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Areal and Laminar Distribution of Neurons Interconnecting the Central Visual Cortical Areas 17, 18, 19, and MT in Squirrel Monkey *(Saimiri)*

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ABSTRACT The retrogradely transported horseradish peroxidase (HRP) method was used to study the areal and laminar distribution of neurons sending their axons to ipsilateral and contralateral visual cortical areas 17, 18, 19, and MT in the squirrel monkey. Further details regarding neuron type (stellate or pyramidal), size class, and spatial grouping of the cells making these corticocortical connections also were obtained.

All interareal connections are reciprocal. Ipsilaterally, such connections exist between areas 17 and 18, 17 and MT, 18 and 19, 18 and MT, and 19 and MT. In addition, areas 18, 19, and MT receive association fibers from the ipsilateral frontal eye field; when combined with previous findings, these results indicate the existence of reciprocal connections between area 18 and the frontal eye field and between area MT and the frontal eye field. Each of areas 18, 19, and MT is also reciprocally connected with each of the contralateral areas 18, 19, and MT. Area 17 has only weak callosal connections. Both the ipsilateral and the contralateral connections are topographically organized such that they obey a hodological principle of visuotopic connectivity: that is, only representations of the same part of the visual field are interconnected.

With regard to layers of origin, the callosal neurons of these visual areas conform to the general concept of corticocortical fibers arising from supragranular layers in that most of them are located in layer IIIb; only a few of them reside at the junction between layers V and VI. On the other hand, for all the visuocortical connections investigated, the anteriormost area of a reciprocally interconnected pair has its association neurons located predominantly in the infragranular layers while the posteriormost area has its association neurons located primarily in layer III.

All callosal fibers and most association fibers arise from pyramidal cells. The callosal cells are larger and reside at a deeper level in layer III than neurons with ipsilateral corticocortical connections. However, some of the association cells at the junction of layers V and VI in area 17 which project to area MT are relatively large and may include the solitary cells of Meynert; but medium-sized pyramidal cells also participate in this projection. In area 17, some association neurons in layers IIIb and IIIc which project to area 18, as well as some in layer IIIc which project to area MT, are most likely stellate cells.

Several different patterns of cell groupings were observed for the central representation interconnections. Neither ipsilateral area MT nor any of the contralateral visuocortical areas had multiple groupings of labeled neurons. The ipsilateral projections from area 17 to 18, 17 to MT, and 18 to 19 were arranged

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similarly according to a plan involving separate, multiple loci of origin for cells projecting to a small and isolated subregion of the central representation in the target cortical area; following larger injections, cells throughout the central representation of the projecting cortex were labeled. On the other hand area 18 appeared to have some subregions devoid of cells destined to project to area MT.

It is a generally accepted hypothesis that each of the distinctly designated visual cortical areas in primates subserves a different set of functions. For example, in area 17 the inputs from the two eyes converge in the infragranular and supragranular layers to form a fused binocular image of the visual world (Hubel and Wiesel, '68); area 18 apparently fuses the images of the two visual hemifields and is also important for stereopsis (Hubel and Wiesel, '70; Poggio and Fischer, '77); area MT may play an important role in processing information about movement within the visual fields as its cells are highly direction selective (Zeki, '74a,b; Baker et al., '81); and area V4 may be especially important to color vision since it appears to exhibit a high incidence of color-selective cells (Van Essen and Zeki, '78). Furthermore, it is well known that some of the neurons of one visual cortical area project their axons into other visual cortical areas (for a recent review, see Van Essen, '79). It seems reasonable, then, that at least some of these connections should underlie, or at least influence, the functional differences exhibited by those areas. One very important aspect, therefore, in the ultimate critical assessment of visual system neurobiology is a clear understanding of the connectional relationships among the neurons comprising it. The present report is of a comprehensive study on the extrinsic connections between the various visual areas designated as areas 17, 18, 19, and MT in a single species, the squirrel monkey.

In addition to understanding the areal interconnections of various parts of the visual cortex, another aspect of great importance is the laminar origin of fibers interconnecting visual areas. It is widely held that neocortex in general has two large blocks of output neurons separated from each other by a receiving layer of cells: The supragranular layers are said to give rise to efferent corticocortical connections, while the infragranular layers are said to project to subcortical structures; intervening layer IV, then, is the recipient of efferents from subcortical sites as well as from other cortical areas. Our own studies with silver impregnation of degenerating fibers in the visual areas of primates have contributed support to this general concept. However, a number of recent primate studies have revealed that the efferents from area 18 to area 17 do not terminate in layer IV but spare it entirely (Tigges et al., '73, '77; Kaas and Lin, '77; Spatz, '77b; Wong-Riley, '78; Rockland and Pandya, '79). A similar termination pattern has been reported for the efferents from area MT to area 17 (Spatz, '77a). Thus, the functional concept of layer IV being a universal recipient of any given afferent fiber system appears to be shattered. These striking exceptions to the general concept of cortical laminar termination patterns give rise to the question of whether these fiber systems also would contradict the generally held concepts regarding laminar origin of cortical efferents. The initial experiments in this series employed the highly sensitive method for detection of retrogradely transported horseradish peroxidase (Mesulam, '78) in order to identify the laminar origin of area 18 fibers to area 17. The results confirmed the above suspicions and encouraged an extension of this type of inquiry in a carefully planned, comprehensive study on the efferents of other visual areas in a single species. Special care was taken to inject the homologous visuotopic area of each visual cortical area studied. In addition, it was possible to take advantage of the sensitivity of the Mesulam method, which not only shows neural somata but also labels the proximal portions of their processes, to determine which type of neuron (pyramidal or stellate) makes a specific connection.

Since corticocortical axon terminals often have been reported to aggregate in vertical groupings, including those in the visual cortices of squirrel monkey (Tigges et al., '77; Wong-Riley, '78, '79), the intriguing question arises as to whether their parent somata likewise would be arranged in clusters. Although the results pertaining to this question were not uniform, several suggestions could be made about the possible arrangement of cells projecting from the various visual cortical areas.

As this study was in progress, some of the pertinent issues were addressed in part in squirrel monkey, marmoset, and macaque (Spatz, '77b; Rockland and Pandya, '79; Wong-

Riley, '79). The present report, however, deals with these issues in a more comprehensive way and thus extends those previous studies. Some preliminary results were presented at a meeting in Braunlage, W. Germany (M. Tigges et al., '80).

MATERIALS AND METHODS

Twenty-one healthy adult squirrel monkeys *(Saimiri)* were used to study the origin of association and callosal fibers to visual areas 17, 18, 19, and MT. Because of the known visuotopic organization within those visual cortical areas, it was decided to concentrate attention on injections made in the central visual field portion of each area studied (hereafter called the "central representation"). In squirrel monkey, the central representation in area 17 is located at the posterior aspect of the occipital lobe. Its delineation is based on both physiological mapping (Cowey, '64) and on experimental-anatomical studies (Tigges et al., '77). The central representation in area 18 has been delineated by both physiological and anatomical studies and is located immediately anterior to area 17. It derives its definition through its input from and association with the central representation in area 17 (Cowey, '64; Spatz et al., '70; Spatz and Tigges, '72; Martinez-Millan and Holländer, '75; Wong-Riley, '79). In the present studies, area 18 is accepted to be a strip parallel to the striate cortex no wider than 5 mm (Wong-Riley, '79) to 6 mm (Cowey, '64; Spatz et al., '70; Spatz and Tigges, '72). The central representation in area 19 is defined by its fiber afferents from the central representation in area 18 and is located immediately adjacent to the anterior border of area 18 (Tigges et al., '74). The central representation in area MT is located in the posterior bank of the dorsal termination of the superior temporal sulcus and is defined by its afferent connections from the central representation in areas 17 and 18. No physiological mapping has been done yet to confirm the anatomical definitions for central representation in areas 19 and MT. The paracentral representation in area MT, on the other hand, is located in the anterior bank of the dorsal termination of the superior temporal sulcus (Spatz et al., '70; Tigges et al., '74; Martinez-Millan and Holländer, '75; Wong-Riley, '79). In the present experiments, all injections (except in brain 17) were made within the central representations of the visual field in areas 17, 18, 19, and MT (Fig. 1). In brain 17, the injection was made in the paracentral representation in area MT.

Sigma (Type VI) horseradish peroxidase (HRP) was administered to a deeply anesthetized (nembutal) squirrel monkey whose head was secured in a stereotaxic instrument. HRP solution of various concentrations was injected either by pressure or by iontophoresis. For pressure injection, HRP was dissolved in distilled water with 1.6 mg hyaluronidase (Hinrichsen, '75). In one instance (brain 19), however, the HRP was dissolved in a 0.9% NaCl solution with 10% lysolecithin (Frank et al., '80). These solutions were dispensed through either a calibrated, disposable 5-μl micropipette (Spagnolia and Hanaway, '79) or a Hamilton 10-μl syringe fitted with a glass micropipette (30–50-μm inner tip diameter). The plunger was driven by a stepping hydraulic microdrive (David Kopf Instruments) programmed so that a single pressure injection lasted from 15 to 30 minutes. For iontophoretic injection, HRP was dissolved in Tris buffer at pH 8.6 (Graybiel and Devor, '74) and ejected from a glass pipette (9–27-μm tip diameter) with 2 μA DC pulses from a constant current source (5 Hz, 50% duty cycle). At each injection site, current was passed for 25 minutes, followed by a 5-minute period during which no current was applied.

An injection was made in each track with the pipette either at one site at a single depth below the pial surface or at two or three different sites along the track. The details for each brain are indicated in Table 1. Typically, each animal received a single injection in only one visual area. In three brains, however, five injection tracks were evenly spaced in a dorsoventral sequential order in an attempt to expose maximally area 19 to the tracer material. Finally, 15 separate injections were made in each of two brains (brains 20 and 21) to "saturate" a strip of cortex extending from area 17 to the posterior portion of area MT.

After a 3-day survival time, each squirrel monkey was reanesthetized, heparinized (2,-500 units), and perfused with 300 ml of phosphate buffer followed by 1,200 ml of 0.5% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH 7.4). The perfusion was continued with 200 ml of chilled 10% sucrose in phosphate buffer (Rosene and Mesulam, '78). The brain was immediately blocked in a stereotaxic instrument and stored in the refrigerator in 25% sucrose in phosphate buffer for 1 to 3 days. The brains were sectioned serially at 60 μm in a cryostat except for brain 19 which was cut by a vibratome at 100 μm. The occipital lobes were cut in the horizontal

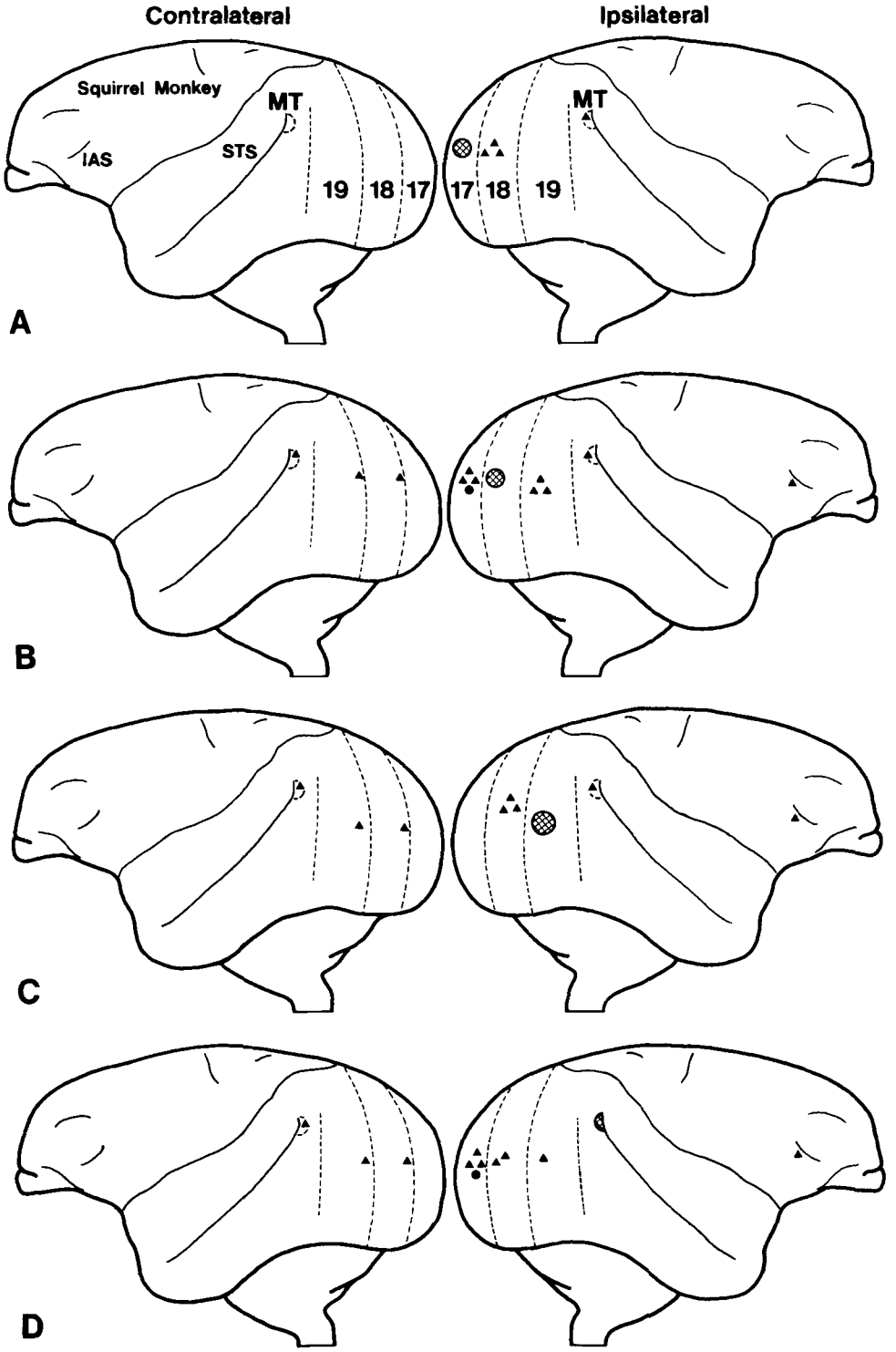


Fig. 1. HRP injection sites (crosshatched regions) in the central visual field representation of area 17 (A), area 18 (B), area 19 (C), and area MT (D), and the areal distribution of labeled cells following such injections. Labeled pyramidal cells are indicated by solid triangles while stellate cells are indicated by solid circles. The number of triangles and circles grossly approximates the relative numbers of labeled neurons. IAS, inferior arcuate sulcus; STS, superior temporal sulcus.

TABLE 1. Details of HRP injections

Brain	Area	Mode of injection[1]	Depth of injection sites (mm)	No. of injection tracks	Conc. of HRP (%)	Total inj. vol. (μl)
1	17	p	1.0	1	50	0.3
2	17	p	1.0	1	50	0.2
3	17	p	1.5	1	50	0.05
4	17	i	0.3	1	30	—
5	17	i	0.5; 1.0; 1.5	1	30	—
6	17	i	0.5; 1.0; 1.5	1	25	—
7	18	p	0.8	1	50	0.05
8	18	i	0.5; 1.0	1	25	—
9	18	i	0.5; 1.0; 1.5	1	30	—
10	18	i	0.5; 1.0; 1.5	1	30	—
11	19	i	1.0	1	25	—
12	19	i	1.0	1	25	—
13	19	i	1.0	5	25	—
14	19	i	1.0	5	25	—
15	19	i	1.0	5	25	—
16	MT	i	1.0	1	30	—
17	MT	i	1.0	1	30	—
18	MT	i	1.0	1	30	—
19	MT	p	1.0	1	30	0.9
20	occ. lobe	p	1.0	15	50	4.5
21	occ. lobe	p	1.0	15	50	4.5

[1] i, iontophoresis; p, pressure injection.

plane, and the frontal lobes were cut in the transverse plane.

Routinely, four of every five sections were processed with tetramethylbenzidine (TMB) for the blue HRP reaction (Mesulam, '78). One of the four reacted sections was counterstained either with thionin (Adams, '80) or safranin to determine the laminar position of HRP-positive neural somata. The fifth section (unreacted) was mounted on a slide and stained with cresyl violet acetate for cell bodies. It should be mentioned that, although the TMB method was very sensitive, the TMB reaction product in the labeled neurons tended to fade with time. Consequently, the distribution and characteristics of labeled neurons had to be recorded soon after processing.

For comparison of labeled neuron morphology in layers V and VI of area 17 following HRP injections in area MT, Golgi material of 1- to 18-day-old infant squirrel monkeys was examined.

All cell measurements are presented as height × width. The height of a cell was measured along a line perpendicular to the pial surface and cell width was measured parallel to the pia. The measurements were not corrected for shrinkage.

RESULTS

The results have been organized according to the cortical areas in which the labeled neurons were found. After injections into the central visual representation in areas 17, 18, 19, or MT, all labeled neurons were located in the central visual representation of the respective areas studied (summarized in Fig. 1). The injection in brain 17 was not in the central representation and results from that experiment are described in the relevant sections below.

As in previous studies from this laboratory the layering scheme for area 17 that subdivides layer III instead of layer IV (Hassler and Wagner, '65; Spatz et al., '70) has been followed. For area 18, the scheme of Valverde ('78) has been adopted.

Labeled neurons in ipsilateral area 17

After area 18 injections. The labeled neurons had accumulated most densely in a broad band in layer IIIa (Figs. 2, 3). Some more loosely packed cells occupied the lower reaches of layer IIIb and the upper reaches of layer IIIc. Only occasionally was there a single labeled cell in the border region between layers V and VI. The vast majority exhibited characteristics typical of pyramidal cells (i.e., the soma had a more or less triangular shape and appeared to have one apical dendrite and a number of basal dendrites). Some neurons in layer IIIb and IIIc, however, failed to have a typical apical dendrite; instead, their dendrites appeared to radiate in all directions (Fig. 4A–C). These dendritic characteristics are usually associ-

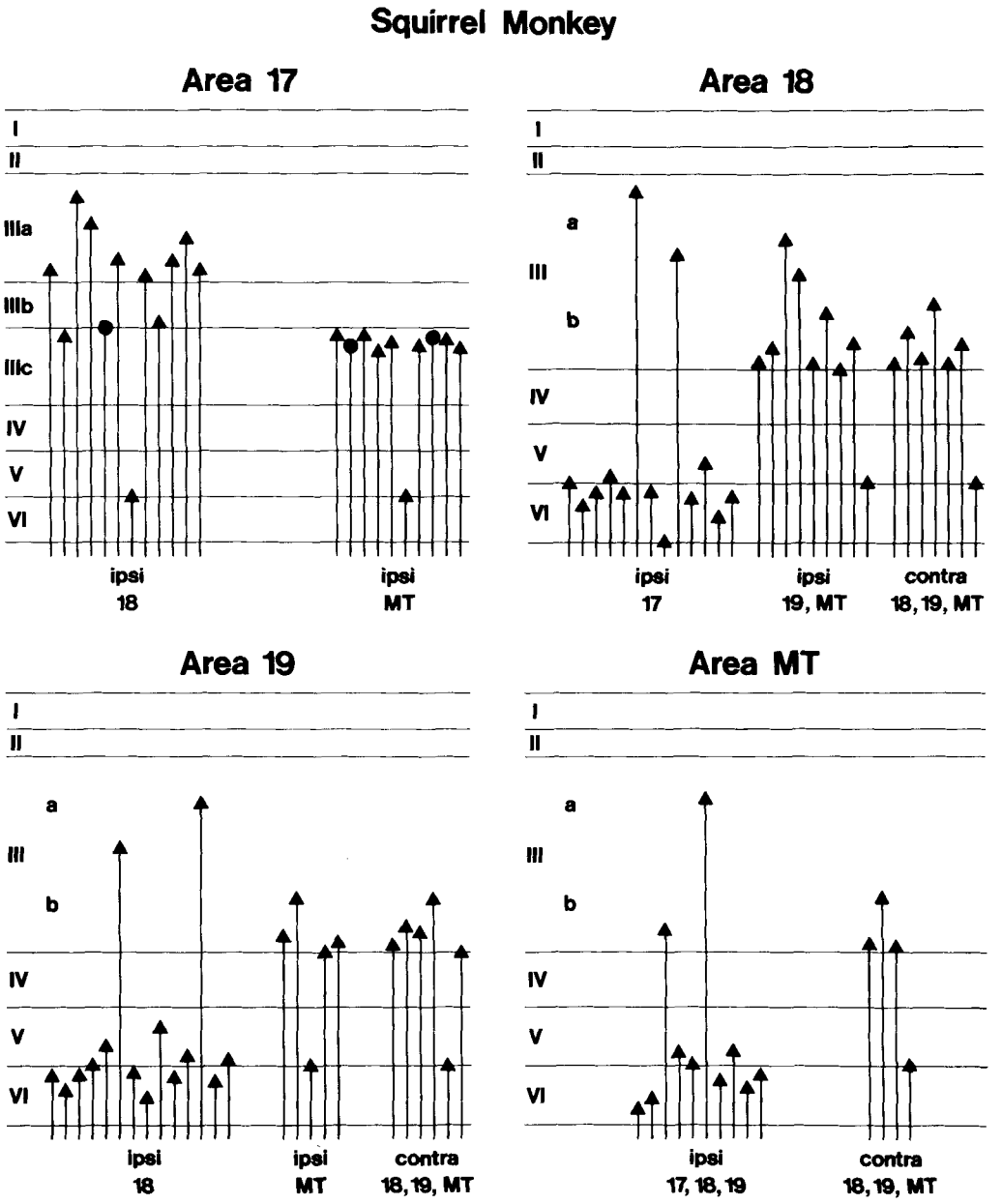


Fig. 2. Summary diagram showing the laminar distribution of labeled neurons in areas 17, 18, 19, and MT projecting to their ipsilateral and contralateral target areas. Triangles indicate pyramidal cells and circles indicate stellate cells. Relative numbers of neurons are approximated by the number of symbols. Relative sizes of neurons are not indicated.

ated with stellate cells; therefore, those neurons have been assessed as such and are indicated to be present among the projection cell population in Figures 1 and 2. The size of labeled cells in layer IIIa ranged from 8.7 × 6.2 μm to 16.2 × 11.2 μm; the average size was 11.5 × 8.5 (n = 100 cells). Because of the small number of labeled cells in the infragranular layers, no measurements were recorded.

After small HRP injections in area 18, the labeled neurons were arranged in groups and the cells in their respective layers of residence

were in vertical register. The center-to-center distance between such vertical groupings varied from 350 to 550 μm. These groupings could be followed only through a few consecutive sections after which the gaps between them either gradually filled in with labeled neurons or two adjacent groupings coalesced. After a large HRP injection, no groupings were found; instead, the labeled cells formed a long, continuous horizontal band in the section.

After area 19 injections. No labeled cells were found in ipsilateral area 17.

After area MT injections. Area MT was the only area where injections were made in two different visual field representations. Figure 1 indicates that when an HRP injection was made in the posterior bank of the superior temporal sulcus (MT's central representation), the accumulation of labeled cells in area 17 was on the posterolateral side of the ipsilateral hemisphere (area 17's central representation). However, after an injection in the anterior bank of the superior temporal sulcus of brain 17 (MT's region of the paracentral visual field representation), the labeled cells were seen in the calcarine fissure (not illustrated) which is where area 17's paracentral visual field is represented.

The overwhelming majority of labeled cells were restricted to the superficial portion of lamina IIIc in area 17 (Figs. 2, 8). Photographs of reacted wet sections revealed the position of myelinated fibers (for references regarding this method see Tigges and Shanta, '69), and Figure 7 clearly shows that these labeled cells are located in the most superficial aspect of the line of Gennari. A few labeled neurons were also found at the junction between layers V and VI (Figs. 8, 9A,B).

Many of the labeled neurons in layer IIIc exhibited characteristics typical of pyramidal cells. However, some pyramidal profiles were distorted in that the apical dendrite grew initially laterally before turning toward the pia (Fig. 13). Other neurons clearly lacked an apical dendrite (Fig. 13) and the proximal dendritic segments radiated in all directions, indicating these neurons most likely to be stellate cells. The average size of the labeled somata in layer IIIc was 12.2 × 12.1 μm (n = 100 cells), with a range from 10.0 × 8.8 μm to 17.5 × 12.5 μm. The large cells were rather rare.

The labeled neurons at the junction of layers V and VI (Fig. 9) ranged in size from 13.8 × 12.5 μm to 25 × 30 μm or 31.3 × 23.8 μm, averaging 20.3 × 19.1 μm (n = 50 cells). The largest cells, as indicated by the two measurements given, varied considerably in geometrical configuration: The axis perpendicular to the pial surface of the brain can be either significantly smaller or significantly larger than the widest diameter parallel to the pial surface. Some of them displayed fleshy apical and basal dendrites (Fig. 10); in other cases, the dendrites were rather thin and threadlike (Fig. 11).

After a small HRP injection, single cells and cell groupings were irregularly spaced in layer IIIc, separated from each other by 50- to 300-μm-wide gaps devoid of labeled cells. After a large injection, they formed an uninterrupted long horizontal band about five cells deep (Figs. 7, 8, 13). Labeled neurons at the junction between layers V and VI were either absent or sparse, and most were only faintly labeled after small injections. They were seen in appreciable numbers and darkly labeled only after a large injection (Fig. 9A).

Labeled neurons in contralateral area 17

In no cases were labeled neurons seen in contralateral area 17 after single injections restricted to individual visual cortical areas. However, after large multiple injections aimed at saturating the central representation of all areas between the occipital pole and the superior temporal sulcus (brains 20 and 21), a few labeled pyramidal cells were found in layers IIIa and IIIb of contralateral area 17 (Fig. 17). It should be noted that these cells were usually within 500 μm of the border between areas 17 and 18, although some were as far away from the border as 1 mm. Because of the large injections in these experiments, the exact termination site at which these pyramidal cell axons were labeled is not known. Therefore, this result was not included in the schematic diagram of Figure 2. The labeled neurons in contralateral area 17 ranged in size from 11.2 × 11.2 μm to 25.0 × 15.0 μm, with an average size of 17.6 × 14.8 μm (n = 50 cells).

Labeled neurons in ipsilateral area 18

After area 17 injections. Most labeled neurons were concentrated in the upper reaches of layer VI (Figs. 2, 5). From there, a gradient of decreasing cell numbers occurred both upward into the lower aspect of lamina V and downward into the lower aspect of lamina VI. A few cells were found in the white matter. A second tier of labeled neurons (but much fewer in number) was seen in layer III, predomi-

nantly in layer IIIa. Since there is no sharp boundary between layers II and III, there is no absolute assurance that none of these labeled neurons might have been in layer II. For example, in Figure 5, a group of neurons (arrow) is shown to be located very close to layer II. An occasional labeled neuron was seen in the uppermost reaches of layer IV. However, they occurred so rarely that it was not felt justifiable to indicate their presence in Figure 2. The labeled neurons in layers V and VI ranged in size from 8.7 × 8.7 μm to 13.7 × 13.7, averaging 10.7 × 10.3 (n = 100 cells). The labeled cells in layer III were generally larger; they ranged from 8.7 × 8.7 μm to 16.2 × 15.0 μm, averaging 12.1 × 11.2 μm (n = 100 cells).

The supragranular neurons most definitely were pyramidal cells. In the infragranular layers, the somata appeared to be pyramidal cells, although some cells were difficult to classify unequivocally. Nevertheless, all the cells observed in these experiments have been designated as pyramidal cells in Figure 2.

After a small iontophoretic injection in area 17, the resultant labeled cells always formed one small group in area 18, in which the cells in their respective layers were in register. After a large pressure injection, the labeled neurons formed a continuous band which stretched approximately 5 mm in the anteroposterior direction.

After area 19 injections. Most labeled neurons were in layer IIIb, although some extended upward into the lower aspect of layer IIIa (Fig. 14). An occasional cell was found in the upper to middle reaches of layer IV and at the junction between layers V and VI. The labeled neurons in layer III ranged in size from 8.7 × 8.7 μm to 13.7 × 12.5 μm, averaging 11.2 × 10.1 μm (n = 100 cells).

Groupings of labeled neurons were observed in several brains but did not seem to conform to any uniform pattern. In one brain, one group of labeled neurons was close to the border between areas 17 and 18, while a second group was located approximately 1 mm anterior to the first group. No labeled cells were found in the space between the two groups. In another brain, up to four groups of heavily labeled cells were detected and were observed to be arranged in an anteroposterior sequence with a center-to-center distance of 300 to 600 μm. The spaces between these groups were filled with weakly labled neurons.

After area MT injections. The labeled cells were located laterally in area 18 where the central visual field is represented. In brain 17, however, where the injection was made in the paracentral visual field representation of area MT (i.e., in the anterior bank of the superior temporal sulcus), the labeled neurons were found in the cortex of the calcarine fissure (which is the presumed paracentral representation of area 18).

The cells were in layer IIIb with a gradient of decreasing numbers toward layer IIIa (Figs. 2, 8). These cells ranged in size from 8.7 × 8.7 μm to 18.7 × 13.7 μm, averaging 13.2 × 11.4 μm (n = 100 cells). There was an occasional labeled cell at the junction between layers V and VI. All labeled neurons displayed characteristics of pyramidal cells.

After a small injection, there was only one small group of labeled neurons in area 18. In contrast, after a large injection in brain 19, the labeled cells were accumulated in two groups with a center-to-center distance of approximately 700 μm (Fig. 8). Unfortunately, the occipital lobe of brain 19 was trimmed for vibratome cutting so there was no cortex left for investigating the region anterior to the two groupings shown in Figure 8.

Labeled neurons in contralateral area 18

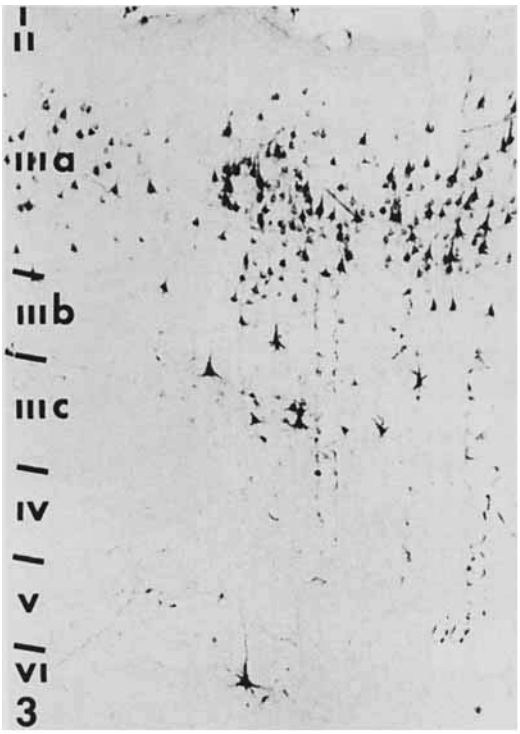
After area 18 injections. The labeled neurons were homotopically located relative to the injection site (Fig. 1). Their accumulation began abruptly at the border between areas 17 and 18 from which fingerlike extensions of labeled cells reached anteriorly for distances of

Fig. 3. Labeled neurons in area 17 following an HRP injection in ipsilateral area 18. Note one conspicuous pyramidal cell at the junction between layers V and VI. × 100.

Fig. 4. Three labeled neurons at the junction between layers IIIb and IIIc in area 17 followling an HRP injection in area 18. A, B, and C are through-focus photographs to show that one cell (arrow) displays a typical apical dendrite; thus, this cell is classified as a pyramidal cell. The other two cells lack an apical dendrite; hence, they are classified as stellate cells. × 250.

Fig. 5. Labeled neurons in area 18 following an HRP injection in ipsilateral area 17. Note the large number of labeled neurons in layers V and VI. Some neurons are even situated in the white matter (WM). The arrow points to a group of relatively large pyramidal cells located very superficially in layer IIIa; some other neurons also may be seen deeper in layer III. Counterstained with safranin. bv, vertically running blood vessel. × 100.

Fig. 6. Labeled neurons in area MT in the posterior bank and bottom of the superior temporal sulcus (STS) following an HRP injection in ipsilateral area 17. Most cells are in the infragranular layers, but a few are in layer III (arrowheads). Counterstained with safranin. × 100.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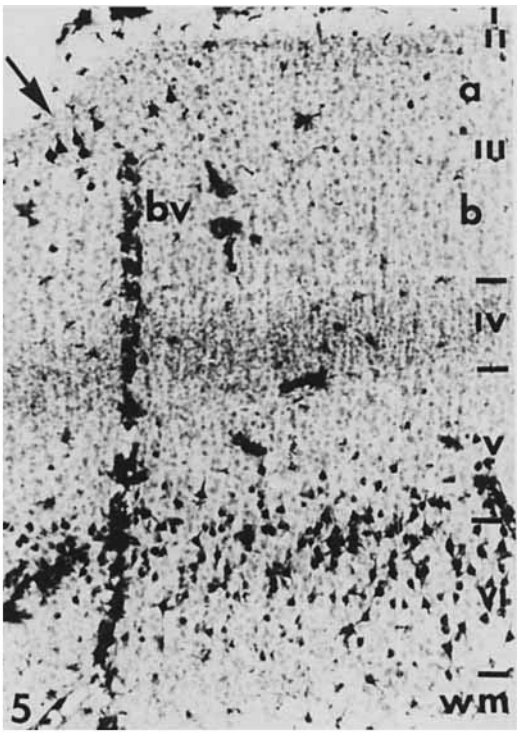
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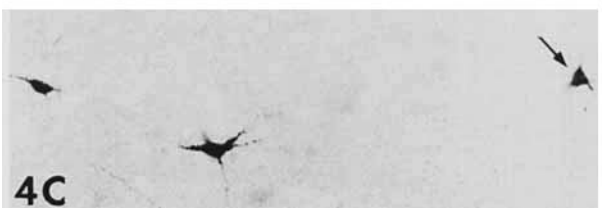
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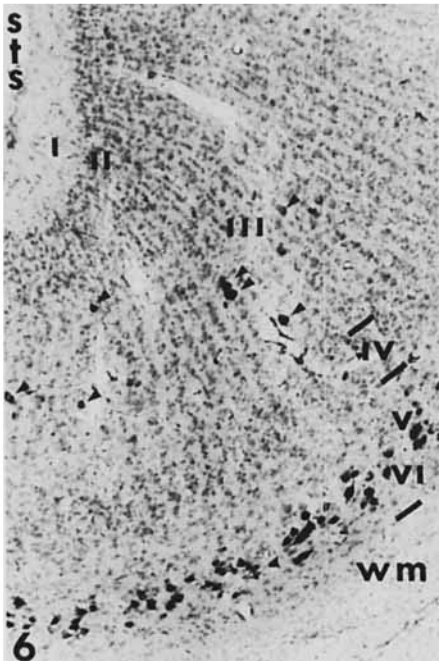
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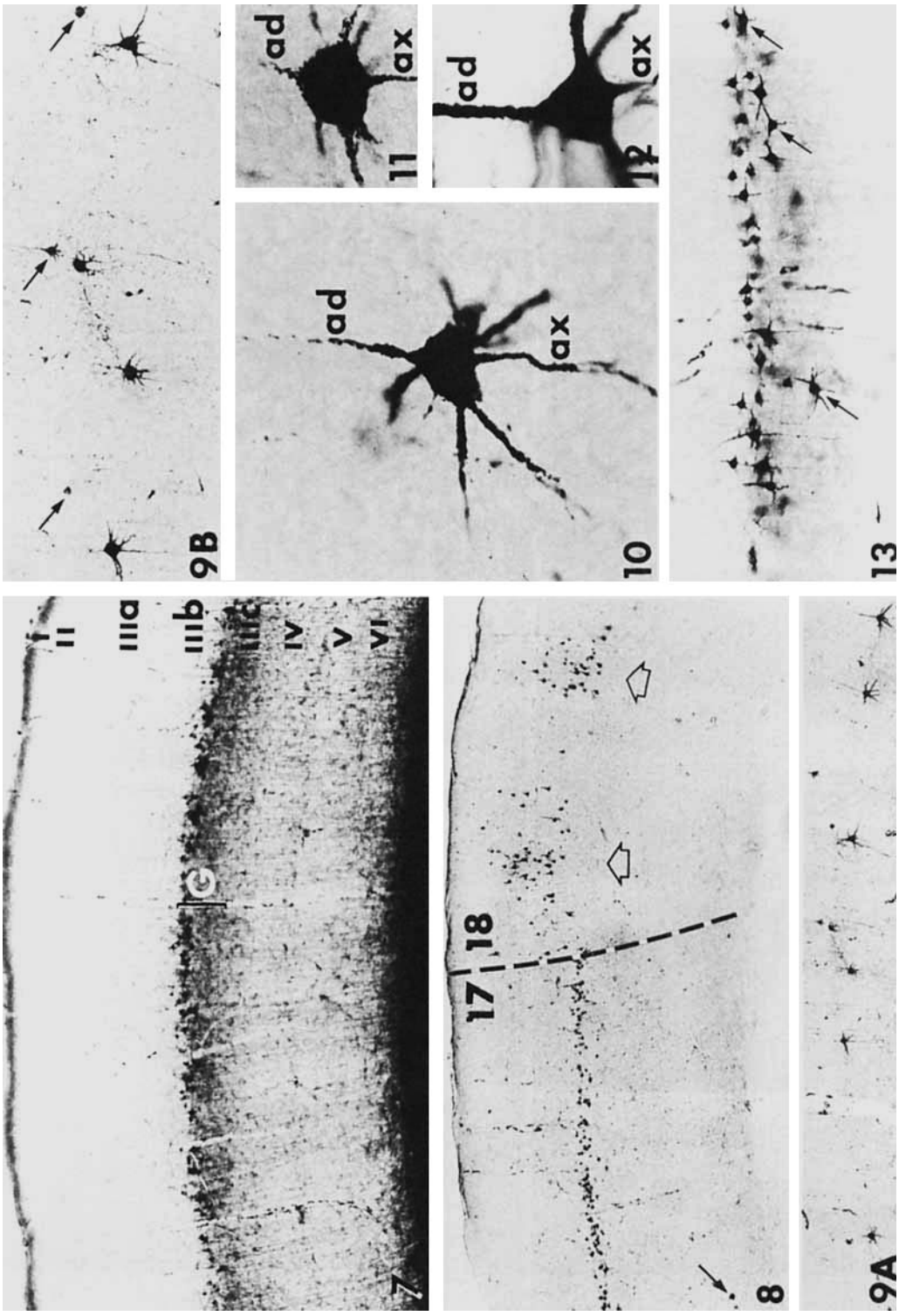
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up to 2 mm. Most cells were located in layer IIIb; a few were found in the superficial tier of layer IV; cells at the junction between layers V and VI were rare. The laminar distribution of cells after a single injection was similar to that after large multiple injections (e.g., in brains 20 and 21), the only difference being that with multiple injections the number of labeled cells was much larger (Fig. 17). All labeled neurons were pyramidal cells.

The small strip of area 18 bordering area 17 is often referred to as OBg because of its characteristic "giant" pyramidal cells in layer III (von Bonin et al., '42). In sections stained for somata, the size of the neurons in OBg ranges from "giant" pyramids of approximately 27 × 18 μm down to small pyramids of only half that size. HRP-filled somata in OBg from the present material ranged from 12.5 × 12.5 μm to 27.5 × 18.7 μm, averaging 19.0 × 13.6 μm (n = 100 cells).

After area 19 injections. These injections resulted in only a few labeled cells, all of which were restricted to lamina IIIb in the contralateral OBg (Figs. 1, 2). Rarely was a cell encountered at the junction between layers V and VI.

After area MT injections. A few labeled neurons were seen in lamina IIIb of the contralateral OBg (Figs. 1, 2).

Labeled neurons in ipsilateral area 19

After area 17 injections. No labeled cells were found in area 19.

After area 18 injections. Most labeled neurons were located near the junction between layers V and VI; fewer cells were found in layer III (Fig. 2). Most of the layer III cells were heavily labeled, whereas the cells in the infragranular layers most often were faintly labeled (Fig. 15). It was also noted that the faintly labeled cells tended to fade soon, which could lead to the impression that there might be only a few cells projecting to area 18. The cells in layer III were typical pyramidal cells while those in the infragranular layers did not always exhibit all features typical of pyramidal cells. The cells in layer III ranged in size from 8.7 × 8.7 μm to 18.7 × 12.5 μm, averaging 12.6 × 11.4 μm, while the labeled cells in layers V and VI ranged from 7.5 × 7.5 μm to 17.5 × 15.0 μm, averaging 10.7 × 10.3 μm (n = 100 cells).

After area MT injections. The labeled cells were located at 6 mm or more anterior to the boundary between areas 17 and 18. Only a few cells were found in layer IIIb. An occasional cell was seen in the upper reaches of layer IV and at the junction between layers V and VI (Fig. 2). The cells in layer III ranged in size from 10.0 × 8.7 μm to 20.0 × 12.5 μm, averaging 13.1 × 10.1 μm (n = 50 cells).

Labeled neurons in contralateral area 19

After area 18 injections. The accumulation of labeled neurons was approximately 5.2 mm or more anterior to the border between areas 17 and 18. Most labeled neurons were found deep in layer IIIb (Fig. 16); some somata, however, occurred regularly in the upper reaches

Fig. 7. Numerous labeled neurons are seen forming an uninterrupted band in the superficial aspect of the line of Gennari (G) of area 17 following a large HRP injection in ipsilateral area MT (brain 19). Photomicrograph of wet section without counterstain. × 40.

Fig. 8. Labeled neurons in areas 17 and 18 following a large HRP injection in ipsilateral area MT (brain 19). In area 17, a large number of neurons is forming an uninterrupted band in layer IIIc and some are at the junction between layers V and VI (arrow). Figure 9 shows further details on some of these deep-lying cells from another section. In area 18, the neurons are arranged in two clusters (open arrows) in layer III. This particular section shows no cells in the infragranular layers of area 18, although they are present in other sections. × 40.

Fig. 9. A. Twelve labeled neurons at the junction between layers V and VI in area 17 following a large HRP injection in ipsilateral area MT (brain 19). Five of these neurons are noticeably smaller than the others. × 40. B. A portion of Figure 9A is shown at higher magnification. Three relatively small neurons are pointed out by arrows. Both the proximal portion of an apical dendrite and the axon are discernible in the cell at the middle arrow. × 100.

Fig. 10. A large, labeled pyramidal cell, probably a solitary Meynert cell, at the junction of layers V and VI in area 17 following a large injection in ipsilateral area MT (brain 19). Among the processes, the axon (ax) and apical dendrite (ad) are clearly discernible. × 400.

Fig. 11. Another large, labeled neuron in the same section as the cell shown in Figure 10. In this case, the soma is well labeled whereas the apical dendrite (ad) and axon (ax) are only weakly labeled. × 400.

Fig. 12. Solitary Meynert cell of an infant squirrel monkey (4 days of age). Compare with Figures 10 and 11. Golgi preparation. × 400.

Fig. 13. Labeled neurons in layer IIIc of area 17 following an HRP injection in ipsilateral area MT. The leftmost arrow points to a cell whose "apical dendrite" emerges from the lateral aspect of the soma; the other two arrows point to cells without apical dendrites. × 100.

of layer IV. An occasional neuron appeared at the junction between layers V and VI (Fig. 2). The labeled cells occurred in one cluster.

After area 19 injections. The labeled cells were found in a strip of cortex approximately 5.0 to 6.5 mm anterior to the border between areas 17 and 18. The laminar distribution and characteristics of cells were similar to those found after an area 18 injection (Fig. 2), but the number of labeled cells was much larger after an area 19 injection. Most prominent in appearance were the generally large and more darkly stained cells; however, it is clear from Figure 16 that a large number of faintly labeled and smaller neurons also was present within the cluster. The labeled neurons ranged in size from 12.5 × 8.7 μm to 25.0 × 15.0 μm, averaging 17.5 × 12.2 μm (n = 100 cells). The callosal neurons in layer III are larger than those with ipsilateral connections, as conveniently can be seen by comparing neurons in Figures 15 and 16 (see also Table 2).

After area MT injections. Location, laminar distribution, and characteristics of labeled cells were similar to those after areas 18 or 19 injections (Fig. 2); however, the number of labeled cells was considerably smaller after area MT injections.

Labeled neurons in ipsilateral area MT

After area 17 injections. The labeled cells appeared in the posterior bank and at the bottom of the superior temporal sulcus (Fig. 1). Most of them were located at the junction between layers V and VI. They ranged in size from 7.5 × 10.0 μm to 18.7 × 12.5 μm, their average size being 12.0 × 11.0 μm (n = 100 cells). A few cells occurred in layer III, particularly in its lower half (Figs. 2, 6). These cells were larger than those labeled in the infragranular layers, ranging in size from 8.7 × 8.7 μm to 19.5 × 13.7 μm with an average of 13.8 × 11.9 μm (n = 100 cells). The laminar distribution was similar to that found in area 18 after an area 17 injection which has been illustrated in Figures 5 and 6. The labeled cells in area MT were always arranged in one continuous band.

After area 18 injections. The location and laminar distribution of labeled neurons was similar to that after an area 17 injection; however, the number of cells labeled after area 18 injections was considerably less (Figs. 1, 2). The cell sizes in layer III ranged from 8.7 × 8.7 μm to 20.0 × 15.0 μm, averaging 14.7 × 12.2 μm. In layers V and VI, the sizes ranged from 8.7 × 8.7 μm to 16.2 × 13.7 μm, averaging 12.1 × 10.3 μm (n = 100 cells).

After area 19 injections. Only a few labeled cells were seen; their location and laminar distribution was similar to that following area 17 injections (Figs. 1, 2).

Labeled neurons in contralateral area MT

After area 18 injections. Only a few faintly labeled cells were seen in the lower portion of layer IIIb (Figs. 1, 2).

After area 19 injections. The results were similar to those after area 18 injections (Figs. 1, 2).

After area MT injections. The labeled cells were located homotopically with respect to the injection site. That is, when the HRP injection was placed in the posterior bank of the superior temporal sulcus, the labeled cells were located in the contralateral posterior bank (Fig. 1); when the cortex in the anterior bank was injected, the cells were found in the contralateral anterior bank (brain 17; not illustrated). The cells were darkly labeled and found deep in layer IIIb. Some cells were seen in the upper reaches of layer IV, and a rare cell was seen

Fig. 14. Labeled neurons in area 18 following an HRP injection in ipsilateral area 19. Most neurons are in layer IIIb. This particular section had no cells in the infragranular layers although they are present in other sections. × 100.

Fig. 15. Labeled neurons in area 19 after an HRP injection in ipsilateral area 18. The largest neurons are in layer IIIb, while smaller neurons appear throughout layer III and in the infragranular layers V and VI. A number of labeled axons are seen. × 100.

Fig. 16. Darkly labeled neurons appear in layer IIIb of area 19 following an injection of HRP in contralateral area 19. Note that the most heavily labeled cells in this figure are, in general, larger than those in Figure 15. Note also the many faintly labeled cells as well. (The lowermost arrow points to faintly labeled neurons in the upper reaches of lamina IV, while the other two arrows point to faintly labeled neurons located more superficially in layer III). × 100.

Fig. 17. Photomicrograph showing the junction between areas 17 and 18 after multiple large injections in the contralateral occipital lobe (brain 20). Two labeled cells (small arrows at the left) are seen in area 17, while labeled cells in the OBg of area 18 are much more numerous. In area 18, most cells are in layer IIIb. Open arrows at the right point to cells in layer IV. Counterstained with safranin. × 100.

Fig. 18. Labeled neurons in layer IIIb and in layer IV (open arrow) of area MT after an HRP injection in contralateral area MT. Counterstained with safranin. × 100.

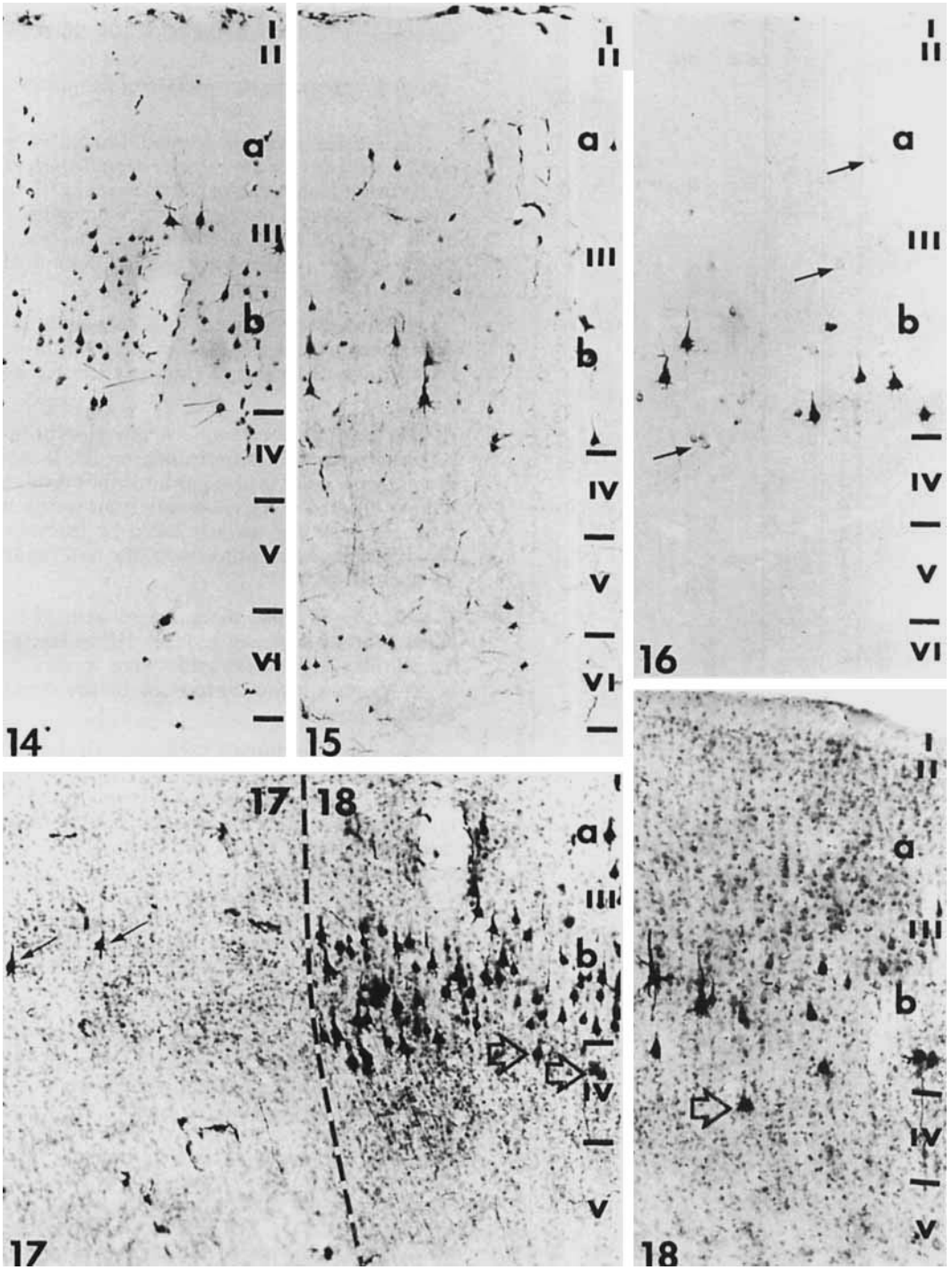
I
II
a
III
b
IV
V
VI
14
15
16
17
18

TABLE 2.

Injection in	Layer	Mean cell sizes (μm) in visual areas and their layers: Ipsi. area 17	Contra. area 17	Ipsi. area 18	Contra. area 18	Ipsi. area 19	Contra. area 19	Ipsi. area MT	Contra. area MT	Ipsi. fr. eye field
area 17	III	—	0	12.1 × 11.2	0	0	0	13.8 × 11.9	0	0
	V, VI	—	0	10.7 × 10.3	0	0	0	12.0 × 11.0	0	0
area 18	III	11.5 × 8.5	0	—	19.0 × 13.6	12.6 × 11.4	...	14.7 × 12.2	...	...
	V, VI	...	0	—	...	10.7 × 10.3	0	12.1 × 10.3	0	0
area 19	III	0	0	11.2 × 10.1	...	—	17.5 × 12.2	...	...	...
	V, VI	0	0	...	0	—	...	...	0	...
area MT	III	12.2 × 12.1	0	13.2 × 11.4	...	13.1 × 10.1	...	—	17.6 × 13.2	...
	V, VI	20.3 × 19.1	0	...	0	...	...	—	...	...
area 17 to area MT	III	—	17.6 × 14.8	—	*	—	*	—	*	16.2 × 13.7
	V, VI	—	0	—	*	—	*	—	*	14.1 × 12.3

– intermediate; 0 no cells were observed; ... too few cells to be measured; * no measurements were made

at the junction between layers V and VI (Figs. 2, 18). The labeled neurons in layer III ranged in size from 12.5 × 8.8 μm to 25.0 × 16.2 μm, averaging 17.6 × 13.2 μm (n = 100 cells).

Labeled neurons in the ipsilateral frontal eye field

This cortical area is located immediately posteroventrally to the inferior arcuate sulcus in the frontal lobe of the squirrel monkey (Sanides, '68). Labeled neurons occurred generally in the ventral bank of this sulcus; however, they were not infrequently seen in the dorsal bank.

After area 18 injections. An occasional labeled pyramidal perikaryon occurred in layer III in the dorsal bank of the inferior arcuate sulcus.

After area 19 injections. A few clearly labeled neurons were seen in lamina III. In addition, some cells in the border zone between lamina V and VI exhibited only faint reaction product which had usually faded by the time the coverslipped sections were dry and ready for photography.

After area MT injections. A number of labeled neurons occurred in layer III; in the infragranular layers, the cells were mostly in layer VI, and a few were seen in the lower aspect of layer V.

After large multiple injections. Brains 20 and 21, which had received large multiple injections, had laminar distribution patterns of labeled cells similar to those seen after single injections in area 18, 19, or MT. Labeled neurons were found in layer III, particularly in its deeper portion, and in layers V and VI. There were slightly more cells in the infragranular layers compared to layer III. The appearance of the labeled neurons in these two tiers was different; cells in layer III were usually larger in size and their apical dendrites were often fleshy, measuring 5 μm or more in diameter. It was often difficult to determine where the soma ended and the apical dendrite began. In comparison, the labeled neurons in the infragranular layers were smaller and often displayed an inconspicuous apical dendrite (Fig. 19). The size of labeled neurons in layer III ranged from 13.7 × 8.7 μm to 28.7 × 18.7 μm, averaging 16.2 × 13.7 μm (n = 100 cells), while those in the infragranular layers ranged from 11.2 × 8.7 μm to 21.2 × 18.7 μm, averaging 14.1 × 12.3 μm (n = 100 cells).

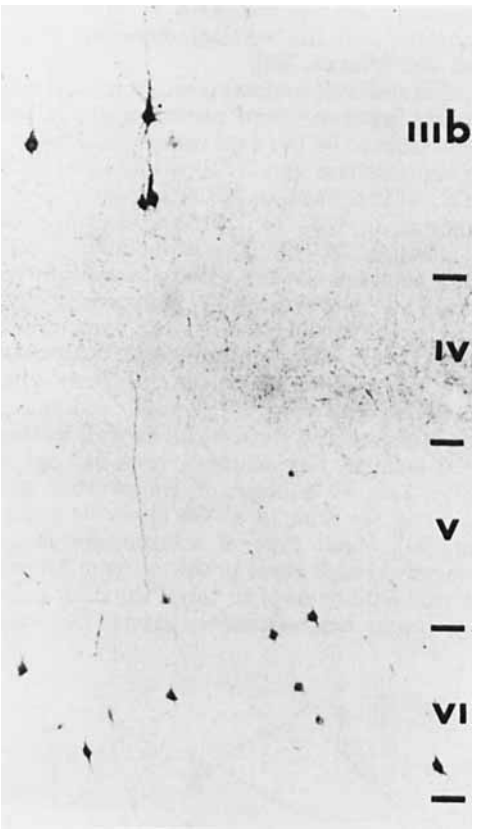

Fig. 19. Labeled neurons in layer IIIb and in layers V and VI of the frontal eye field after large multiple injections in the ipsilateral occipital lobe (brain 20). Note the particularly prominent apical dendrite of the layer IIIb neurons. Note also the large accumulation of anterogradely transported HRP in layer IV. × 100.

DISCUSSION

Areal distribution of labeled cells

All HRP injections (except in brain 17) were made in the central visual field representation in areas 17, 18, 19, and MT. The axons terminating there always arose from neurons located also in the central representations of the respective projecting ipsilateral and contralateral visual areas (Fig. 1). In brain 17, a single HRP injection in the anterior bank of the superior temporal sulcus (the site of the paracentral representation in area MT) resulted in HRP-positive neurons in areas 17 and 18 in the ipsilateral calcarine fissure, and in contralateral area MT in the anterior bank of the superior temporal sulcus (each of which is the respective site of paracentral representation in those areas). This demonstrates that the ipsilateral and contralateral connections of these areas are precise, topographically organized, and, it should be emphasized, between loci that deal with the same portion of the visual field. In fact, most of the anatomical studies on visual areas in primates similarly report a topographical organization of connections (for a recent review, see Van Essen, '79). Thus, the visuocortical areal interconnections seem *always* to obey a hodological principle of visuotopic connectivity in which only representations of the same part of the visual field are connected. This principle may be less clearly expressed in area MT of marmoset and in area STS (homologue of area MT) of macaque as Spatz ('77a) reported some "degree of overlap of the projections of area 17 upon area MT" and Montero ('80) found in macaque that "this partial overlapping follows an orderly, although diffuse, topographical trend." However, to date, in no visuocortical study has such a principle been expressly violated.

The present experiments have revealed in a single species the existence of ipsilateral reciprocal connections between areas 17 and 18, areas 18 and 19, areas 17 and MT, areas 18 and MT, and areas 19 and MT (Fig. 20). This is in agreement with the collective results of previous reports on several monkey species (Tigges et al., '73, '77; Spatz, '77a,b; Rockland and Pandya, '79, '81; Wong-Riley, '78, '79). Further, homotopic callosal connections were revealed between areas 18, between areas 19, and between areas MT; obviously any homotopic interhemispheric connection must be a reciprocal one. The reciprocal callosal connections between areas 18 in squirrel monkey have been described before (Tigges et al., '74; Wong-Riley, '74) and are in agreement with findings in cat (Innocenti, '80). The heterotopic reciprocal callosal connections found in the present study between visuotopically equivalent parts of area 18 and contralateral areas 19 and MT, and between area 19 and contralateral area MT have not been described previously. The callosal reciprocity between heterotopic visual areas may be unique since heterotopic connections were apparently absent in the somatosensory and motor cortices of primates (Jones et al., '79).

The relative number of labeled cells seen in the various visual cortical areas after HRP injections varied from area to area. If the number of labeled cells is a valid indicator of the strength of connections between various areas, then some statements can be made about the

relative strength of the interconnections. The strongest ipsilateral connections in both directions are those between ipsilateral areas 17 and 18, between areas 17 and MT, and between areas 18 and 19; the connections are weak between areas 18 and MT, and are even weaker between areas 19 and MT. The most numerous callosal projections are between homotopic loci in the two hemispheres; among them, the cells interconnecting the two OBg's are most prominent. In comparison, callosal cells interconnecting heterotopic points are relatively few in number. The fewest callosal cells were found in area 17. In fact, they were revealed only in animals 20 and 21, each of which had received multiple injections of HRP (Fig. 17). Certainly one interpretation of the appearance of a small population of labeled cells following large injections of tracer material (especially when small injections failed to reveal them) might be the spurious labeling of fibers of passage or the spread of tracer material by diffusion out of the intended injection site. On the other hand the scarcity of area 17 callosal projections in the present study is in sharp contrast to findings in a prosimian, *Galago senegalensis*, in which area 17 contributes a substantial projection to the contralateral hemisphere (Weyand and Swadlow, '80). Innocenti et al. ('77) have provided evidence for the postnatal decline of an originally widely distributed callosal fiber system in the cat visual system. If a similar situation exists in the squirrel monkey, the small number of callosal cells in area 17 might be the relics of such a projection.

The callosal reciprocal connections between the two OBg's are thought to join the two visual hemifields, since the vertical meridian is represented along the border between areas 17 and 18 (Choudhury et al., '65; Hubel and Wiesel, '67). The reciprocal callosal connections between the posterior portions of areas MT may subserve a similar function, since, according to mapping in the related owl monkey (Allman and Kaas, '71), these portions are very close to the representation of the vertical meridian in area MT. However, neither the callosally interconnected anterior portions of areas MT nor the callosally interconnected regions of areas 19 represent the vertical meridian. Thus, some of the callosal connections must have functions other than joining the visual hemifields, as was pointed out for callosal connections of visual area DM in owl monkey (Wagor et al., '75). This view is supported by findings in the well-mapped visual areas of owl monkey where profuse callosal connections are not restricted to various representations of the vertical meridian (Newsome and Allman, '80).

Ipsilateral and contralateral reciprocal connections between visual cortical areas in primates seem to be the rule rather than the exception (Graham et al., '79; Kaas et al., '77; Rockland and Pandya, '79, '81; Spatz, '77a,b; Tigges et al., '73, '74, '77; Wagor et al., '75; Wong-Riley, '78, '79). Their functional significance remains obscure. There is speculative agreement, however, that reciprocally connected areas modulate or control each other's internal processing and signal output. Because of the complex interrelations, all these areas are potentially controlling and modulating each other's output in a serial as well as nonserial fashion. For example, area MT might control area 17 directly, or, conceivably, also indirectly via area 18 or via areas 19 and 18 (Fig. 20). What type of information is exchanged through these pathways is unknown, but it should be kept in mind that the information may not necessarily always be visual

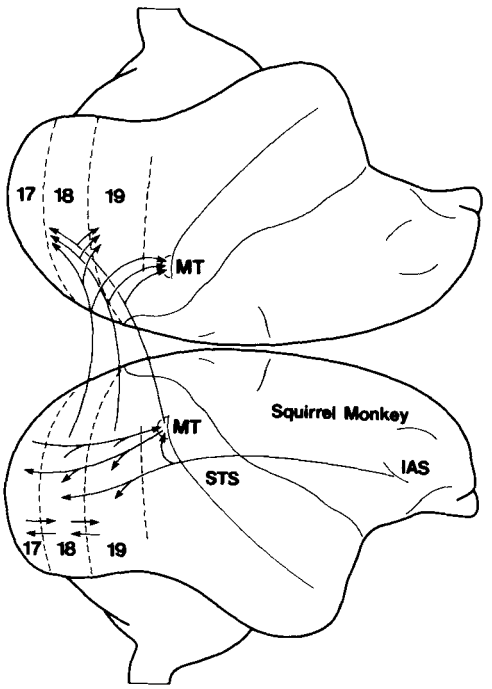

Fig. 20. Summary diagram indicating areal origin and termination of all association and callosal fiber systems uncovered in the present investigation. Most anteriorly a projection arises from the frontal eye field just ventrally to the inferior arcuate sulcus (IAS).

in character. The possibility for influx of nonvisual messages from cortical areas is further complicated by the fact that there are connections to visual cortical areas from definitely nonvisual subcortical structures (J. Tigges et al., '80).

Reciprocities also exist between visual areas and the ipsilateral frontal eye field. The known projection from area 18 to the frontal eye field (Tigges et al., '74) is complemented by the present finding of a connection in the reverse direction. Similarly, the known association from area MT to the frontal eye field in the marmoset (Spatz and Tigges, '72) would appear to be complemented by the reverse connection found in the present study on squirrel monkey.

Laminar distribution of cells

The overwhelming majority of labeled cells in contralateral areas 18, 19, and MT were located deep in layer IIIb. These cells often conveyed the impression that they were "riding" on layer IV. A few cells even dropped into the upper reaches of layer IV. Labeled cells at the junction between layers V and VI were rare. Thus, the callosal fibers, whether connecting homotopic or heterotopic loci in the visual areas, arise almost exclusively from cells deep in layer III, thereby conforming to the general concept that corticocortical fibers arise from supragranular layers. Similar results have been obtained in the sensorimotor cortex (Jones and Wise, '77). These findings in monkeys are in great contrast to those obtained in rabbit (Swadlow, '79) and in cat (Sanides, '79; Keller and Innocenti, '80), in which visual callosal neurons appear to have a much wider laminar distribution. In fact, in the case of rat somatosensory cortex, the origin of callosal fibers interconnecting homotopic loci appears to be from pyramidal cells in all cortical layers (Wise and Jones, '76). The obvious conclusion from a comparison on the available data in primates and nonprimate mammals is that a more restricted laminar distribution of contralaterally projecting corticocortical neurons develops as one ascends the phylogenetic scale.

While the laminar origin of some of the ipsilateral corticocortical connections revealed in squirrel monkey conforms similarly to the general concept (i.e., these fibers arise from supragranular layers), those of some other fiber systems clearly do not. Take for example the reciprocal connection between areas 17 and 18: While fibers to area 18 arise predominantly from layer III and to a minor degree from infragranular layers, the fibers to area 17 arise predominantly from infragranular layers and to a minor degree from layer III. Hence the laminar origin of one fiber system involved in a reciprocity appears to be the inverse of the other. Similar reversals were revealed for the reciprocities between areas 18 and 19, areas 17 and MT, areas 18 and MT, and areas 19 and MT. Without exception, the anteriormost area had its association neurons located predominantly in the infragranular layers, while the posteriormost area of a reciprocally interconnected pair had its association neurons residing primarily in layer III. The results of the present study are clearly in disagreement with those of Spatz ('77a; marmoset) and of Wong-Riley ('79; squirrel monkey), who reported that fibers to area 17 from area MT arise mainly from supragranular neurons. This discrepancy may be the result of the less sensitive diaminobenzidine method employed in their studies in contrast to the much more sensitive TMB method used in the present study. The present findings are similar to those reported by Rockland and Pandya ('79; macaque), who also used the TMB method. Some of our findings are also in agreement with those reported for cat where injections into area 17 and 18 labeled cells in layer VI of the Clare-Bishop area (Gilbert and Kelley, '75).

The present results on visual areas show remarkable similarities with findings obtained in somatosensory and motor cortices of monkeys (Jones and Wise, '77). In all these systems, there is a sublaminar organization of efferents: Contralaterally projecting corticocortical neurons lie deeper in layer III than the ipsilaterally projecting corticocortical cells.

The target layers of reciprocal fiber systems are quite different also. For example, in all monkey species studied, there is general agreement that efferents from area 17 to areas 18 and MT terminate in layer IV and the lower aspect of layer III (Spatz et al., '70; Tigges et al., '74; Martinez-Millan and Holländer, '75; Spatz, '77b; Rockland and Pandya, '79; Ungerleider and Mishkin, '79; Weller and Kaas, '78; Wong-Riley, '79). There is also agreement that the efferents from areas MT and 18 back to area 17 spare layer IV and lower layer III (Kaas and Lin, '77; Spatz, '77a; Tigges et al., '73, '77; Wong-Riley, '78; Rockland and Pandya, '79). When the results obtained with anterograde and retrograde axonal transport methods in squirrel monkey are combined, the following picture emerges. Efferents from area 17 to areas 18 and MT originate mainly from

layer III (although from different subdivisions) and to a smaller extent from the infragranular layers. They terminate predominantly in layer IV and to some degree in lower layer III. The fibers from areas MT and 18 back to area 17 originate mainly from the infragranular layers and terminate in all layers except in layer IV and lower layer III. Since similar relationships have been revealed in marmoset (Spatz, '77a) and macaque (Rockland and Pandya, '79), they can be assumed to be universal in monkeys, and perhaps even in all primate species. Thus, one fiber system of a reciprocally interconnected pair conforms to the general concept that corticocortical fibers terminate predominantly in layer IV with a decreasing gradient in layer III, while the other fiber system spares these termination sites in its target area.

The striking restriction of labeled neurons to layer IIIc in area 17 following an HRP injection in area MT is unique and has no parallel in any other visual area investigated. Dow ('74) found a directionally selective class of neurons concentrated in the myelinated line of Gennari where the labeled cells in the present study were located (also, Spatz, '77a). Other studies show that area MT neurons are also strongly directionally selective, more so than those in other visual areas (Baker et al., '81; Zeki, '74a,b, '80). This clearly raises the possibility that the characteristics of an entire area might be defined by the input from a restricted population of cells with special physiological properties located in another area. The Clare-Bishop area in cat (which is often thought to be the homologue of area MT of primates), however, receives input from neurons throughout the supragranular layers of area 17 (Gilbert and Kelly, '75). This discrepancy between cat and monkey might be interpreted as evidence against the existence of a homology between the Clare-Bishop area and area MT.

In areas where corticocortical fibers arise mostly from supragranular layers, some fibers usually originate also from cells lying at the junction between layers V and VI. What might be the significance of such a multilayered source for these projections? It is conceivable that these infragranular neurons simply failed during embryonic development to migrate from their places of origin near the ventricular surface to their final positions in supragranular layers (Rakic, '76); if so, they might be considered "displaced" supragranular layer neurons. However, after an injection into area MT, some of the labeled neurons in the infragranular layers of area 17 were among the largest cells observed in that area; furthermore, the largest of these cells were clearly larger than any neuron found in the supragranular layers in the present material. If this size difference is a valid basis for discriminating separate neuronal classes, then it may reasonably be concluded that those few cells in infragranular layers of area 17 genuinely "belong" there and are not just spatially displaced members of a single class of neuron which normally reside in the supragranular layers. Another possibility is that neurons in both the infragranular and the supragranular layers contribute heavily to corticocortical connections during early phases of life, but that later in postnatal development, the infragranular or supragranular layers would experience a severe reduction in the number of neurons contributing to a corticocortical connection. This speculation is not without foundation, for Innocenti and Frost ('80) have uncovered in cat a gradual postnatal reduction of a more widespread juvenile population of callosal neurons to the final adult distribution. Finally, it is entirely possible that two functionally different projections to the same area exist and that one of their differentiating characteristics is their position at different layers within the same cortical area. There would seem to be a need for more studies involving the physiological characterization of neurons in order to resolve these matters.

In summary, the present study reports three exceptions in visual areas (in addition to that in the frontal eye field) to the concept that corticocortical connections arise exclusively from supragranular layers. Whether this is a widespread phenomenon or is restricted to visual cortex and its associated areas can only be determined with more experimentation. However, on the basis of the present study, it appears possible that a more widespread origin of corticocortical fibers may obtain and that the residence of their cells of origin in *supragranular* or *infragranular* layers may depend on their participation in "feed forward" or "feed backward" information processing, respectively. In the light of this, one wonders whether the current concept of corticosubcortical projections arising strictly from infragranular layer is in for a revision, too. So far, though it seems that this concept still holds, without exception, all cells projecting to subcortical structures both from visual areas (Lund et al., '75; Glickstein et al., '80) and from sensorimotor areas (Jones and Wise, '77; Jones et al., '77; for

corticospinal references, see Tigges et al., '79) have been found to reside in infragranular layers.

Types and sizes of labeled neurons

All callosal connections clearly were made by pyramidal cells; this was especially apparent in brains 20 and 21, which had received 15 injections each and thus exhibited an abundance of well-labeled contralateral cells. Even the few labeled neurons in the upper reaches of layer IV were pyramidal cells. This is in contrast to findings in cat where many visual callosal fibers originate from layer IV stellate cells (Sanides, '79; Garey and Hornung, '80; Innocenti, '80).

In ipsilateral areas, the labeled supragranular cells were probably all pyramidal neurons, except for some of the area 17 efferents in layers IIIb and IIIc to area 18 and some of the layer IIIc efferents to area MT. Since these latter cells lacked a definitive apical dendrite (the most widely accepted characteristic of pyramidal cells) it was concluded that some efferent association fibers of area 17 originate from stellate cells. Indeed, Polyak ('57) and Lund ('73) reported the presence of stellate cells in their lamina 4B in macaque. Preliminary results of an electron microscopic study show that some of the labeled cells in layer IIIc exhibit ultrastructural characteristics of stellate cells (Tigges et al., '81). Therefore, in primates, as well as in cats, stellate cells no longer can be regarded as merely local interneurons.

The overwhelming majority of HRP-positive neurons in the infragranular layers of ipsilateral areas 17, 18, and 19 were pyramidal cells. Some of the remainder could have been stellate neurons. It was especially difficult to classify the labeled neurons in the infragranular layers of ipsilateral area MT because the superior temporal sulcus had distorted the shapes of the somata. Because of this uncertainty, a conservative view was adopted in compiling Figure 2 so that only pyramidal cells are indicated to project from the infragranular layers.

Spatz ('75) reported that the cells in layers V and VI of area 17 which send axons to area MT belong to one distinct population of neurons. These, he concluded, were identical with the solitary cells of Meynert. In agreement with Spatz, Lund et al. ('75) found that only the largest pyramidal neurons of layer 6 in area 17 were labeled after area STS (MT) injections. The present study would suggest that both of the above reports are incomplete statements of the full projection. In agreement with the above reports, large neurons in layers V and VI were labeled. In fact, there is great probability that some of these cells are Meynert cells since the largest of the HRP-labeled cells match the size of the largest cells (29 × 31 μm) seen in Golgi material of infant squirrel monkeys (Tigges and Tigges, unpublished data). However, the solitary cell of Meynert occurs infrequently, and yet the number of large labeled cells seen in a single section of the present material (Fig. 9A,B) is fairly great. Even though the classification of large cells is not without hazards (Chan-Palay et al., '74), analysis of our Golgi material indicated that there are a number of the large pyramidal cells in layers V and VI which could be classified into different categories. Likewise, the large HRP-labeled cells of the present material comprised a heterogeneous group. For example, the cell shown in Figure 10 displays fleshy apical and basal dendrites (the characteristics of solitary Meynert cells) while the cell shown in Figure 11 is of a similar size but exhibits only threadlike processes. Thus, the Meynert cells may comprise only one class of the large labeled cells. Finally, it should be noted that, in addition to the large neurons described by Spatz ('75) and Lund et al. ('75), there are a number of labeled cells which are considerably smaller (Fig. 9B). To insure that these cells were not just portions of large cells sliced with the microtome, measurements were made only on neurons which clearly exhibited proximal portions of their axons and apical dendrites. In summary, it is concluded that the cells at the junction between layers V and VI of area 17 which make an efferent connection with area MT range in size from medium to large and probably include the solitary cells of Meynert.

The OBg of area 18 in primates is characterized by some large pyramidal cells in layer III (von Bonin et al., '42) which have been implicated in callosal connections (Shoumura et al., '75; Glickstein and Whitteridge, '76). The present study adduced more evidence in support of this view. First, a size analysis of labeled cells in the contralateral OBg following HRP injections in area 18 showed a number of large pyramidal cells in the sample taken. Second, following multiple large injections in the occipital lobe (brains 20 and 21), the sections counterstained with thionin revealed that almost all the large pyramidal cells in the appropriate location in the contralateral OBg were HRP-positive. Of course, the medium-

sized pyramidal cells labeled with HRP by far outnumbered the large cells.

In general, for all visual areas studied, the neurons in layer III making contralateral corticocortical connections were larger than those making ipsilateral connections (Table 2); a similar observation has been made in the somatosensory and motor cortices of primates (Jones and Wise, '77).

Cell groupings

Modern tracing techniques have revealed that projecting cortical neurons and their axon terminals can occur in groupings (or clusters or columns) separated from each other by gaps devoid of labeled neurons or terminals. The present study produced some evidence pertinent to this general concept. The clearest example of labeled cell groupings occurred in area 17 following a small HRP injection in area 18. The results in these cases can be construed to mean that a restricted portion of area 18's central representation receives converging input from several specific and separately placed cell clusters throughout area 17's central representation. After large injections (presumably covering area 18's central representation), however, no grouping patterns remained in area 17 and all labeled cells formed a long continuous band (or slab) in layer IIIa. One reasonable interpretation of these data is that separate subdivisions of area 18's central representation are connected with different multiple loci which are interlaced throughout the entire area 17 central representation. A similar arrangement, although organized at a much grosser level, was seen in the association projection of area 18 to area 19. Much less-regularly spaced cells or groupings of cells (with gaps between them ranging from 50 to 300 μm wide) were observed in layer IIIc of area 17 following small HRP injections in area MT. But after injections large enough to include all of area MT's central representation, these gaps also disappeared leaving the labeled cells to form one long and uninterrupted dense band.

An entirely different arrangement would appear to exist for the association projections from area 18 to area MT. Although a single, spatially isolated group of cells was labeled in area 18 after a small injection in area MT, when a large injection was made in area MT, at least two distinct groupings were still present in area 18. Thus it would appear that there are specific subregions of area 18's central representation that *do not* project to ipsilateral area MT at all.

Finally, there were cases in which multiple groupings never occurred. In no cases were callosal neurons observed in multiple groupings. Likewise, after injections in either areas 17 or 18 or 19, the labeled cells in ipsilateral area MT were always arranged in one single group no matter what size the injections were. It may be that some sort of functional arrangement does exist but that the spatial restrictions in area MT are such that anatomical arrangements into groups separated by gaps cannot be resolved. At any rate, these areas are interconnected with area MT grossly in a topographically ordered fashion (Spatz et al., '70; Spatz, '77a, b; Tigges et al., '74; Martinez-Millan and Holländer, '75; Wong-Riley, '79; present report). According to this interpretation, one might expect an anterogradely transported label also to occur in *one* grouping only; in fact, this *is* our interpretation of the labeling in area MT following an area 18 injection of tritiated leucine which was depicted in Figure 17 of Wong-Riley's ('79) study (although she interpreted this accumulation of silver grains as being indicative of *two* radioactively labeled groupings). A similar argument regarding the relatively small volume of area 19 visual cortex (as compared to area 18) might also be made for area 19; this area never showed more than one grouping following an HRP injection in area 18. The dorsoventral extent of area 19 has not been fully explored in squirrel monkey (Tigges et al., '74): If area 19 is homologous to area DL in owl monkey, and if it has a similar configuration (Allman and Kaas, '74), then it is probably too small to reveal readily anatomical groupings. As was suggested for area MT above, in such small areas the groupings would be fused and it is a matter of either spatial resolution or individual interpretation as to whether one considers them separated by gaps. In addition to the size of a visual area, there are many other factors such as its configuration, the arrangement of the representations of the meridians, and the order of transformation of the visual field which could play a role in defining output cell groupings and terminal bouton clusterings.

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