Concluding Remarks
The picture of the orbito-insular region that emerges from this and a parallel study (Clascá et al., 1997) is that of extreme heterogeneity, both in terms of histological architecture and neural connections. Each area has a unique set of cortical and thalamic links (Fig. 17). To a large extent, the various areas seem to have closer ties to other frontal, parietal or temporal cortical regions than to each other. Cortical and thalamic connections of areas GI and DI strongly suggest that they may participate in the cortical control, respectively, of the face, neck and upper limbs, or of the mouth and upper digestive tract. Area AIV is associated with limbic cortices and thalamic nuclei. Area AIV appears to be chiefly linked to olfactory telencephalic regions. The area Pi connections are compatible with the notion that it may be chiefly linked to olfactory telencephalic regions. The area Pi connections are compatible with the notion that it may be primarily linked to olfactory telencephalic regions. The area Pi connections are compatible with the notion that it may be predominantly linked to olfactory telencephalic regions.

Finally, the connections of area AS suggest that it may be a complex auditory–visual field participating in the modulation of eye and head orientation responses.

Notes
1. Unlike the anterior half of the dorsal bank of the anterior ectosylvian sulcus, which is a largely monomodal somatosensory field (SIV) (Clemo and Stein, 1984; Claye and Irvine, 1990), neurons in the posterior half of the bank and the caudal ‘pouch’ of this sulcus have been shown to respond mainly to auditory and/or somesthetic stimuli (Meredith and Clemo, 1989; Claye and Irvine, 1990; Wallace et al., 1992; Rauschecker 1996). Avendaño et al. named this zone the posterodorsal anterior ectosylvian field (PAE) (Avendaño et al., 1988). The cytoarchitecture and connections of PAE are different from those of SIV (Burton et al., 1982; Roda and Reinoso-Suárez, 1983; Burton and Kopf, 1984; Reinoso-Suárez and Roda, 1985; Avendaño et al., 1988; Meredith and Clemo, 1989) as well as from neighboring auditory fields (Clarey and Irvine, 1990). The fundus and ventral bank of the anterior ectosylvian sulcus is referred to in this study as the ventral anterior ectosylvian field (VAE). This field would include the physiologically identifiable but poorly localized ‘visual ectosylvian area’ (Mucke et al., 1982; Olson and Graybiel, 1987) as well as ventrally and rostrally adjacent sulcal cortex that is architectonically and hodologically similar but contains, amidst a majority of visually responsive neurons, a significant proportion of cells responding to somatic and auditory stimuli (Clarey and Irvine, 1990; Wallace et al., 1992). Transition between PAE, SIV and VAE is gradual, and relatively variable in position (Rauschecker, 1996). For our analysis, however, we took coronal plane +14 as the conventional limit between SIV and PAE in the dorsal bank of the sulcus, and plane +12 as the border between VAE and PAE in the fundus and ventral bank of the sulcus (Fig. 1).

2. We call the cortex in the anterior thalamic sulcus rostral to plane +19 the agranular orbital area (AO). Like the agranular insular areas, this cortex is cytoarchitectonically transitional between the isocortex and the olfactory allocortex. Thalamic connections of AO, however, are different from those of the agranular insular cortex (Craig et al., 1982; Clascá et al., 1997).

3. Anatomical and physiological studies (Woolsey, 1960; Reale and Imig, 1980; Updyke, 1986; Bowman and Olson, 1988a,b) suggest that the posterior ectosylvian gyrus may be understood as consisting of three main parallel anatomo-functional zones or ‘belts’: (i) a rostral belt of tonotopically organized auditory cortex (fields Vp and D); (ii) a central belt of monomodal auditory cortex with no clear tonotopy (here labeled fields EP and DP); and (iii) a belt of visual or visual–auditory cortex near the anterior lip of the suprasylvian sulcus, which we refer to as EPP (Updyke, 1986).

4. Following the [14C]2-deoxyglucose study by Vanduffel et al. (Vanduffel et al., 1995), we distinguish a relatively large, visually related field in the ventral bank and lip of the splenial sulcus called the cingulate visual area (CVA). The splenial visual field (Kalia and Wüteridge, 1973; Rosenquist, 1985), which is dorsally adjacent to CVA, was not labeled in our experiments.

5. Applying a nomenclature proposed by Demeter et al. (Demeter et al., 1990) for the macaque monkey to the cat, we distinguish, within the splenium of the corpus callosum, a dorsal portion that is laterally continuous with the dorsal branch of the forceps major, and a ventral portion that is laterally continuous with the ventral branch of the forceps major. These two splenial portions can be readily delineated in mid sagittal sections of the cat’s cerebral commissures (Fig. 14). In the cat, the dorsal hippocampal and ventral splenial fiber bundles are not clearly separated (Jouandet et al., 1986), but, rostrally to AP +5, ventral splenial fibers become progressively separated from the body of the callosum by non-commisural fimbria-fornix fibers (F. Clascá, unpublished observations). We thus took AP +5 as the conventional limit between the ventral splenium and dorsal hippocampal commissure.

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