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Callosal and Prefrontal Associational Projecting Cell Populations in Area 7A of the Macaque Monkey: A Study Using Retrogradely Transported Fluorescent Dyes

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ABSTRACT

The spatial interrelationship of neurons in area 7a in the inferior parietal lobule that project through the corpus callosum to the corresponding field in the contralateral hemisphere or to the ipsilateral prefrontal cortex has been analyzed in macaque monkeys by using double-labeling procedures with retrogradely transported fluorescent dyes. The populations of callosal and associational projecting neurons have similar laminar distributions and are topographically intermingled. Less than 1% of the neurons were double-labeled, thus suggesting that the two populations are largely separate. Two-dimensional reconstructions of the distribution of labeled cells made on flattened reconstructions of the inferior parietal lobule revealed that the areal distribution of the two cortico-cortical output arrays is complex. Although each pattern of labeling showed some discontinuities in density, there was no obvious periodicity within or between the spatial distributions of the two projecting populations. It was consistently observed that the cortex of the lateral wall of the intraparietal sulcus, adjacent to area 7a, projects more heavily to the prefrontal cortex than does area 7a itself.

Key words: inferior parietal lobule, prefrontal cortex, cerebral cortex, cortico-cortical connections, cortical columns

The posterior parietal cortex is a critical node in a widely distributed network of connections that includes a number of cortical and thalamic structures that are involved in the highest levels of neural processing. These areas include the prefrontal cortex, the cortex of the superior temporal sulcus, the cingulate cortex, and the medial pulvinar. All of these areas are reciprocally connected to each other and to the posterior parietal cortex (Baleydier and Maugiuere, '77; Divac et al., '77; Jacobson and Trojanowski, '77; Jones and Powell, '70; Mesulam et al., '77; Pandya and Kuypers, '69; Pandya et al., '81; Pearson et al., '78; Trojanowski and Jacobson, '76). Clinical studies have revealed that lesions in any of these regions in humans can result in spatial and attentional disorders similar to those seen with lesions to the posterior parietal cortex. These results suggest that the entire complex of structures either represents or contains a distributed functional system concerned (among other things) with spatial perception and attention (Critchley, '53; Heilman and Valenstein, '72; Mesulam, '81). Data from single unit recordings also indicate that at least some of these regions have marked functional similarities (Benevento et al., '81; Bizzi, '68; Goldberg and Bushnell, '81; Hyvarinen and Poranen, '74; Lynch et al., '77; Mikami et al., '79; Mohler et al., '73; Mountcastle et al., '75; Robinson et al., '78; Sakata et al., '80,'83; Suzuki and Azuma, '77; Yin and Mountcastle, '77).

For a more complete understanding of the functional organization of the posterior parietal cortex, it is necessary to clarify further the structural and functional relationships of its various inputs and outputs. Single unit recording

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experiments have provided evidence for a columnar organization of functional properties within the posterior parietal cortex (Mountcastle et al., '75). At some level, this columnar organization must be reflected in its pattern of connections. In the present series of studies, we wished to determine whether or not different radially organized modules or columns in the posterior parietal cortex receive their afferents from, and project to, different regions of the brain. We also wished to determine the laminar distribution of these outputs and inputs and how they might vary according to the destination or source of the projection. Finally, we were interested in whether individual neurons in the posterior parietal cortex have collateral projections to more than one of its known projection fields, or whether the various projecting populations are separate.

To address these issues we used the retrogradely transported fluorescent dye technique. This method enabled us to directly visualize in individual monkeys the spatial relationships of different populations of projecting neurons and, in double-labeling experiments, to determine the extent of collateralization of the various projections.

In this study we examined the structural relationship between the homotopic callosal projection to the contralateral posterior parietal cortex and the associational projection of the posterior parietal area to the ipsilateral prefrontal cortex. We concentrated our attention on a single subdivision of the posterior parietal cortex, area 7a, which is located medially on the lateral convexity of the inferior parietal lobule. This area was chosen because most single unit recordings in the inferior parietal lobule (Hyvarinen and Poranen, '74; Mountcastle et al., '75,'81; Robinson et al., '78; Sakata et al., '80,'83; Motter and Mountcastle, '81; Andersen and Mountcastle, '83; Andersen and Essick, '84) have been made in this subdivision. The prefrontal projection sites that were examined include area 8a (the frontal eye fields) and areas 46 and 45 of Walker ('40). Together, these three areas represent the total set of frontal cortical fields that are reciprocally connected with the inferior parietal lobule. In a parallel series of experiments, we examined the spatial relationships of the divergent projections to area 7a and the prefrontal cortex from the cingulate gyrus, the superior temporal sulcus, and the medial pulvinar; these results will appear in subsequent reports.

Our principal finding is that the populations of callosal and associational projecting neurons in area 7a are largely intermingled, but only a small percentage of neurons gives rise to both callosal and associational projections. The two projections have also been found to have a similar laminar distribution. Their overall tangential distribution within the inferior parietal lobe is rather complex and shows none of the periodicities in these patterns that is a feature of certain other projection systems. Furthermore, the cortex on the lateral wall of the intraparietal sulcus (the lateral intraparietal area, LIP) has been found to project much more strongly to the prefrontal cortex than does area 7a.

METHODS

Experimental and histological procedures

Cynomolgus monkeys (*Macaca fascicularis*) were used in these experiments. All surgical procedures were performed with full aseptic precautions with the animals anesthetized with sodium pentobarbital (35 mg/kg). Craniotomies were made over the posterior parietal area and prefrontal cortex in separate procedures, and the dura were resected to allow direct visualization of the relevant cortical areas. The tracer injections (500 nl each) were made by pressure with 1 μl Hamilton syringes, usually at a rate of about 50 nl/minute. Single or multiple injections were made in area 7a; in the prefrontal cortex multiple injections were made across areas 8a, 45, and 46. The various tracers were dissolved in sterile saline. Fast blue or true blue (5% solution) was injected into area 7a; nuclear yellow (2% solution) was injected into the prefrontal cortex. Survival times were 2 weeks for the fast blue and true blue injections and 3–5 days for the nuclear yellow injections. A mixture of tritiated amino acids (^{3}H-proline and ^{3}H-lysine concentrated to 100 μCi/μl), from New England Nuclear was also injected into these animals for anterograde tracing experiments. After the survival period the animals were perfused transcardially with three solutions in the following order: (1) cold, heparinized saline, (2) 10% formalin in 0.1 M phosphate buffer (pH 7.5), and (3) an 8% sucrose, 10% buffered formalin solution. After removal, the brains were cut into four blocks, placed in 15% sucrose in phosphate-buffered saline and then sectioned, generally within 36 hours.

The tissue blocks were sectioned on a freezing microtome at 30 μm. Every ninth section was mounted (i.e., at intervals of 270 μm) except for brain 83A8, in which every sixth section was used (180 μm intervals). The intervening sections were stored in the buffered formalin solution for subsequent autoradiography (Cowan et. al., '72). The sections to be examined under a microscope were immediately mounted from water after sectioning and were dried with circulating air to prevent tracer diffusion. The sections were viewed with a Leitz Orthoplan fluorescence microscope. Color photomicrographs were made of many of the sections, and the labeled neurons in each section, or in some cases every other section, were plotted under direct observation. Cell counts were made from the sections themselves or from photomicrographs. Control checks of the counts made directly from the sections and from photomicrographs of the same sections showed that both methods gave essentially the same results.

Two-dimensional reconstructions

To facilitate the analysis of the distribution of the callosal and associational projecting populations, flattened reconstructions of the posterior parietal and prefrontal cortices were made according to the method of Van Essen and Maunsell ('80). Since only the relevant areas were flattened, the degree of areal distortion was kept to a minimum. Figures 1a and 2a indicate the extent of the prefrontal and posterior parietal cortex that was flattened. Figure 1b illustrates that the flattening of area 7a and the adjoining fields can be thought of as pulling the superior temporal sulcus out like an accordion, raising the lateral wall of the intraparietal sulcus, and opening up the lateral fissure by separating the lips of the sulcus. The shaded area in Figure 1c indicates that a large amount of the cortex of interest lies buried in sulci. Figure 1d shows the major cortical subdivisions recognizable in our material. Area PG (von Bonin and Bailey, '47) contains two cortical fields, areas 7a and LIP, that can be differentiated quite clearly on the basis of their connections. The middle temporal area was identified by using a modification of a myelin stain (Gallya, '79) as suggested by Van Essen et al. ('82). Figure 2 shows the same analysis for the prefrontal cortex; the various frontal cortical fields were identified cytoarchitectonically following the criteria given by Walker ('40).

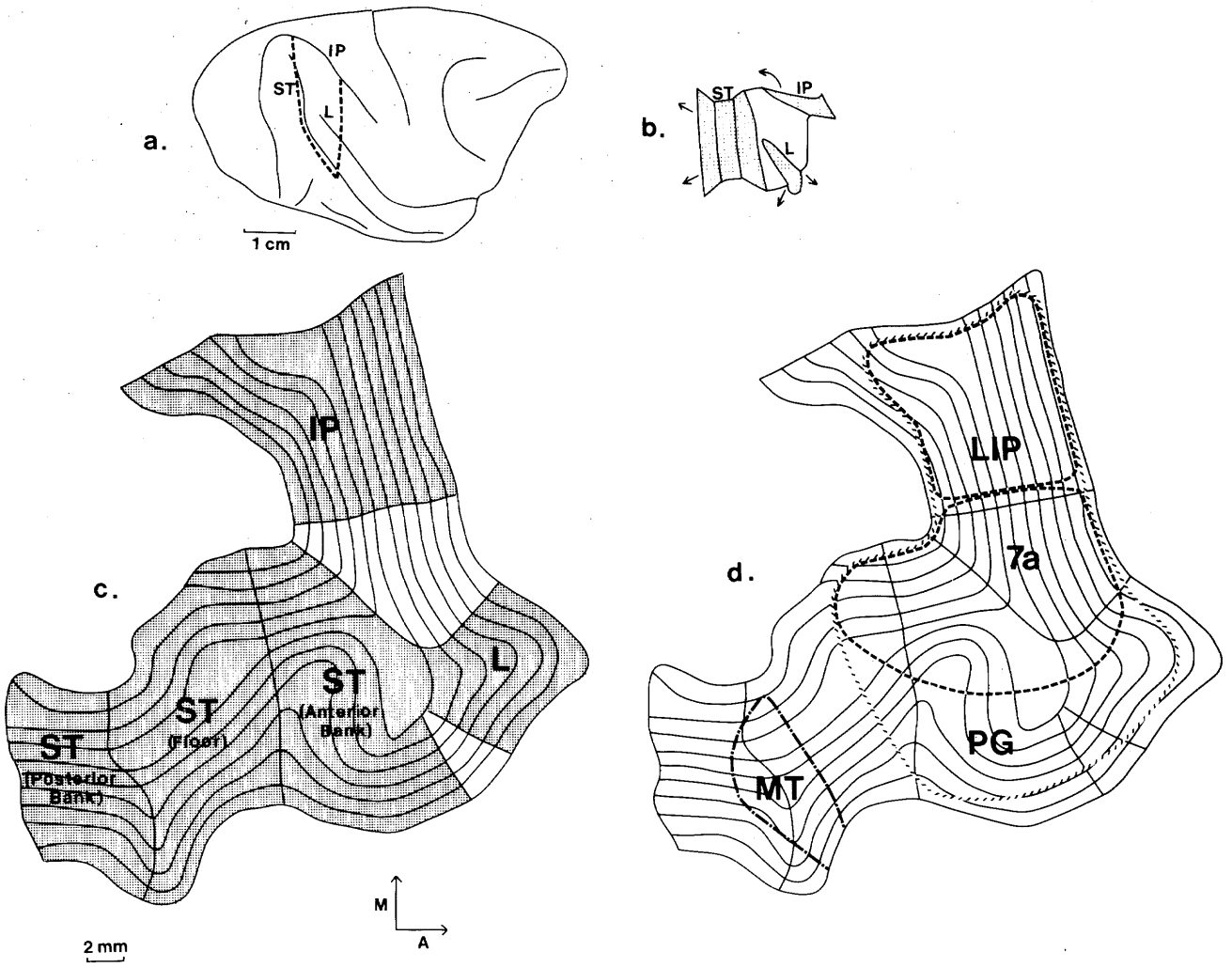


Fig. 1. Method of flattening the caudal inferior parietal lobule. a. Surface view of the dorsolateral aspect of the right hemisphere of a macaque monkey. The borders of the area to be flattened are indicated by the dotted line: Note, the dorsal border is along the fundus of the intraparietal sulcus and is hidden from view. IP, intraparietal sulcus; L, lateral sulcus; ST, superior temporal sulcus. b. The steps involved in flattening the cortex. The figure is again a dorsolateral view, but now only layer IV is shown; the stippled areas represent cortex buried within sulci. Layer IV of the intraparietal sulcus is folded dorsally, the cortex within the superior temporal sulcus is pulled out caudally, and the lips of the lateral fissure are spread apart. c. Example of a flattened inferior parietal lobule and the adjoining region of the temporal lobe; the shading again indicates cortex buried within sulci. Note that much of the cortex of interest is located within sulci. The parallel lines represent the contours of layer IV taken from individual coronal sections. Medial is up and anterior is to the right. d. The same flattened cortex as in c with the borders of the different cortical fields indicated. The extent of area PG was determined using the cytoarchitectural criteria of von Bonin and Bailey ('47). Contained within PG are the lateral intraparietal area (LIP) and area 7a; the boundaries of these areas were determined on the basis of their connections. The middle temporal area (MT) was identified by its dense myelination as seen in preparations stained by a modification of the Gallyas ('79) technique (Van Essen et al., '82).

We also made a three-dimensional wire reconstruction of layer IV for each of the brain regions illustrated in Figures 1 and 2. Soft wire was molded to the outlines of layer IV as drawn from magnified projections of the Nissl-stained sections. These wire outlines were then soldered together in sequence, and the resulting skeleton was covered with plastic tape, painted with latex, and cured in an oven. The contours of the sections were drawn on the hardened sheet of latex; then the sheet as a whole was removed from the wire mold. By making a few small cuts in the sheet, and then flattening it, we were able to get a fairly direct measure of the amount of areal distortion in our reconstructions. These areal distortions were found to be quite small—under 5% for both the prefrontal and posterior parietal reconstructions. The absence of appreciable areal distortion is due to the fact that rather small sections of the cortex were flattened, and angular distortions were not minimized. Thus we are confident that the plots of the spatial distributions of the labeled neurons that we made on these flattened reconstructions are quite accurate.

RESULTS

Three monkeys were used for the main phase of these experiments. In each animal two sets of dye injections were made; one dye was injected into the posterior parietal cortex and a second dye was injected into the contralateral

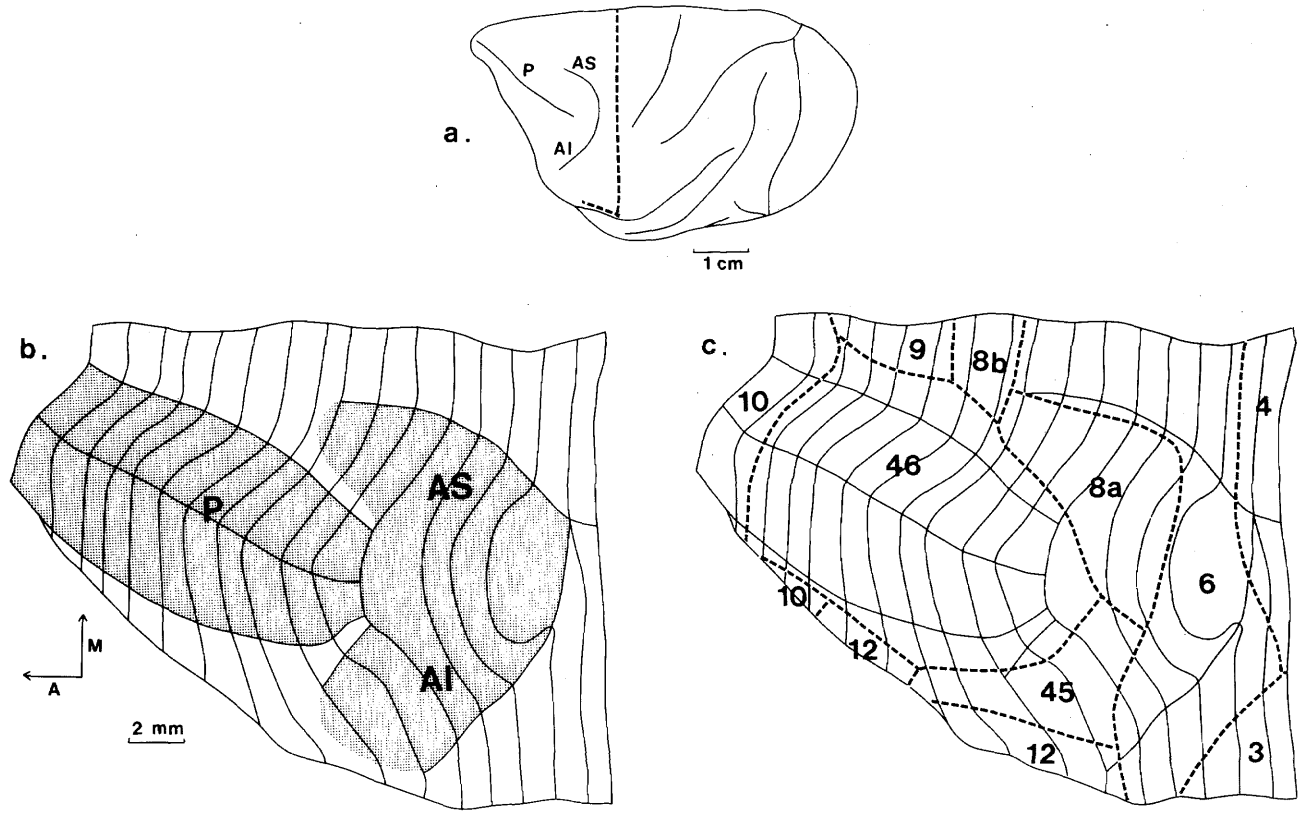


Fig. 2. Method of flattening the dorsolateral prefrontal cortex. a. A dorsolateral view of the left hemisphere of a macaque monkey. The dotted line indicates the caudal limit of the region to be flattened. AS, superior arcuate sulcus; AI, inferior arcuate sulcus; P, principal sulcus. b. Flattened reconstruction of the dorsolateral prefrontal cortex. The parallel lines are the outlines of area IV taken from individual Nissl-stained sections. The shaded areas represent cortex buried within sulci. c. The same flattened reconstruction as in b but showing the boundaries of the different cortical fields determined by the cytoarchitectonic criteria of Walker ('40).

prefrontal cortex. The findings in all three cases were essentially identical. In Figure 3 we have reconstructed the locations of the injection sites. In each case the prefrontal injections included parts of areas 8a, 45, and 46. In cases 83A-4 and 83A-8 there was some fluorescence from the tracer injected into the prefrontal cortex in part of area 6; however, we do not believe that sufficient tracer was present in area 6 to label axon terminals in this field, because none of the areas known to project to area 6 were labeled in these brains. In case 83A-4 a single dye injection was made into area 7a; in case 83A-5 three tightly spaced injections were made near the center of area 7a; and in case 83A-8 20 injections were made so as to label almost half of area 7a. In 83A-8 the injection site also included parts of areas 7b and LIP, but in the other two cases the injections were strictly confined to 7a. In general the labeling patterns seen on the contralateral side were consistent and differed only in that the area of cortex labeled increased more or less in proportion to the number of injections. In two of the monkeys (83A-4 and 83A-5) single injections of a mixture of tritiated amino acids were also placed in area 7a on the side contralateral to the 7a dye injection.

In a further group of seven monkeys single injections of labeled amino acids (seven hemispheres) or fluorescent dyes (three hemispheres) were made at different locations in areas 7a, 7b, and LIP. These brains were used to define the boundaries of the three areas, based on their afferent and efferent connections, and to provide additional information about the ipsilateral prefrontal and the contralateral callosal projections.

Spatial distribution of the prefrontal and callosally projecting neurons

Figure 4 shows the distribution of labeled neurons in representative cross sections through the ipsilateral prefrontal and contralateral posterior parietal cortices in case 83A-5. The solid triangles represent groups of neurons labeled by fast blue injections into the posterior parietal

Fig. 3. Flattened reconstructions of the injection sites (indicated by shading) in three of the brains studied. On the left are represented the dye injections made into the right prefrontal cortex, and on the right the injections made into the left posterior parietal cortex are shown. In each case the multiple injections of nuclear yellow made into the prefrontal cortex included much of areas 8a, 45, and 46. For the posterior parietal cortex a single injection of true blue was made into area 7a in case 83A: in case 83A-5 a cluster of three injections of the dye fast blue were made at a small locus in 7a; and in case 83A-8 multiple injections of fast blue were made into areas 7a and 7b.

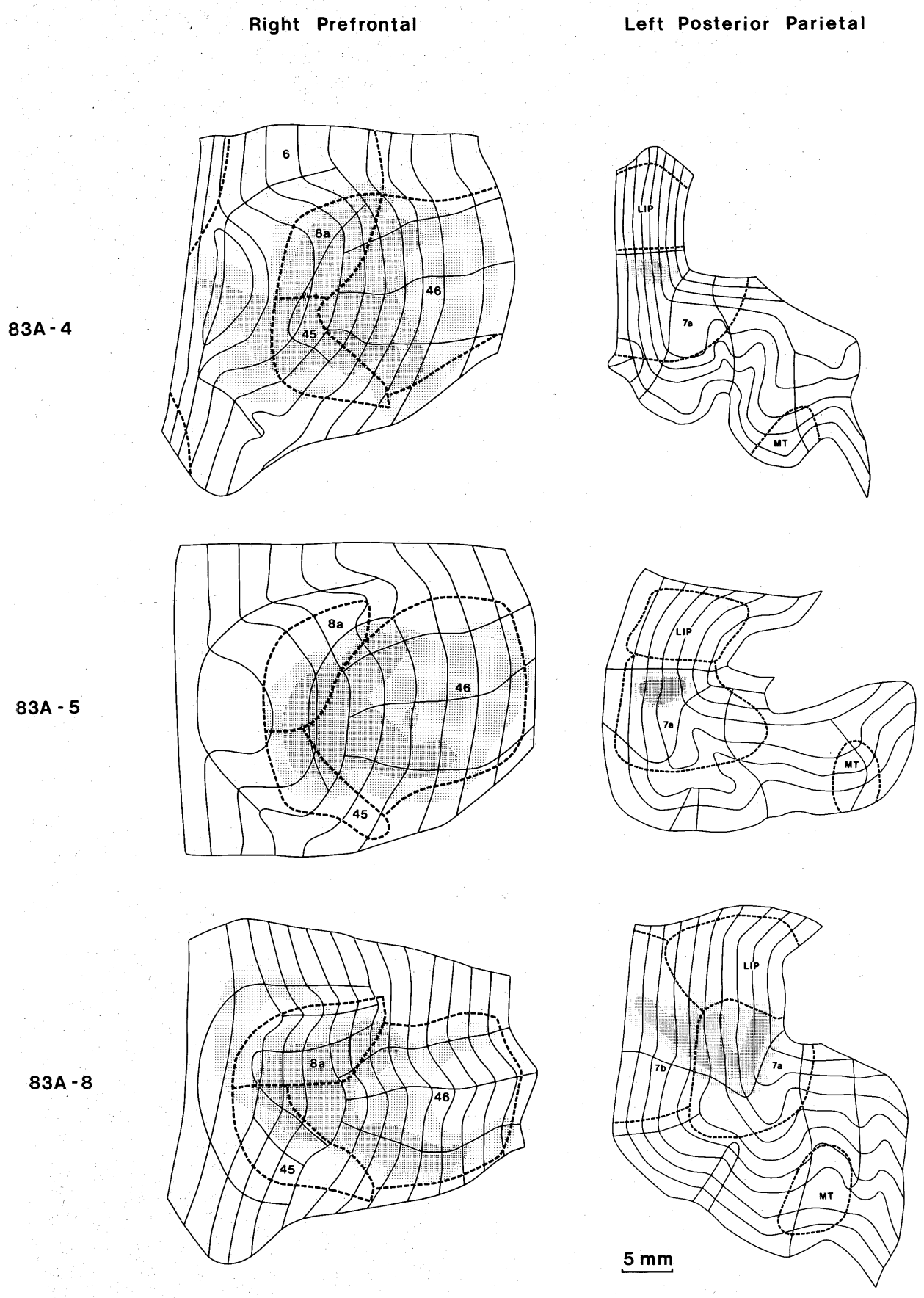
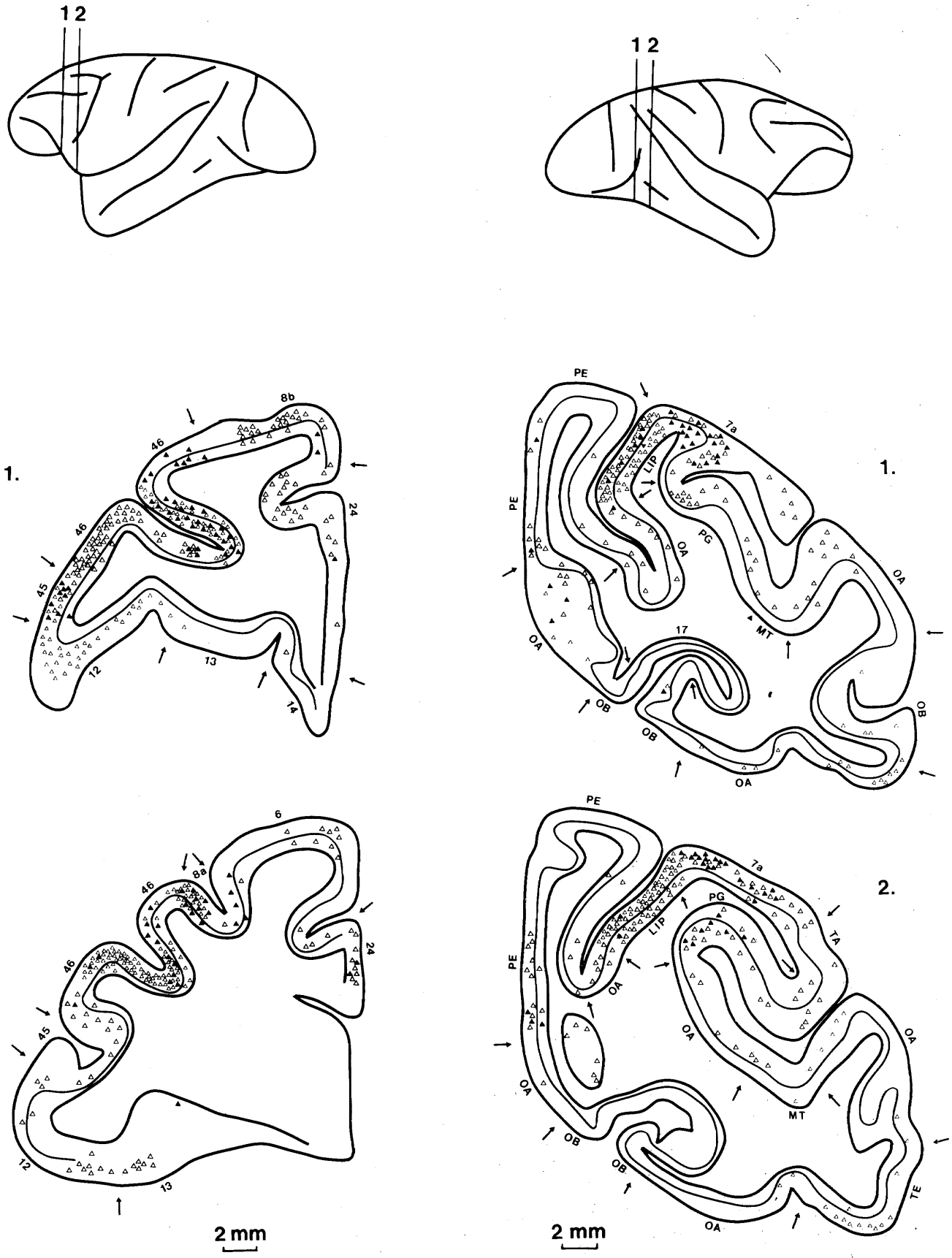

Right Prefrontal
Left Posterior Parietal
83A-4
83A-5
83A-8
6
8a
46
45
LIP
7a
MT
7b
5 mm

Figure 3


1 2
1 2
1.
8b
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24
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Figure 4

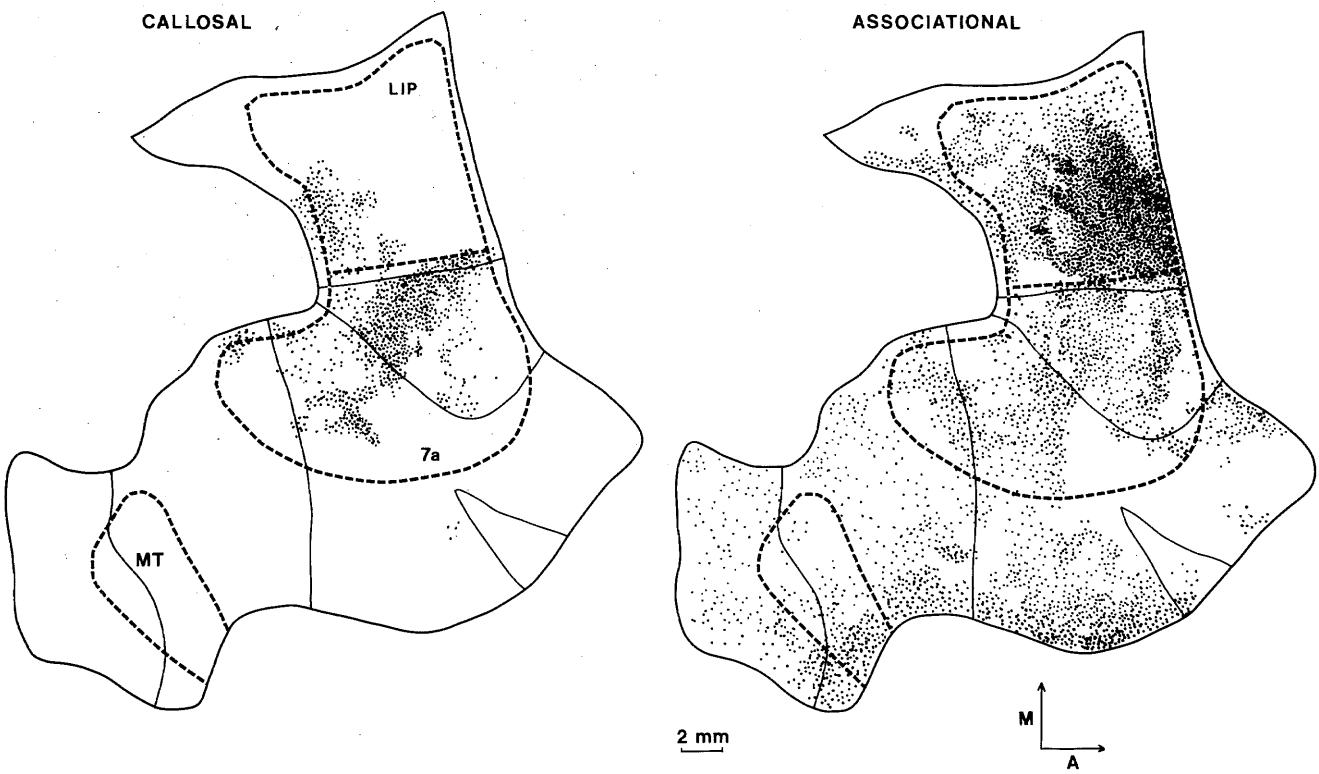

Fig. 5. The spatial distribution of callosally projecting posterior parietal cells (left) and prefrontal associationally projecting neurons (right) in the caudal inferior parietal lobule, reconstructed from the same sections. Case 83A-5.

cortex, while the open triangles represent groups of neurons labeled by nuclear yellow injections into the prefrontal cortex. Each triangle represents a substantial number of neurons; generally many hundreds of neurons were labeled per section. It is evident that in both the prefrontal cortex and the posterior parietal cortex, the populations of callosally and associationally projecting neurons are largely intermingled.

The two-dimensional reconstructions of the relevant cortical areas afford a direct view of the spatial relationships of the two projecting populations in this case. Figure 5 is one such reconstruction for the posterior parietal cortex. In this experiment a tight cluster of three fast blue injections had been made into the prefrontal cortex. The reconstruction on the left illustrates the distribution of callosally labeled neurons, with each dot representing several labeled neurons. The labeled neurons were rather evenly dispersed in area 7a. The central zone of heaviest labeling was about 4 mm by 3 mm in diameter and corresponds, in its position, to the location of the injections in the mirror-symmetrical region on the contralateral side. In other experiments in which we made single injections of a retrograde tracer (three cases) or of labeled amino acids (nine cases) in different parts of area 7a, we always found that the heaviest labeling on the opposite side was in a region corresponding to the principal focus of the injection. Two or three clusters of heavier labeling were often seen at the margins of the labeled regions; however, these varied from animal to animal, so it is difficult to know if they are to be attributed to some inhomogeneity in the disposition of tracer at the injection site or if they represent anatomically significant variations in the callosal projection.

The reconstruction to the right in Figure 5 illustrates the populations of neurons in the parietal cortex that project to the ipsilateral frontal lobe. These associational projections were always heaviest in LIP.[1] One or two zones of heavy

Fig. 4. Representative coronal sections through the prefrontal cortex (left) and posterior parietal cortex (right) from case 83A-5, to show the extent of overlap of the callosal and associational projections of the two regions. For the prefrontal sections, each hollow triangle represents several nuclear-yellow-labeled neurons that projected to the contralateral prefrontal cortex, and each filled triangle represents several fast-blue-labeled neurons that projected to area 7a of the same side. In the drawings of the posterior parietal sections the filled triangles represent fast-blue-labeled neurons that project callosally to 7a, and the open triangles indicate nuclear-yellow-labeled neurons that project to the ipsilateral prefrontal cortex.

[1]In general, the borders of LIP are defined by a consistent pattern of efferent projections seen when anterograde tracers are placed in this region, and by the extremely dense projection from this area to the frontal lobe. The boundaries of area 7a are determined both by its efferent projections and the heavy intracortical labeling that results from injections of retrograde tracers in the area. In case 83A-5 (Fig. 5) the borders of area 7a were established by the intracortical labeling pattern in the contralateral hemisphere and by an injection of labeled amino acids near the intraparietal sulcus in the ipsilateral field on the lateral convexity of the gyrus that produced a characteristic pattern of efferent projections. LIP in case 83A-5 was determined by the configuration of the frontal lobe projection.

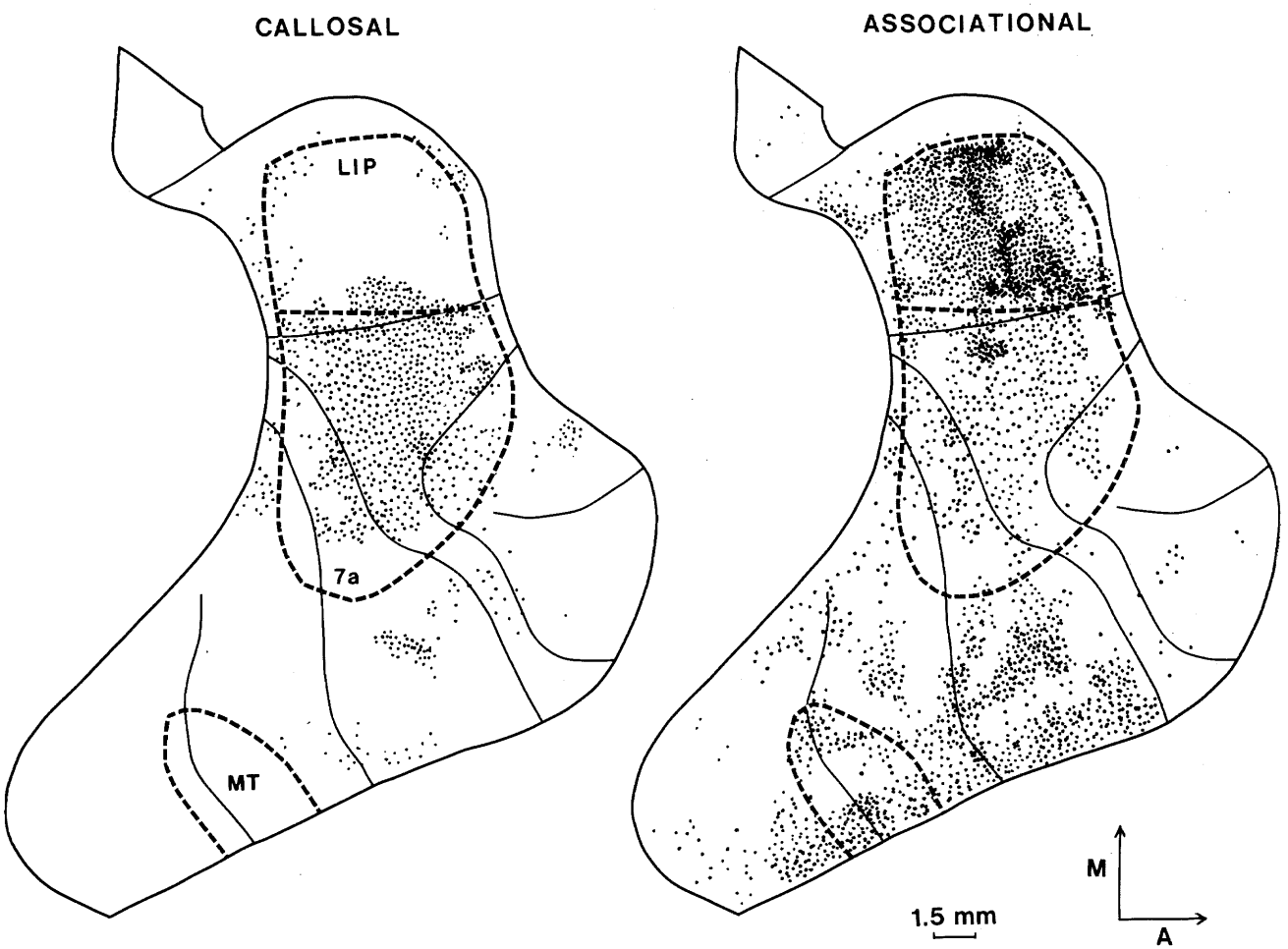

Fig. 6. Another illustration prepared in the same way as Figure 5, to show the spatial distribution of callosal and prefrontal associational projecting neurons in the caudal inferior parietal lobule in case 83A-8.

labeling were always found to extend from LIP into the medial aspect of area 7a that borders LIP; however, even in these small areas of relatively heavy labeling in area 7a, the density of labeled neurons was usually less than half that seen in LIP. Lighter, but quite distinct, labeling was seen throughout area 7a and along the posterior aspect of the superior temporal sulcus, including the middle temporal area. The labeling in the middle temporal area was discontinuous in every brain examined. The cortex along the more rostral part of the superior temporal sulcus was very heavily labeled, and only the posterior tip of the lateral fissure was devoid of labeled neurons.

A comparison of the two reconstructions in Figure 5 (which were made from the same sections) indicates that there is a substantial degree of overlap in the populations of neurons that project to the opposite side or to the frontal lobe. The greatest degree of overlap was found in the medial-anterior part of area 7a; this was true both for the cases with single dye injections in area 7a of the opposite side (as illustrated in Fig. 5) and for those cases with multiple dye injections that spanned a much larger extent of area 7a (as illustrated in Fig. 6). Figures 5 and 6 also show that there are no obvious systematic patternings in the distributions of these two projections, such as interdigitating or overlapping stripes or patches.

Figure 7 shows the same type of spatial analysis for the prefrontal cortex in experiments 83A-5. The reconstruction on the left shows the distribution of the neurons that project to area 7a of the ipsilateral side. The projections to this region were strongest from areas 8a and 46, but there was also a weaker projection from area 45. There were two separate foci of heavy labeling in areas 8a and 46, respectively. There were also two rostrocaudally oriented bands of labeled neurons in area 46. These bands were extensive—up to 14 mm in the case of the band at the base of the principal sulcus. The reconstruction on the right, drawn from the same sections, is of cells that projected through the corpus callosum to areas 8a, 45, and 46 of the opposite side. Again, a comparison of the two reconstructions indicates that the projecting populations overlapped, at least partially, and that there was no obvious systematic patterning of patches or stripes in these two rather complexly

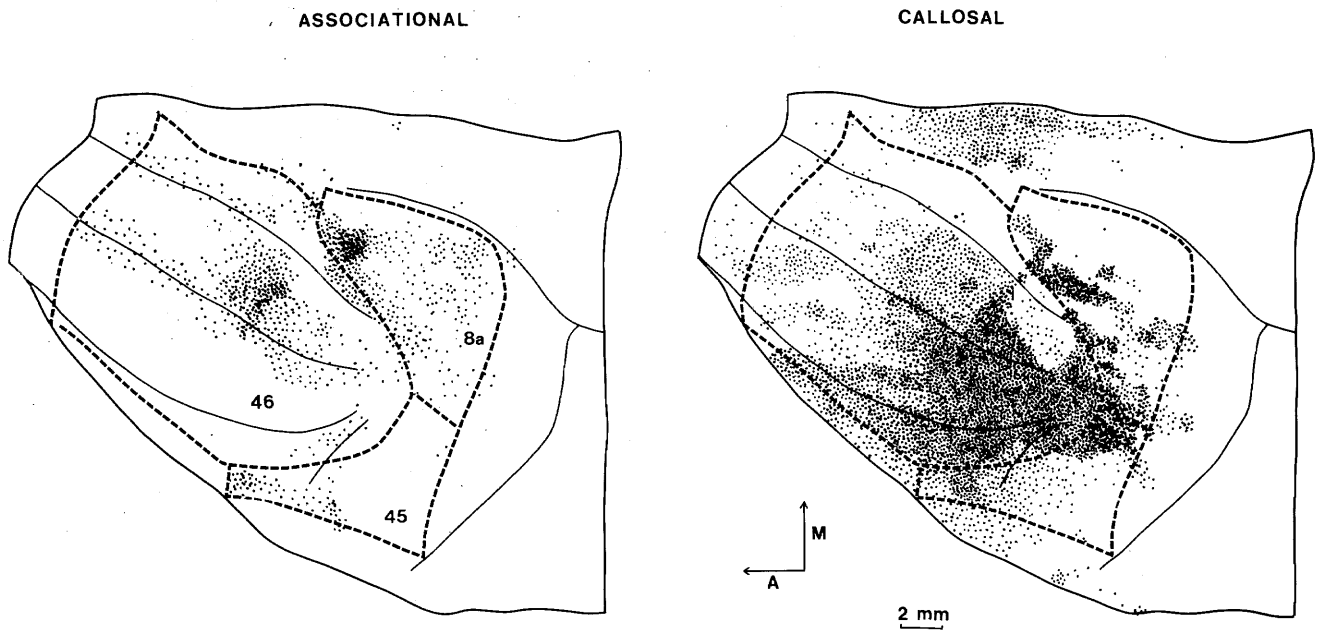

Fig. 7. The spatial distribution of neurons in the dorsolateral prefrontal cortex that project to the posterior parietal cortex (left drawing) and contralateral prefrontal cortex (right drawing) in case 83A-5.

arranged projecting populations. A good deal more cells were callosally labeled than associationally labeled in the frontal lobe. Also, larger numbers of cells were labeled callosally with multiple injections in the frontal lobe than with multiple injections into the inferior parietal lobe.

Collateral projections of single neurons

In general, the retrogradely labeled cell populations in area 7a that projected callosally were quite separate from those that projected to the ipsilateral frontal lobe. Figure 8a is a photomicrograph of fluorescently labeled cells in layer III of area 7a. The arrows indicate cells whose nuclei were labeled after the injection of nuclear yellow into the ipsilateral frontal cortex. The other labeled neurons in the photomicrograph were labeled with fast blue, following the injection into area 7a of the contralateral side. It is clear that the two populations of labeled neurons are intermingled but quite separate. Only occasionally were double-labeled neurons seen in our experiments; however, even at their most numerous, such cells constituted less than 1% of the total population of labeled neurons. The arrow in Figure 8b shows one such double-labeled cell; in the original color photomicrograph the nucleus was bright yellow, while the cytoplasm showed a characteristic blue fluorescence. Near this neuron are two out-of-focus single-labeled cells; to the right is a nuclear-yellow-labeled neuron, and below and to the right there is a fast-blue-labeled cell. The double-labeled neurons were very easy to pick out from the general population of single-labeled cells in our material. Double-labeled cells were only found in layer III and were usually only seen along the medial-anterior aspect of area 7a, where the greatest numbers of single-labeled cells were also found. In eight separate counts from different sections involving all cortical layers only 0.71% of the neurons were found to be double-labeled. In an additional 13 sections in which only the labeled cells in layer III were counted, 1.03% of the labeled neurons were double-labeled.

The survival times used for these experiments were chosen to optimize the labeling in the posterior parietal area. This meant that the survival time was generally too long for the callosal projections of the prefrontal cortex. As a result some of the nuclear yellow dye had diffused out of the nuclei into the cytoplasm of these cells and possibly into neighboring cells. In spite of this problem, very few neurons in areas 8, 45, or 46 were double-labeled in our material.

Laminar distribution

The laminar distributions of the callosal and associational projecting neurons in area 7a were very similar (see Table 1). The listed percentages were determined from counts of the numbers of labeled neurons in a given layer, divided by the counts of the total numbers of neurons in that layer (made from the same sections after counterstaining). Our total cell counts agree well with those of Rockel et al. ('80); they found that there were 114.6 $\pm$ 9.9 cells in a 25 by 30 μm strip of cortex extending from pia to white matter; our estimates for the same cortical volume were 109.97 $\pm$ 7.35. The counts given in Table 1 were made on 30 μm sections, over strips 335 μm wide that extended from the pia to the subcortical white matter. Eight counts were made on different sections through area 7a, and seven counts were made on different sections in the lateral intraparietal area. Counts were made at locations with high concentrations of labeled cells.

In our material, many more cells in area 7a appeared to project to area 7a of the opposite side than to the frontal lobe. However, the laminar distributions of the two project-

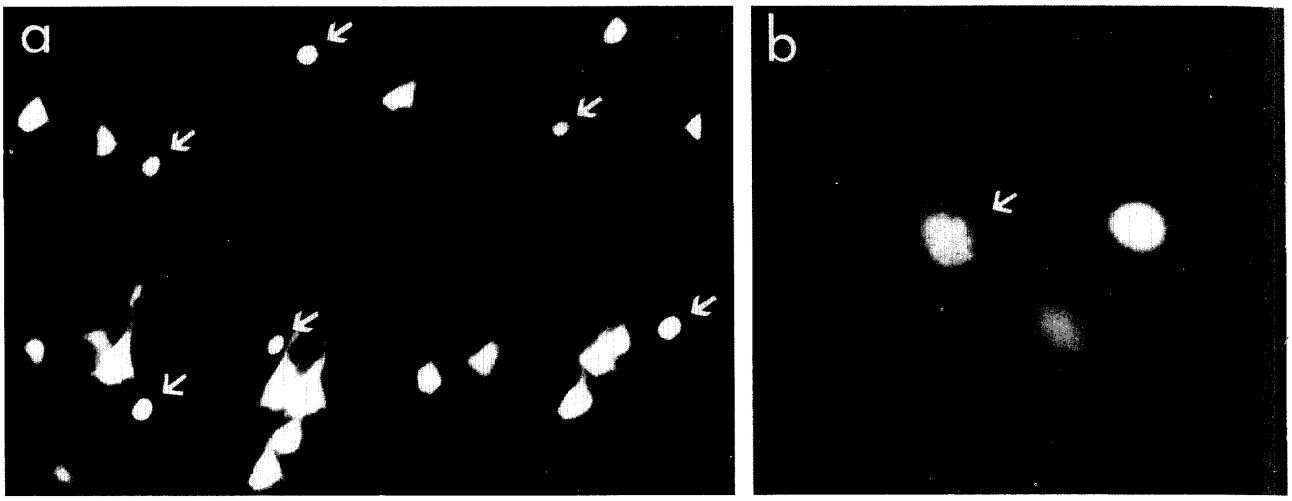

Fig. 8. Fluorescence photomicrographs of labeled neurons in area 7a. a. The arrows indicate the cell nuclei in layer III labeled with nuclear yellow after injections of the dye into the ipsilateral prefrontal cortex. The other labeled profiles are neurons labeled with fast blue that project to area 7a of the contralateral side. Note that the two labeled populations are intermingled. b. The arrow in this figure indicates a single double-labeled neuron. Under the fluorescent microscope the nucleus appeared bright yellow, while the cytoplasm was a brilliant blue. There are also two single-labeled neurons in the photomicrograph: to the lower right is an out-of-focus fast-blue-labeled neuron, and to the right an out-of-focus nuclear-yellow-labeled neuron.

TABLE 1. Percentages of Labeled Neurons, by Cortical Layer, for Area 7a and the Lateral Intraparietal Area (LIP)

	Callosal[1]		Associational[2]	
Layer	%	SE[3]	%	SE
Area 7a				
II	0.83	0.28	0.35	0.17
III	20.01	1.90	6.16	0.35
IV	0.42	0.28	0.00	0.00
V	6.73	1.00	2.61	0.69
VI	2.91	0.42	4.48	1.05
Area LIP				
II	0.00	0.00	0.14	0.13
III	3.50	1.27	29.53	4.83
IV	0.09	0.10	0.19	0.12
V	0.72	0.34	9.40	1.75
VI	0.96	0.44	7.30	1.35

[1]Percentage of labeled neurons per cortical layer following dye injection into the contralateral area 7a.
[2]Percent of labeled neurons per cortical layer following dye injection into the ipsilateral prefrontal cortex.
[3]Standard error of the mean. Each count was made from different sections. Eight counts were made for the area 7a data and seven counts for the LIP data.

ing populations were similar, most cells being located in layer III, followed by layers V and VI; only very few cells were in layer II. Callosal and associational projecting neurons predominated in the lower part of layer III where together they often accounted for as much as 50% of the neurons. Just deep to this zone, an occasional callosally labeled pyramidal cell was seen mixed in with the granule cells of layer IV. More callosally projecting neurons were seen in layer V than in layer VI ($P = .01$), but the opposite was true for the associationally projecting neurons ($P = .20$). In fact, it was only in layer VI that more associational than callosally projecting neurons were seen.

In the lateral intraparietal cortex, we found many more labeled neurons that projected to the frontal cortex; this, of course, was to be expected since the injections into the contralateral hemisphere were made into area 7a and not into LIP. But what is of interest is the finding that there were about four times as many neurons in LIP that projected to the ipsilateral frontal lobe than in area 7a. This observation was consistent in every brain examined.

DISCUSSION

One of the most significant recent advances in our understanding of the functional organization of the cerebral cortex has come from the discovery that the outputs of different cortical regions tend to be anatomically segregated. For example, certain projections are now known to arise from different cortical layers; this laminar segregation is particularly evident for the various subcortical projections but is also evident for a number of cortico-cortical projections (see Jones, '81; Maunsell and Van Essen, '83, for reviews). Other systems of connections, and especially cortico-cortical connections, tend to be segregated into radially oriented, periodic discontinuities within the cortex (see Mountcastle, '78, for review). In monkeys these discontinuities generally range in width (half period) from about 0.4 to 1 mm, and their occurrence in many cortical areas has been cited as additional evidence for a general modular or columnar organization for all neocortex (Mountcastle, '78). However, more recent work has made it clear that there are a number of exceptions to the above, including the findings of this study. We have seen no indication of a laminar segregation of the callosal or associational projections of area 7a. Although there are discontinuities in the two projection arrays, the patterns of variation in labeling are extremely complex. Thus we have seen no evidence for a periodic order to these discontinuities. Hedreen and Yin ('81), who have also examined the distribution of callosally projecting neu-

rons in area 7 using the retrogradely transported marker horseradish peroxidase, have similarly found no evidence for a simple periodicity in the discontinuities in the callosal projection to the inferior parietal lobule of the opposite side. Additionally, we have found the spatial relationships between the patterns of callosal and associational labeling to be extremely complex. By and large the two projection populations were intermixed with no simple phase relationship between the variations in density of the two labeled arrays. These results are not indicative of a periodic unit-structure, since such a structure would require a phase relationship between discontinuities.

These results suggest that not all cortico-cortical connections are organized in a periodic fashion. By themselves, of course, these findings do not imply that some periodic form of connection does not exist in the posterior parietal cortex at some level. It is conceivable that using large injections may have obscured more local periodic patterns. Connections of area 7a with cortical areas other than the ones we examined may have a periodic pattern. It is also worth mentioning the possibility that a modular segregation of connections may be subtle, especially in view of Lane's ('83) quantitative analyses of these connections. Using computer-assisted reconstructions and statistical methods, he has found evidence for a "waxing and waning" in the numbers of callosally and associationally projecting neurons in the inferior parietal lobule. However, since in Lane's experiments large grids of multiple injections of horseradish peroxidase were made, it is difficult to know whether the small fluctuations in the densities of the labeled neurons he reported are real features of the organization of the outputs of the inferior parietal lobule or whether they are simply the result of an uneven distribution of the tracer at the injection site.

In single unit recording studies in behaving monkeys, Mountcastle et al. ('75) reported that in electrode penetrations perpendicular to the cortex of the lateral convexity of the inferior parietal lobe all cells encountered tended to have the same functional characteristics, a finding which is consistent with a columnar organization in this area. In a later study Lynch et al. ('77) reported that these columns were not segregated by functional types into particular parts of the inferior parietal lobule. Taken together these results suggest that the repeating units of each functional class are rather evenly distributed across the inferior parietal lobe. However the data of Lynch et al. ('77) must be regarded with some caution since the results were pooled from several different brains and referenced to sulcal patterns that vary considerably from animal to animal.

Although a good deal more work is required to determine the functional organization of the inferior parietal lobule, our results do not support the notion of these functional columns being evenly distributed across the entire inferior parietal lobule. If such a structure existed, it would be quite likely that the different columns would have different contributions to the callosal and associational projections, especially considering that the functional differences of the columns are quite distinct—some being somatosensory and others visual in nature. Thus periodic discontinuities in the projections and phase relationships between the discontinuities of the two projections would be predicted to be readily apparent. No such structure in the projections was found.

Moreover, some of the discontinuous nature of the labeling appears to conform to the boundaries of cortical fields. Thus LIP projects much more strongly to the frontal lobe than does area 7a, and area 7b gives a weaker prefrontal projection than does area 7a (Andersen et al., '84). Recent functional mapping experiments of the inferior parietal lobule in behaving monkeys (Andersen et al., '84) indicate that at least some of the presently recognized functional classes (Mountcastle et al., '75; Lynch et al., '77; Robinson et al., '78; Andersen and Essick, '84) are in fact segregated into entire cortical fields that are also recognized on the basis of differential connections. Thus, different strengths of the associational and callosal projections may be useful in defining the locations of general functional properties. However, these properties appear to be segregated into cortical fields and not into periodic modules that are distributed throughout the inferior parietal lobule. The organization of functional properties within these cortical fields is yet to be determined and likely will include a columnar organization of certain features. Given the observation that the callosal and prefrontal projections essentially arise from separate neuronal populations, it is probable that these neurons with different projections have different, as yet unknown, functional properties. If this should prove to be the case, and considering that these two populations are extensively intermingled, then at least some aspects of the functional segregation must occur on a much more local level than the framework of 0.4–1 mm diameter cortical columns.

Cortex of the lateral intraparietal sulcus

The cortex of the lateral intraparietal sulcus has been identified as a separate cortical field (area PO*a*) by Seltzer and Pandya ('80), in large part on the basis of the finding that this area alone among the structures in the posterior parietal cortex receives a projection from the prelunate gyrus. Our anterograde axonal tracing experiments confirmed their observations; however, we found that the major part of this projection is restricted to the caudal aspect of the lateral bank of the intraparietal sulcus (Andersen et al., '84). In addition, we found that the prelunate gyrus also sends a robust projection to the anterior bank of the superior temporal sulcus and a lesser projection to the medial aspect of the lateral convexity of the posterior parietal lobe (medial aspect of area 7a). Our material further suggests that the cortex in the caudal aspect of the lateral wall of the intraparietal sulcus can be distinguished on the basis of its other connections. For example, we observed that this area projects to the superior colliculus (whereas area 7a does not), and it also projects much more heavily to the prefrontal cortex than does area 7a. Barbas and Mesulam ('81), using retrograde labeling techniques, found that the cortex along the caudal aspect of the lateral bank of the intraparietal sulcus is the only region within the inferior parietal lobule that projects to the frontal eye fields. Based on the extent of its connections with the prefrontal lobe, with area 7a, and with the prelunate sulcus, we estimate that the surface area of this region is between 80 and 100 mm^2. We are designating this region the lateral intraparietal area (LIP) to distinguish it from the ventral intraparietal area (Maunsell and Van Essen, '83) which abuts upon LIP's ventral border. It is contained within the caudal half of area POa.

Relation to previous studies

Schwartz and Goldman-Rakic ('82), using essentially the same dye-labeling technique as ours, recently examined the relationship of the callosal projection from the lateral intra-

parietal cortex and the ipsilateral projection from the same area to a portion of area 46 on the dorsal bank of the principal sulcus. In general their findings are similar to ours: they found relatively few double-labeled neurons and an extensive intermingling of the two populations of single-labeled cells. Taken together with our findings on a different cortical field in the posterior parietal cortex, it seems reasonable to suggest that the same arrangement of callosal and associational connections probably holds true for all parts of the inferior parietal lobule. In other experiments, using the same double-labeling techniques, we examined the divergent associational projections from the cingulate gyrus and the cortex of the superior temporal sulcus to area 7a and the prefrontal cortex, and also the thalamocortical projections to these various fields from the medial pulvinar (Asanuma et al., '84; Andersen et al., '84). In every instance we found that the projecting populations are at least partially intermingled, but with only an occasional neuron (always under 1% of the total population) projecting to more than one area. The similarity of all these results suggests that (at least on the gross level revealed by our large injections) the extensive overlapping of projection populations is a general rule of connectivity that is applicable to the large reciprocal network of projections that interconnects these areas.

The percentage of double-labeled neurons observed probably represents the lower limit of the number of neurons with collateral projections of this type. The reasons for this are largely technical and have to do with the exact placement of the dye injections, the duration of the survival period, the local concentration of the dyes at and immediately around the injection site, and the general sensitivity of the fluorescent methodology (Swanson, '83). It is thus impossible to make any sweeping statements about the absolute degree of collateralization in this large system of connections. Despite this reservation, we suspect that there are, in fact, relatively few neurons in these areas that have widely divergent projections. Certainly the degree of collateralization is substantially less than in many other neural systems. Indeed, in a series of preliminary experiments on the rat sensorimotor cortex which were carried out to establish the optimum survival times for our dye injections, we found that as many as 20% of the neurons in the caudal part of the somatosensory cortex were double-labeled after injections into the rostral motor cortex and the homotopic sensory area of the contralateral side. And in experiments in monkeys in which two fluorescent dyes were injected at different, but closely spaced, locations in area 7a, we also found a high percentage of double-labeled neurons in the cortical fields that project to area 7a. It seems likely, therefore, that if there are significant numbers of neurons with divergent axons in area 7a, we should have labeled many of them in our experiments. The fact that we consistently failed to find large numbers of double-labeled cells suggests that the true number of cells with divergent projections in the posterior parietal cortex is low.

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