0270-6474/81/0109-0956$02.00/0

The Journal of Neuroscience
Vol. 1, No. 9, pp. 956–980
September 1981

THE VISUAL CLAUSTRUM OF THE CAT

I. Structure and Connections[1]

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Abstract

The cat's dorsocaudal claustrum was studied in Golgi preparations, by electron microscopy, and by anterograde and retrograde tracer techniques. It receives a convergent retinotopic projection from several visual cortical areas, including areas 17, 18, 19, 21a, and PMLS (posteromedial lateral suprasylvian area). The projection arises from spiny dendrite cells (pyramidal and fusiform) in the middle of cortical layer VI. As shown by a double label experiment, they form a separate population from those projecting to the lateral geniculate nucleus. There are also inputs from the lateral hypothalamus, from the nucleus centralis thalami, and probably from the locus coeruleus, but not from the sensory nuclei of the thalamus. Non-visual cortical areas do not project to the visual claustrum, but many of them are connected to other parts of the nucleus. For example, the splenial (cingulate) gyrus projects to a claustral zone just ventral to the visual area, and regions anterior to the visual area are connected with somatosensory and auditory cortex.

The commonest cell type in the claustrum is a large spiny dendrite neuron whose axon leaves the nucleus after giving off local collaterals. Small spine-free cells, with beaded dendrites and a locally arborizing axon, are found also. Electron microscopy of the claustrum after ablation of the visual cortex showed degenerating type 1 axon terminals synapsing on spines and beaded dendrites, suggesting a direct cortical input to both cell types.

The visual claustrum projects back to the visual cortex, to the same areas from which it receives an input. The return projection is predominantly ipsilateral, but there is, in addition, a small crossed projection. The claustrocortical axons terminate in all cortical layers but most heavily in layers IV and VI. The majority of the cells in the visual claustrum project to the cortex, and retinotopy is maintained throughout the entire corticoclaustral loop. No subcortical projections from the claustrum could be identified.

Among the nuclear masses of the telencephalon, the claustrum is probably the least well understood, whether in terms of its origin, its connections, or its function. A likely reason for its neglect is a common conception of it, based on standard myelin preparations of human forebrain, as a barely discernible strip of grey matter serving only to separate two obscure fiber tracts, the external and extreme capsules. Yet, if size is to be a criterion of likely functional significance, it should be pointed out that regions of the claustrum are considerably expanded in thickness; in carnivores, in fact, the dorsal part is so enlarged as to rival or exceed the size of the adjacent putamen.

The claustrum receives its major input from the cerebral cortex. Corticoclaustral projections were demonstrated initially from the frontal and temporal lobes in monkeys (Mettler, 1935; Hirasawa et al., 1939; Whitlock and Nauta, 1956). Further work in the rabbit (Carman et al., 1964) and cat (Druga, 1966b, 1968) demonstrated claustral projections arising from numerous cortical regions, each terminating in a distinct zone within the nucleus. The corticoclaustral projection has been confirmed by the use of the autoradiographic tracing method (Sanides and Buchholtz, 1979; Jayaraman and Updyke, 1979; Olson and Graybiel, 1980; Squatrito et al., 1980) and by horseradish peroxidase injections into the claustrum (Carey et al., 1980). This last study (on the tree shrew) showed, in addition, that the corticoclaustral projection arises from pyramidal cells in layer VI, a layer whose principal subcortical target is the thalamus.

The main output of the claustrum is a return pathway to the cortex. This was suggested first by Narkiewicz (1964), who found that ablations of various parts of the cortex led to neuronal degeneration, apparently retro-

[1] We thank R. Leibowitz for histological assistance, M. Peloquin for photography, T. Cotner for providing ^{125}I-wheat germ agglutinin, and M. Cynader for suggesting the horseradish peroxidase/D-[^{3}H]aspartate double label experiment. We are grateful to T. Wiesel, C. Gilbert, and J. Horton for suggesting improvements in the manuscript. This work was supported by National Institutes of Health Grant EY-R01-1960 to S. L.

grade in nature, in localized segments of the claustrum. The claustrocortical projection was confirmed by peroxidase transport experiments in a number of species (Kievit and Kuypers, 1975; Spatz, 1975; Norita, 1977; Riche and Lanoir, 1978; Carey et al., 1979; Olson and Graybiel, 1980) and by autoradiography after [^{3}H]proline injections into the claustrum (Flindt-Egebak and Olsen, 1979; Carey et al., 1980).

In this paper and the companion papers (LeVay and Sherk, 1981; Sherk and LeVay, 1981b), we describe the organization of the dorsocaudal claustral zone that is known to be connected to the visual cortex. We refer to this region as visual claustrum. In this paper, we describe its connections, as demonstrated with anterograde and retrograde tracing techniques, and some aspects of its internal structure (as shown by the Golgi method and electron microscopy) that may be relevant to an understanding of its functional organization. In the following paper (LeVay and Sherk, 1981), we investigate the question of a retinotopic organization within the visual claustrum, using a combination of anatomical and physiological mapping methods. In the third paper (Sherk and LeVay, 1981b), we describe the responses of claustral neurons to visual stimulation, with the hope of providing some insight into the function of the corticoclaustral loop.

Brief reports of the major findings have been published previously (LeVay and Sherk, 1980a, b; Sherk and LeVay, 1980; Sherk and LeVay, 1981a).

Materials and Methods

A total of 45 cats were used in the anatomical experiments. Most of these animals received injections of anterograde tracers ([^{3}H]proline or [^{14}C]proline) or retrograde tracers (horseradish peroxidase (HRP), ^{125}I-wheat germ agglutinin (^{125}I-WGA), or D-[^{3}H]aspartic acid) into the claustrum, the cerebral cortex, or other structures whose connections with the claustrum were to be investigated. Three cats were used for Golgi studies and two were used for electron microscopy. A number of these cats also were used in the physiological investigations to be described in the following papers (LeVay and Sherk, 1981; Sherk and LeVay, 1981b).

Cats were anesthetized with ketamine (20 mg/kg, i.m.) followed by a surgical dose of pentobarbital and maintenance doses at a rate of 2 mg/kg/hr; following surgery, they were paralyzed with gallamine and mechanically ventilated. End tidal CO_2 and rectal temperature were monitored. The pupils were dilated with atropine and the nictitating membranes were retracted with phenylephrine. Contact lenses of appropriate curvatures were fitted to focus the cat's eyes on a wall at a distance of 145 cm. Eyes were refracted and retinal landmarks were plotted by the fiber optic method of Pettigrew et al. (1979).

In most experiments, the region to be injected was first mapped with a tungsten electrode. This was particularly important for injections of extrastriate visual cortical areas, where the determination of receptive field positions at a single site will not necessarily identify the cortical area that is being injected. To locate the visual claustrum, a standard procedure was adopted: an initial vertical penetration was made into the center of the lateral geniculate nucleus (LGN) and a second was made near the anterolateral edge of the LGN. Any mismatch between the stereotaxic coordinates of this recording site and those of the equivalent site in a standard projection of the visual field onto the LGN (Fig. 8 of Sanderson, 1971) was noted. Then the center of the visual claustrum was reached in a third penetration made at nominal coordinates of AP +11.5, lateral 12.0, corrected by the amount of the previously determined error. Further penetrations were made as necessary based on our knowledge of the layout of the visual field in the claustrum (see LeVay and Sherk, 1981). The tungsten electrode was then replaced with a glass micropipette containing a few microliters of the tracer with mineral oil above. Electrical contact was made via a fine wire that was passed down through the oil to the tracer solution so that recordings could be continued with the micropipette until the desired injection site was reached. We used a simple pressure injection system consisting of a plastic 10-ml syringe with a thumb hole (for quick release of pressure) drilled near the end. The syringe was connected to the micropipette by a length of polyethylene tubing. The wire emerged from the pressure system at the junction of pipette and tubing, which was made air-tight with a drop of cyanoacrylate adhesive.

For the anterograde transport studies, we injected 0.03 to 3.0 μl of L-[2,3-^{3}H]proline (specific activity, 20 to 40 Ci/mmol) at a concentration of 25 to 50 μCi/μl, or 0.1 to 0.2 μl of uniformly labeled L-[^{14}C]proline (specific activity, 250 mCi/mmol) at a concentration of 2.5 Ci/μl (most injected volumes were at the low end of the stated ranges). The labeled compounds were evaporated to dryness and redissolved in 1.8% NaCl. Injections were made at a rate of 5 nl/min, as measured with the aid of the eyepiece micrometer of an operating microscope focused on the air/oil interface. After completion of the injection, the cat was resuscitated (in all cases, it remained deeply anesthetized for several hours after recovery from paralysis) and allowed to survive for from 1 to 7 days. It was then re-anesthetized and perfused through the left ventricle with 4% paraformaldehyde or with 10% formol-saline. Blocks of brain were removed stereotaxically, sunk in 30% sucrose, and cut as frozen sections. These were then processed for autoradiography using Kodak NTB2 emulsion and Kodak D-19 developer.

Injections of retrograde tracers were made in a generally similar way. HRP (Boehringer grade 1) was used at a concentration of 25 to 30% in 1.8% NaCl. In later experiments, α-L-lysophosphatidylcholine (lysolecithin) was added at a concentration of 3% (Frank et al., 1980). After a 2-day survival, the cats were perfused with 0.1 M phosphate buffer followed by a mixture of 3% glutaraldehyde and 1% paraformaldehyde. Usually, blocks were sectioned immediately with a Vibratome at 50 μm, but, in some cases, frozen sections were cut after immersion of the blocks in 30% sucrose in 0.1 M phosphate buffer overnight. Sections were incubated with diaminobenzidine (DAB, diphenyltetra-amine, MCB Reagents) and hydrogen peroxide in cacodylate buffer at pH 5.1 (Malmgren and Olsson, 1978). The DAB solution was filtered before use, and the reaction time was 0.5 to 2 hr at room temperature.

D-[^{3}H]Aspartic acid (Streit, 1980) was used as a second

retrograde tracer in combination with HRP. D-[2,3-^{3}H]Aspartic acid (specific activity, 16 Ci/mmol) was injected at a concentration of 200 μCi/μl and a volume of 0.3 μl, giving a total delivery of 60 μCi. After a 2-day survival, the cats were perfused with 2.5% glutaraldehyde and 0.5% paraformaldehyde. Blocks were immersed for a few hours in the same fixative, transferred to 30% sucrose in phosphate buffer for about 12 hr, and frozen-sectioned at 16 to 20 μm. The sections were reacted for peroxidase as described above. Then they were mounted, defatted, dipped in 2% gelatin as a precaution against chemography, and processed for autoradiography. Differentiation of the two labels was accomplished easily on the basis of the different colors and their location above or within the section. For the purpose of illustration, however, it was necessary to photograph the sections before and after removal of the silver grains. Subsequently, they were counterstained with cresyl violet. The silver grains were removed by treatment of the sections with 7.5% potassium ferricyanide for 10 min followed by fixation and washing (Rogers, 1973). Because of the limited range of the β particles from tritium, only those labeled cells that lay in about the upper half of the section thickness were revealed by the autoradiography.

Golgi preparations were made from the claustra of three adult cats. One was perfused with 10% formol-saline and processed by the Golgi rapid method (Lund, 1973), and the other two were perfused with 3% glutaraldehyde and processed by the Golgi-Kopsch procedure as modified by Colonnier (1964). The blocks were embedded in Epon (Nevin et al., 1978) and cut at 100 μm. The block face was warmed before each section was cut. The two types of preparation showed the same cell types, but the Golgi-Colonnier preparations were of better quality. Cells were drawn using a microscope equipped with a $\times$ 40 oil objective and a camera lucida attachment.

Electron microscopic observations were made on the claustra of two cats. One was a normal adult, and the other had been subjected to a widespread aspiration of areas 17 and 18 on one side 4 days prior to death. These animals were pressure-perfused without prior buffer rinse with a mixture of 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer via a cannula implanted retrogradely in the abdominal aorta. The claustra were sectioned with a Vibratome at 200 μm; blocks excised from the visual region were osmicated, stained with uranyl acetate and phosphotungstic acid, and embedded in Epon. Thin sections were stained further with lead citrate.

Results

Anatomy of visual claustrum. In coronal sections stained with cresyl violet, the cat's claustrum stands out as a densely staining nucleus with a characteristic comma-shaped outline. Figure 1*A* shows such a section taken at a level about 2 mm anterior to the caudal tip of the nucleus. The expanded dorsal part underlies the cortex of the anterior ectosylvian sulcus from which it is separated by a substantial band of white matter. Ventrally, the claustrum narrows to a thin, curved strip that is squeezed between the fundus of the pseudosylvian sulcus laterally and the putamen medially. Two narrow fiber bands, the extreme and external capsules, delineate the ventral claustrum from the cortex and putamen, respectively.

The claustrum is greatly elongated in the anteroposterior direction. Figure 1*B* shows the outline of the left cerebral hemisphere and the positions of the claustrum and the lateral geniculate nucleus (LGN) within it viewed from above. It may be seen that the caudal tip of the claustrum is situated near the anterolateral border of the LGN. From there, it runs forward and medially, following the outline of the hemisphere. The visual region extends from the caudal tip of the claustrum to a level about 4 mm further forward.

A better idea of the shape of the caudal claustrum is offered in Figure 1*C*. This is a view, from a posterolateral direction, of a three-dimensional model of the caudal claustrum constructed from serial coronal sections. At its caudal pole, the claustrum is extremely narrow. Further forward, the dorsal part begins to expand but still has a rounded upper edge. Toward the front of the visual region, the upper edge becomes wider and flattened and is tilted lateral side down. Thus, the anteroposterior level of any coronal section can be estimated fairly accurately from the shape of the claustral outline.

Only the expanded dorsal part of the caudal claustrum is visual; the boundary defined physiologically (LeVay and Sherk, 1981) is indicated by the ***heavy line*** in Figure 1*C*. No obvious landmarks or changes in cytoarchitecture mark this functional boundary.

The cellular picture in the claustrum, as seen in Nissl preparations, is of large, angular, and densely staining somata. Near the edges of the claustrum, these tend to be elongated parallel to the edge, but elsewhere, there is no orderly alignment of cells like that characteristic of cerebral cortex.

In Golgi preparations, two common cell types and a third rare type were seen (Fig. 2). The first and most frequent type was a large, spiny dendrite cell (15 to 29 μm in perikaryal diameter). The dendrites of this cell type often radiated out in all directions (as in the example shown in Fig. 2, *cell 1*). Other cells of this type had elongated dendritic fields, resembling those of pyramidal or fusiform cells of cerebral cortex. The dendritic tree of spiny dendrite cells could span as much as 0.5 mm in any direction (the entire width of the claustrum does not exceed 2.5 mm). As with spiny dendrite cells in the cortex or striatum, the proximal dendrites tended to be free of spines. Spiny dendrite cells had stout axons that, in the visual region, were directed toward the dorsomedial corner of the claustrum and sometimes could be followed into the white matter there. Often, though, the axon ceased to be impregnated near the cell body as if entering a myelin sheath. Many of these axons issued collaterals that ramified in the general neighborhood of the cell body. On the other hand, we could not identify any axon collaterals running to more distant parts of the nucleus.

The second cell type was a typical Golgi type 2 cell or interneuron (Fig. 2, *cell 2*). It was very small (10 to 15 μm) and its dendrites were beaded and lacked spines. Their total spread was rarely more than 150 to 200 μm. The axon was fine and (to judge by the consistent impregnation) lacked myelin. It underwent repeated di-

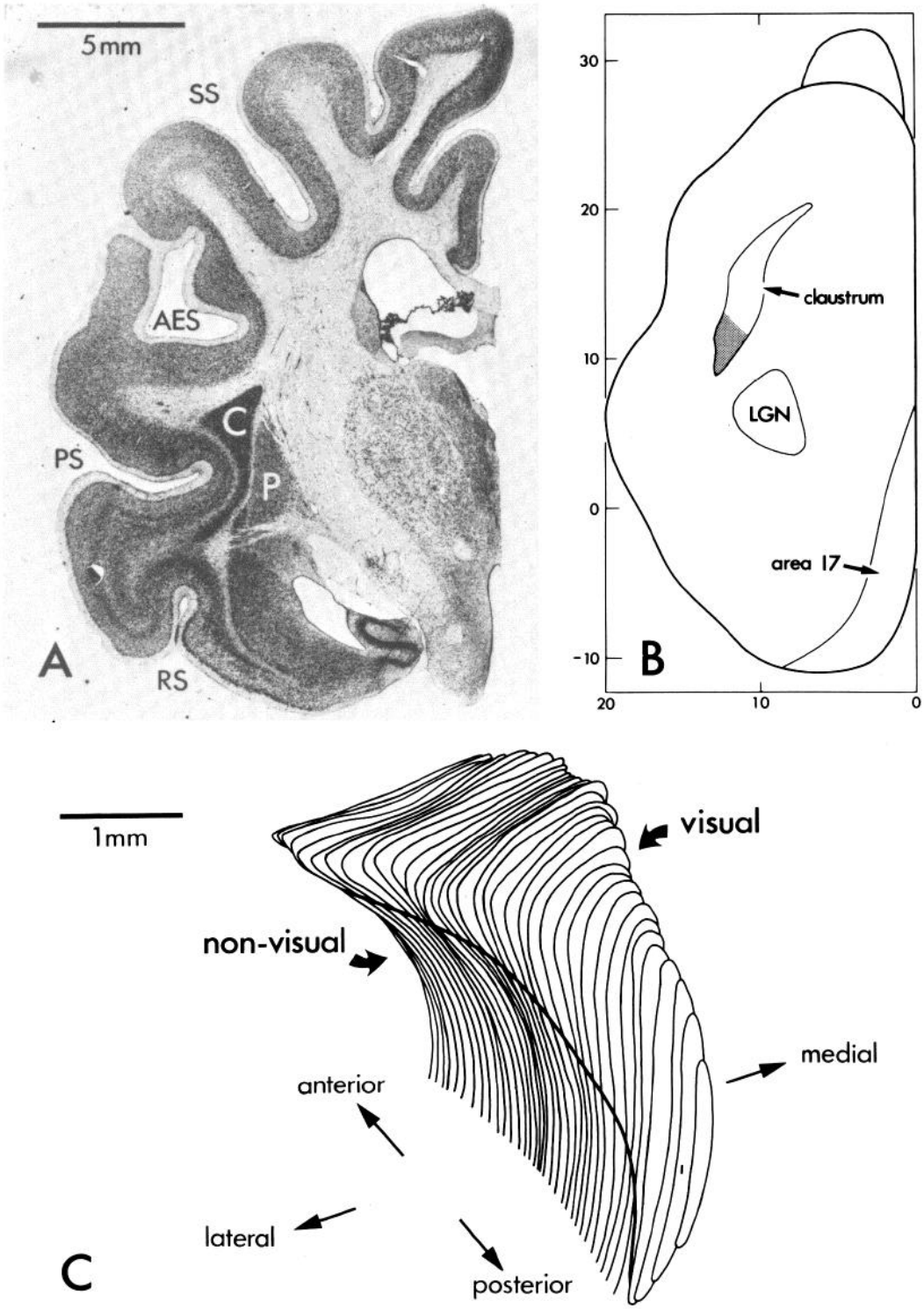

Figure 1.[2] Shape and position of the left claustrum. *A*, Coronal section at about AP +11.0 stained with cresyl violet. *B*, Dorsal view of the hemisphere to show the stereotaxic position and orientation of the claustrum, derived from the coronal sections in the atlas of Reinoso-Suárez (1961). The *shaded portion* of the claustrum indicates the visual area. *C*, A model of the caudal 4 mm of the claustrum constructed from serial 50-μm sections and viewed from a posterolateral direction. The *heavy slanting line* indicates the approximate boundary between visual and non-visual claustrum. The more rostral parts of the claustrum, not shown here, are non-visual.

[2] The abbreviations used on the figures are: AES, anterior ectosylvian sulcus; aud, auditory cortex; C, claustrum; Ce, nucleus centralis thalami; CG, central grey matter; CL, nucleus centralis, pars lateralis; CM, nucleus centralis, pars medialis; CP, cerebral peduncle; Ctx, cortex; d, degenerating terminal; den, dendrite; lat, lateral; LD, dorsolateral nucleus; LGN, lateral geniculate nucleus; LH, lateral hypothalamus; LP, lateral posterior nucleus; LP-P (or LP-pulv.), lateral posterior-pulvinar complex; LSA, lateral suprasylvian area; MB, mammillary body; MD, dorsomedial nucleus; med, medial; mes. V, mesencephalic trigeminal tract; MGN, medial geniculate nucleus; OT, optic tract; P, putamen; PN, posterior nucleus; PS, pseudosylvian sulcus; PT, pretectum; R, nucleus reticularis thalami; RS, rhinal sulcus; SCP, superior cerebellar peduncle; SG, supragenicuiate nucleus; SN, substantia nigra; sp, spine; splen., splenial gyrus; SS, suprasylvian sulcus; VL, ventrolateral nucleus; VM, ventromedial nucleus; VPL, ventroposterolateral nucleus; WM, white matter; ZI, zona incerta; 3, third ventricle.

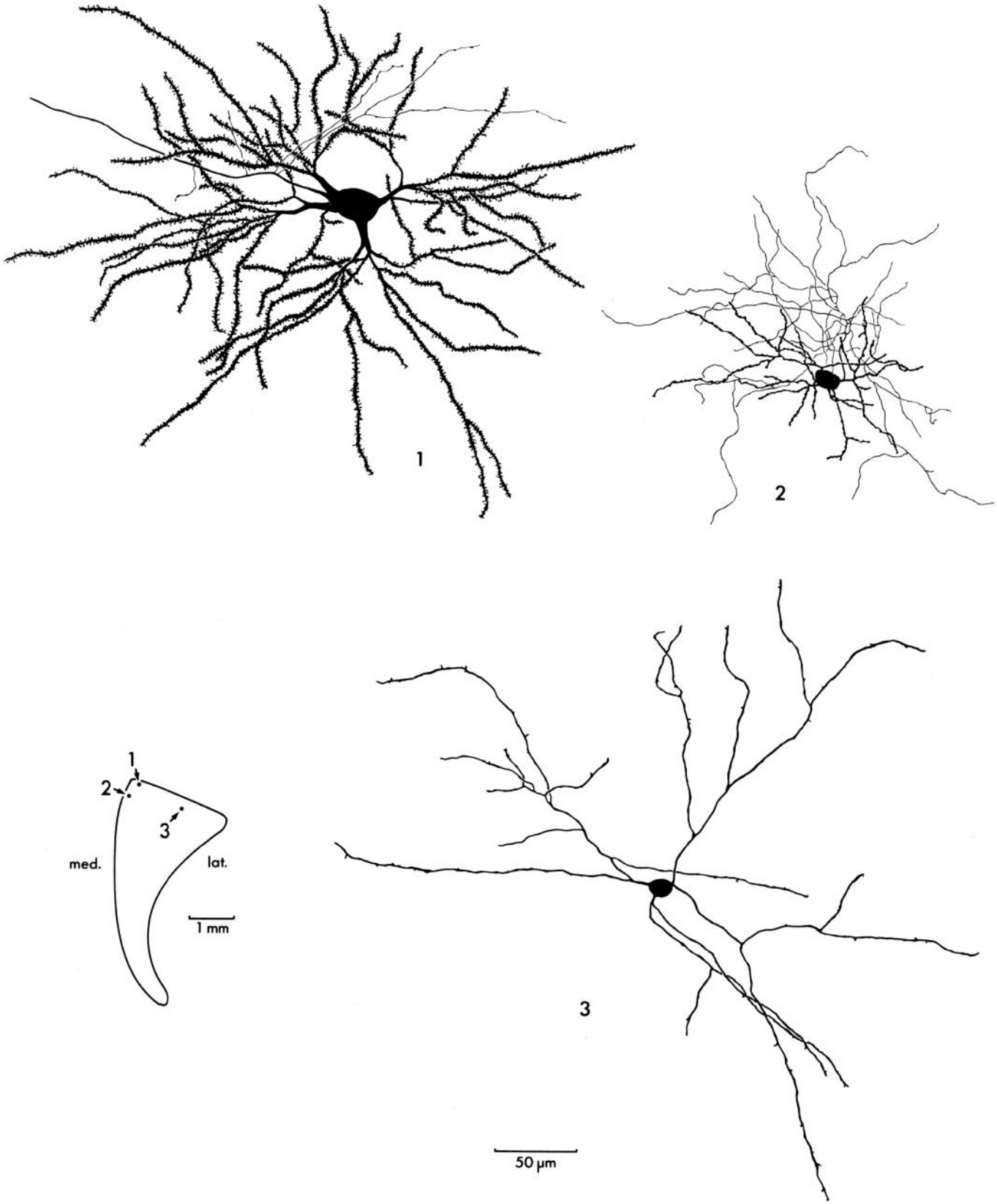

Figure 2. Camera lucida drawings of representative Golgi-impregnated neurons in the visual claustrum. *Cell 1* is a large, spiny dendrite cell whose axon leaves the claustrum after giving off recurrent collaterals. The majority of claustral neurons are of this type. *Cell 2* is a small, spine-free cell with beaded dendrites and a locally arborizing axon. *Cell 3* is a rare type of spine-free cell with a small cell body and a very wide, sparsely branched dendritic tree. An axon was not identified, but it might well have failed to impregnate. The *diagram* (*lower left*) shows the locations of the three illustrated cells in the claustrum.

chotomous branching in the immediate neighborhood of the cell body: few branches strayed far beyond the cell's dendritic tree. The axonal branches bore small boutons that sometimes contacted dendrites of other impregnated type 1 or type 2 cells. These probable interneurons were found throughout the claustrum, but they seemed distinctly more common near its edges; some, in fact, were situated just within the adjacent white matter.

Only three examples of the third cell type (Fig. 2, *cell 3*) have been seen, but these were distinct enough from the previous two types to warrant description. They had small cell bodies, like the type 2 cells, and spine-free dendrites, but the dendrites spread very widely (about 0.5 mm in total extent) with only modest branching. Their axons, if any, were not impregnated. These cells somewhat resembled the claustral cells which have been shown to be immunoreactive to antisera against vasoactive intestinal polypeptide (Sims et al., 1980).

Cortical afferents to claustrum. The obvious first step in determining the sources of afferents to the visual claustrum is to inject it with a tracer, such as horseradish peroxidase, and subsequently to examine the rest of the brain for retrogradely labeled cells. Because the visual claustrum is small, however, and is directly apposed to non-visual claustrum and to three fiber tracts, it is probable that the tracer will label some populations of neurons whose axons merely terminate near or pass by or through the visual claustrum. Rather than attempt very small injections, with the risk of missing important sources of input, we decided to make large injections that would be likely to label all sources of afferents, as well as some that actually projected to adjacent structures, and then to examine each of the candidate projections in turn by the reciprocal technique of [^{3}H]proline autoradiography.

Peroxidase was injected unilaterally into the claustra of six cats. Although the injections were centered at known sites in the claustral visual field representation, the enzyme spread beyond the limits of the nucleus in all cases (Fig. 3). It did not reach the lateral geniculate nucleus or the optic radiation above the LGN, except in one cat in which the front of the LGN was faintly colored. The best retrograde labeling in the cortex was obtained in two animals in which lysolecithin was added to the tracer solution, but all six cats showed a very similar distribution of labeled cells in the brain.

Figure 4*A* charts the distribution of retrogradely labeled cells in the ipsilateral visual cortex and neighboring cortical areas in a single coronal section at a level close to Horsley-Clarke A5. Beginning medially in the splenial (cingulate) gyrus, the cells formed a continuous band across the suprasplenial, lateral, and suprasylvian gyri and onto the ectosylvian gyrus laterally. On the contralateral side (not illustrated), only the splenial gyrus rivaled its partner in labeling density; areas 17 and 18 were almost free of labeled cells, but scattered cells occurred more laterally.

We have not attempted to chart the distribution of labeled cells in all known visual cortical areas because the position of many such areas cannot be determined reliably without physiological mapping. In addition, some of these areas project to structures that, being close to the claustrum, were partially involved in the peroxidase injection. Instead, we have relied on [^{3}H]proline autoradiography to investigate which visual cortical areas project to the claustrum (see below).

The great majority of labeled cells, on both sides of the brain, were found in layer VI, but smaller numbers were seen in other layers, especially at the border of layers III and IV. These were most common in the lateral suprasylvian area. A few very large layer V pyramidal cells were labeled also. The laminar distribution of the labeled cells is shown more clearly in Figure 4*B*, a dark-field micrograph of a portion of ipsilateral area 17. The labeled cells in layer VI did not occupy the full thickness of the layer but were concentrated at approximately the middle of its depth. This sublaminar organization was particularly obvious where layer VI was increased in thickness (at the crests of gyri) and was harder to discern in regions where it was stretched thin (at the bases of sulci).

Peroxidase-labeled cells were counted in areas 17 and 19 in counterstained sections. Although these counts were confined to regions where such cells were most numerous (see below), they were found to make up only a small proportion of neurons in layer VI: in area 17, a maximum of 3.5% of the cells in layer VI were labeled, and in area 19, 4% were labeled.

Labeled cells were commonest in the representations of the peripheral visual field. This distribution was seen

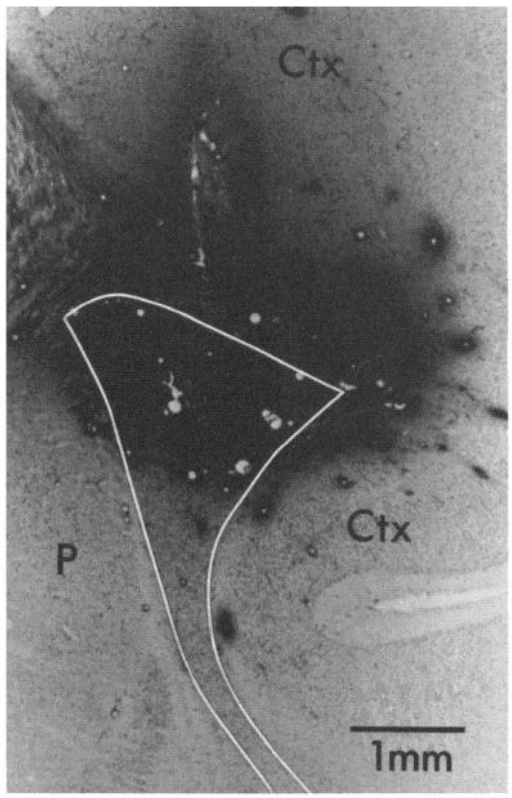

Figure 3. Peroxidase injection site in the right claustrum (lateral is to the *right*; dorsal is *up*). The entire visual (dorsal) portion of the claustrum is labeled heavily, and the enzyme has also spread to adjacent fiber tracts and to the deep layers of the overlying cortex. There is little involvement of the putamen.

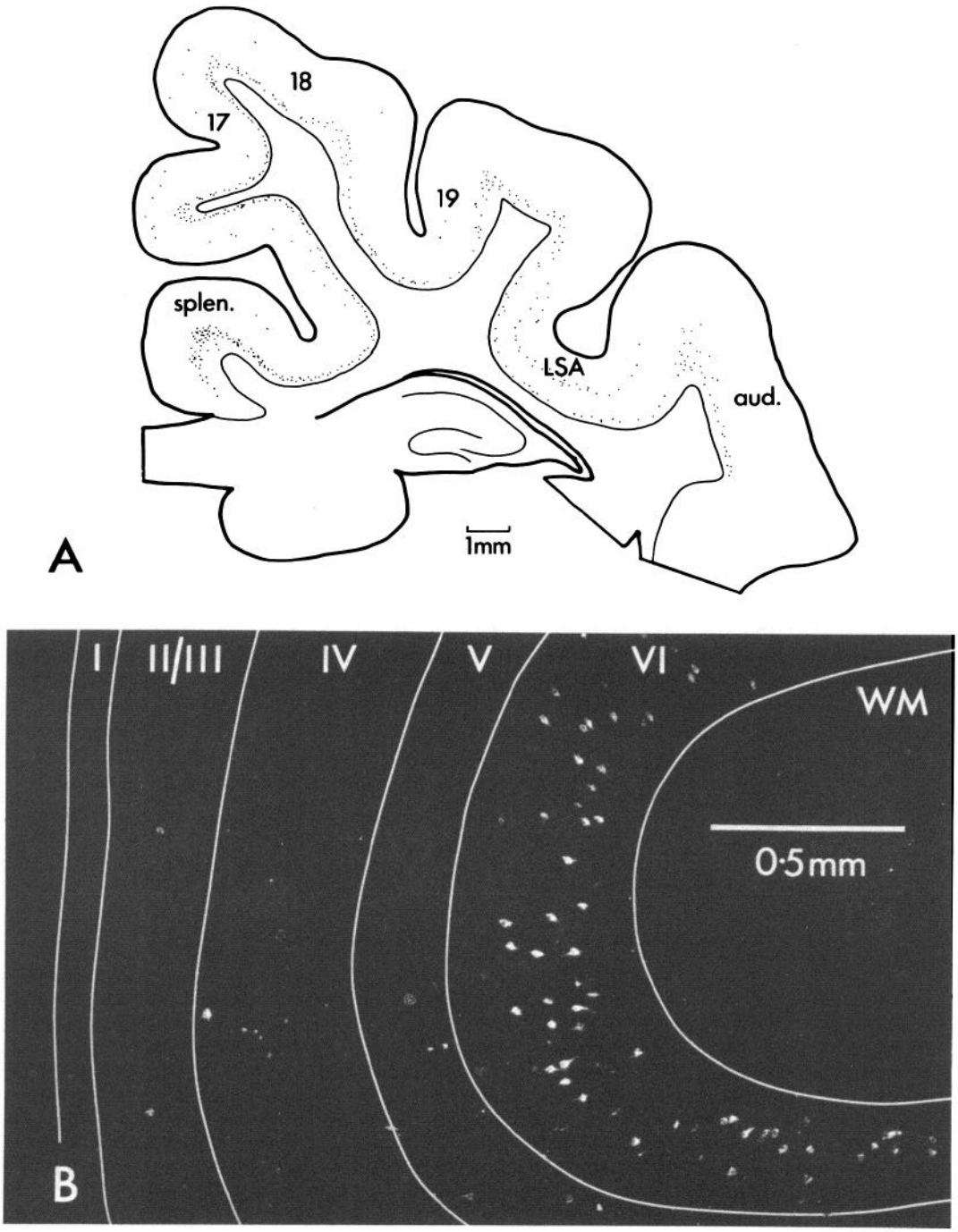

Figure 4. Retrograde labeling in the ipsilateral cortex after a claustral HRP injection. *A*, Camera lucida drawing of a coronal section at about AP +5.0. The labeled cells (*dots*) form a continuous band, deep in the cortex, stretching from the splenial gyrus through the visual areas to the auditory cortex where they end. (We are uncertain as to whether they extend into the primary auditory area, whose boundaries are not easily defined histologically.) Note the scattered cells in the upper layers, especially in the lateral suprasylvian area. *B*, Dark-field micrograph showing a part of area 17 (suprasplenial gyrus). Note that the labeled cells are concentrated in the middle of the thickness of layer VI. In addition, scattered cells are found in the other layers.

in all six cases, including those in which the injection site in the claustrum was centered close to the representation of the vertical meridian. Cell counts were made across area 17 in one cat; the maximum density of labeled cells in the periphery was about 3.5 times the density of such cells at the area 17–18 border.

In the cases where lysolecithin was added to the peroxidase, a solid, almost Golgi-like filling of cortical cells was obtained, allowing us to examine their shapes in some detail. Figure 5 is a camera lucida drawing of some representative solidly filled cells. These cells could be either pyramidal or fusiform in shape, but, in all cases

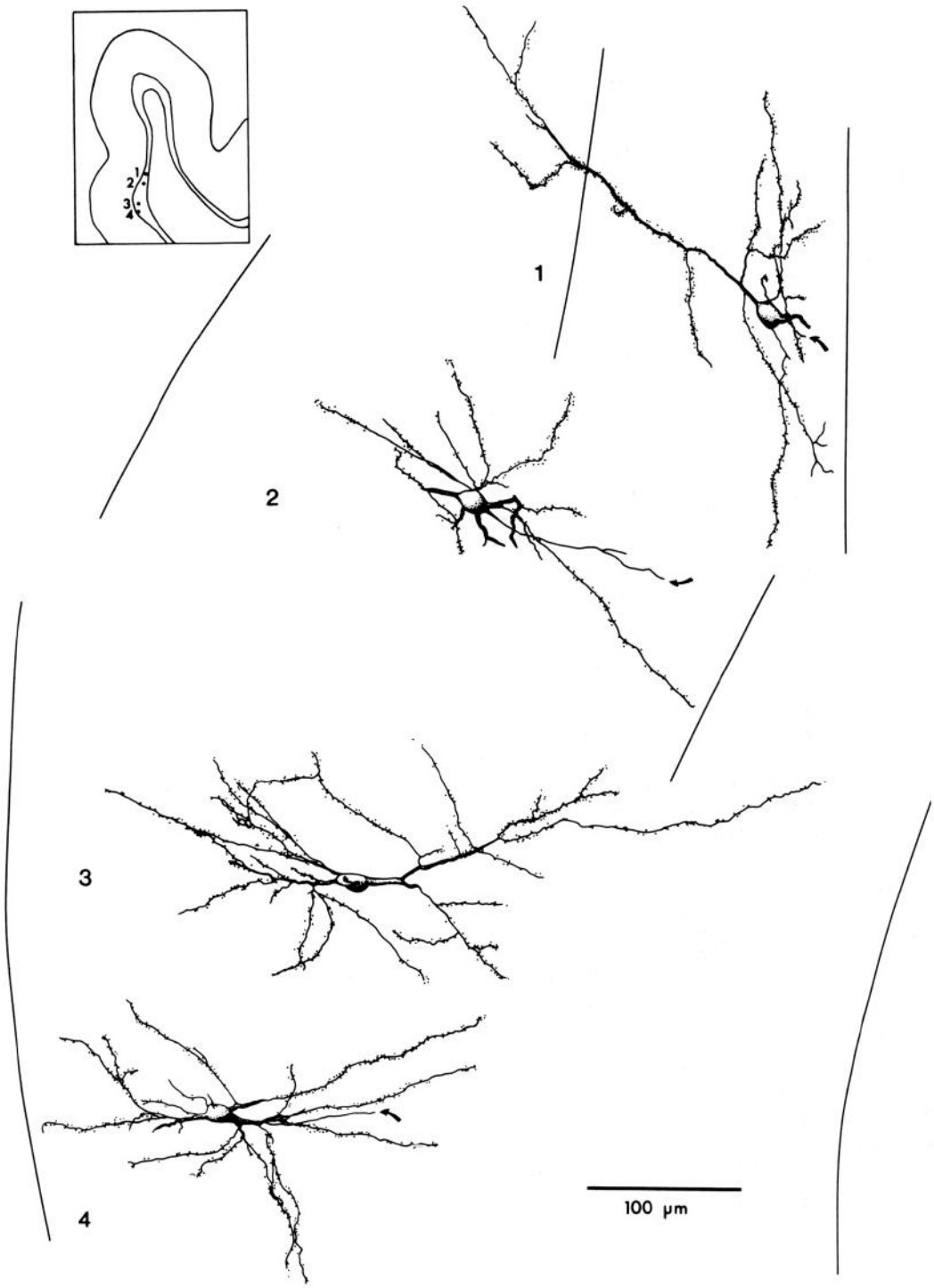

Figure 5. Camera lucida drawings of 4 cells in area 17 bulk-filled with HRP from an injection in the claustrum. The *solid lines* indicate the upper and lower borders of layer VI. The drawings were made from single 50-μm sections so that numerous processes, especially the apical dendrites, have been truncated. The *dots* along the dendrites represent spines. The axons are indicated by *arrows*. The *diagram* (*upper left*) shows the relative positions of the 4 cells in area 17.

where solid filling was obtained, abundant dendritic spines could be seen. This finding is in keeping with the principle that cortical output neurons are all of the spiny dendrite class, while spine-free cells are cortical interneurons (O'Leary, 1941).

The major target of layer VI cells in areas 17 and 18 is the lateral geniculate nucleus (Gilbert and Kelly, 1975). At least half of the neurons in layer VI project to the LGN, and these occupy the full thickness of the layer. We therefore wished to know whether the cells that

projected to the claustrum also had axon branches to the LGN, or whether they formed a separate population. To answer this question, we performed a double label experiment: HRP was injected into the claustrum, and D-[^{3}H]aspartic acid was injected into the LGN, at corresponding retinotopic loci. D-Aspartate is known to be transported retrogradely by cortical cells projecting to the LGN (Baughman and Gilbert, 1980; see also Streit, 1980). In sections of area 17 reacted for peroxidase and subsequently processed for autoradiography, the zones of cells marked by the two labels overlapped extensively, yet no examples of double-labeled cells could be found out of many hundreds of cells examined (Fig. 6).

Two kinds of controls were done. First, we performed the converse experiment, injecting peroxidase into the LGN and D-[^{3}H]aspartate into the claustrum. The result in this case was very similar except that many more cells were labeled with peroxidase, and fewer with silver grains, as was to be expected from the much larger number of layer VI cells that project to the LGN. This experiment showed that cortical cells projecting to the claustrum are indeed capable of transporting D-[^{3}H]aspartate. The second control consisted of injecting a mixture of HRP and D-[^{3}H]aspartate into the LGN. In this case, large numbers of double-labeled cells were found in area 17 (Fig. 7), ruling out the possibility that one of the labels in some way interfered with the transport of the other. Nevertheless, an appreciable fraction—perhaps a quarter—of the peroxidase-labeled cells were not labeled autoradiographically, even though they lay near the surface of the section (see "Materials and Methods"). It may be that these cells had axons that passed through the injection site without terminating, since peroxidase can be taken up by damaged axons, while the uptake of D-aspartate may be limited to the synaptic region. We cannot exclude, however, the possibility that there exists a population of layer VI cells that project to the LGN but do not transport D-aspartate. If such cells exist, we can say nothing about whether they have branching axons. With that proviso, the double label experiment provides strong evidence that the corticogeniculate and corticoclaustral pathways arise from two distinct populations of neurons that are intermixed in layer VI.

Cells projecting to the LGN and to the claustrum also may differ in their intracortical projections. In agreement with the findings of Baughman and Gilbert (1980), injections of the LGN not only labeled perikarya in layer VI but also produced a diffuse band of label in the same layer and another more prominent one in layer IV (Fig. 8*A*). Baughman and Gilbert interpreted the layer IV band as labeling of the terminals of axon collaterals of corticogeniculate neurons, which are thought to arborize in layer IV (Gilbert and Wiesel, 1979). After the claustral injection, on the other hand, no such layer IV band was seen (Fig. 8*B*). There was, however, a weak band in layer VI, intermingled with the labeled perikarya but also extending some distance tangentially beyond the zone of cellular labeling. This finding suggests that, unlike the LGN-projecting cells, those projecting to the claustrum distribute axon collaterals predominantly within layer VI and not to layer IV.

The termination of the cortical afferents in the claustrum was studied by the autoradiographic tracing technique. The result of an injection of [^{3}H]proline at the area 17–18 border, within the representation of the area centralis, is shown in Figure 9. A single focus of terminal labeling was found in the ipsilateral claustrum, situated about 0.8 mm below the dorsal border of the nucleus and in the middle of its mediolateral extent. This labeled zone is at the ventral border of the visual claustrum. Projections to the visual claustrum from areas 18, 19, 21a, and the Clare-Bishop area (the posteromedial lateral suprasylvian area (PMLS) of Palmer et al., 1978) also were demonstrated. These autoradiographic results will be presented in greater detail in the following paper (LeVay and Sherk, 1981) which explores the retinotopic organization of the corticoclaustral projections.

[^{3}H]Proline injections of two visual cortical areas, 20a (Tusa and Palmer, 1980) and the posterolateral lateral suprasylvian area (PLLS) (Palmer et al., 1978), labeled a region of the claustrum that appeared to be just ventral to the visual area. The injection site in area 20a and the resulting claustral labeling are illustrated in Figure 10. The labeling pattern resembled that described by Jayaraman and Updyke (1979) after a similar injection. The projection from PLLS lay more rostrally, underlying the anterior part of the visual area but also extending well forward of it. Areas 20a and PLLS thus appear to differ from other visual cortical areas in that they lack projections to the dorsocaudal zone that we have defined as visual claustrum.

In all cats in which we made [^{3}H]proline injections in visual cortical areas, we looked carefully for the presence of label in regions adjacent to the claustrum. Our concern was that, in the peroxidase experiments described above, some of the HRP-positive cells in the cortex—especially those outside of layer VI—had been labeled artifactually by spread of the enzyme beyond the limits of the nucleus. None of the injections in area 17 gave rise to labeling in adjacent fiber tracts, in the nearby cortex, or in the putamen. There was, however, a tenuous band of labeled fibers that coursed downward past the claustrum, about 1 to 2 mm from its medial edge, in the internal capsule. These fibers could be traced further into the cerebral peduncle and evidently were destined for the pons. [^{3}H]Proline injections of the other visual areas did lead to more widespread labeling of structures adjacent to the claustrum: areas 18, 19, PMLS, PLLS, and 21a projected to the putamen at sites close to visual claustrum, and the lateral suprasylvian area had, in addition, a heavy projection to the anterior ectosylvian sulcus immediately above the claustrum. The implications of these findings will be taken up under "Discussion."

In all of the autoradiographic experiments, the silver grains were dispersed over the neuropil of the claustrum, suggesting a termination on dendrites, and did not form pericellular nests, such as have been reported with use of the Nauta method (Druga, 1966b). To examine the manner of termination of the corticoclaustral pathway more closely, we turned to electron microscopy. In normal claustrum (not illustrated), the synaptic architecture was very similar to that seen in the cerebral cortex: most synapses were simple axospinous or axodendritic contacts, and of these, the majority contained round synaptic

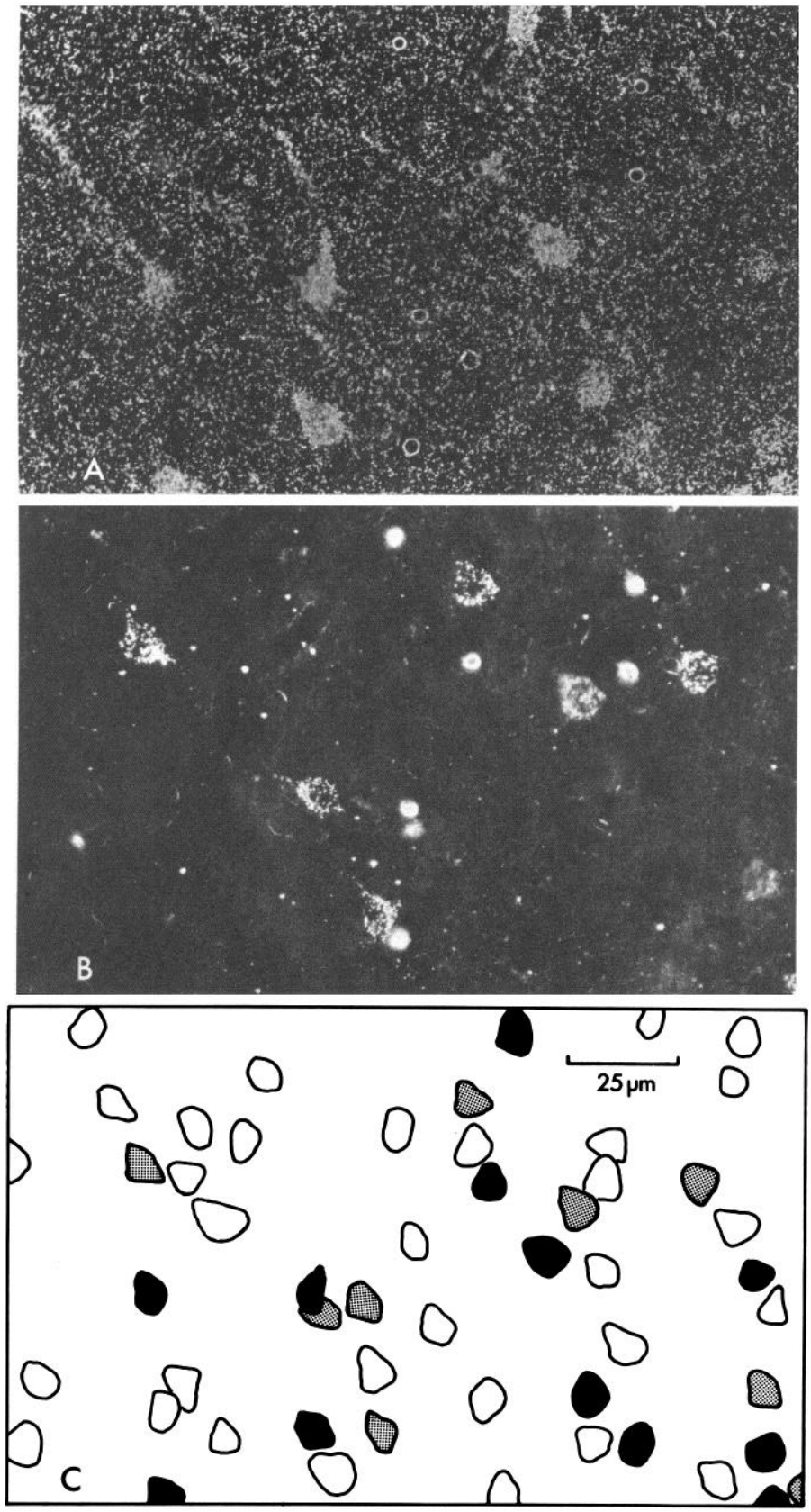

Figure 6. A 16-μm section of area 17 (layer VI) from an experiment in which HRP was injected into the claustrum and D-[^{3}H]aspartate was injected into the LGN. The section was processed both for autoradiography and for peroxidase histochemistry. *A*, Micrograph focused on the emulsion layer. There are about 10 cells that are labeled heavily and that therefore must have projected to the LGN. *B*, Micrograph of the same field after removal of the silver grains and now focused on the upper part of the tissue. There are 8 cells that contain HRP reaction product and that therefore projected to the claustrum. *C*, Camera lucida drawing of the same field after counterstaining with cresyl violet. Every neuron in the upper half of the section thickness has been *outlined*. The cells labeled autoradiographically are *black*, while the HRP-labeled cells are *shaded*. No cells are double labeled. Note that many cells carry neither label. Some of these may in fact project to the LGN, since D-aspartate does not appear to label the entire population of corticogeniculate neurons (see text and Fig. 7).

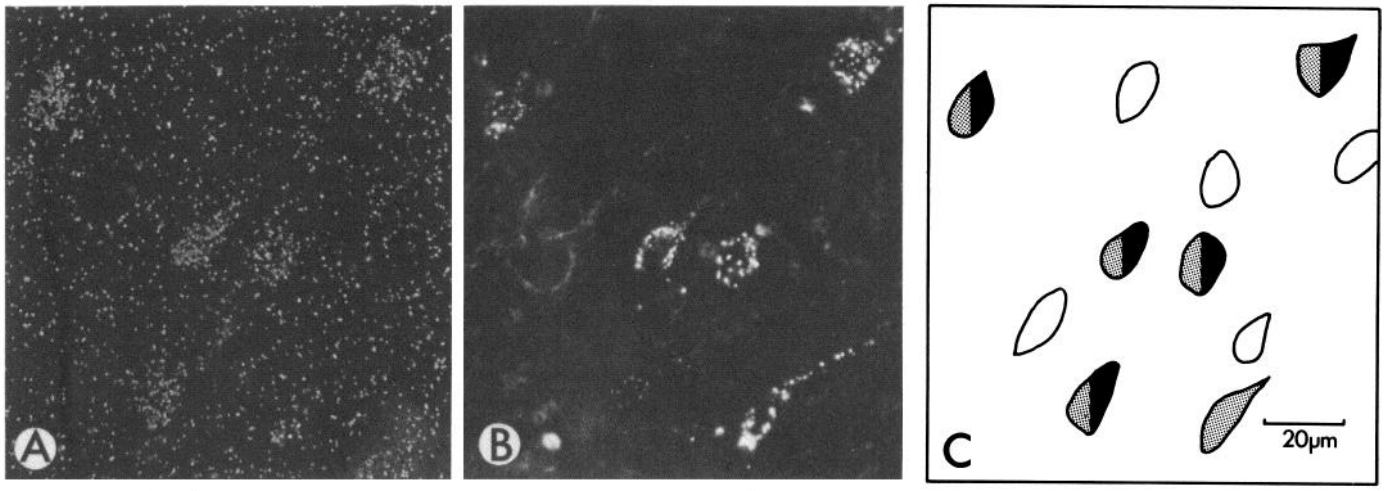

Figure 7. This figure is made up in the same way as Figure 6, but it is from a control experiment in which a mixture of HRP and D-[^{3}H]aspartate was injected into the LGN. The field contains 4 double-labeled cells, 5 unlabeled cells, and 1 cell (*bottom right*) that contains HRP reaction product but is not labeled autoradiographically. The apparent slight labeling of this cell in *A* is an artifact caused by the presence of bright shining HRP granules in the tissue very close to the emulsion. This cell therefore had an axon that terminated in or passed through the geniculate injection site, but it failed to transport a detectable amount of D-[^{3}H]aspartate.

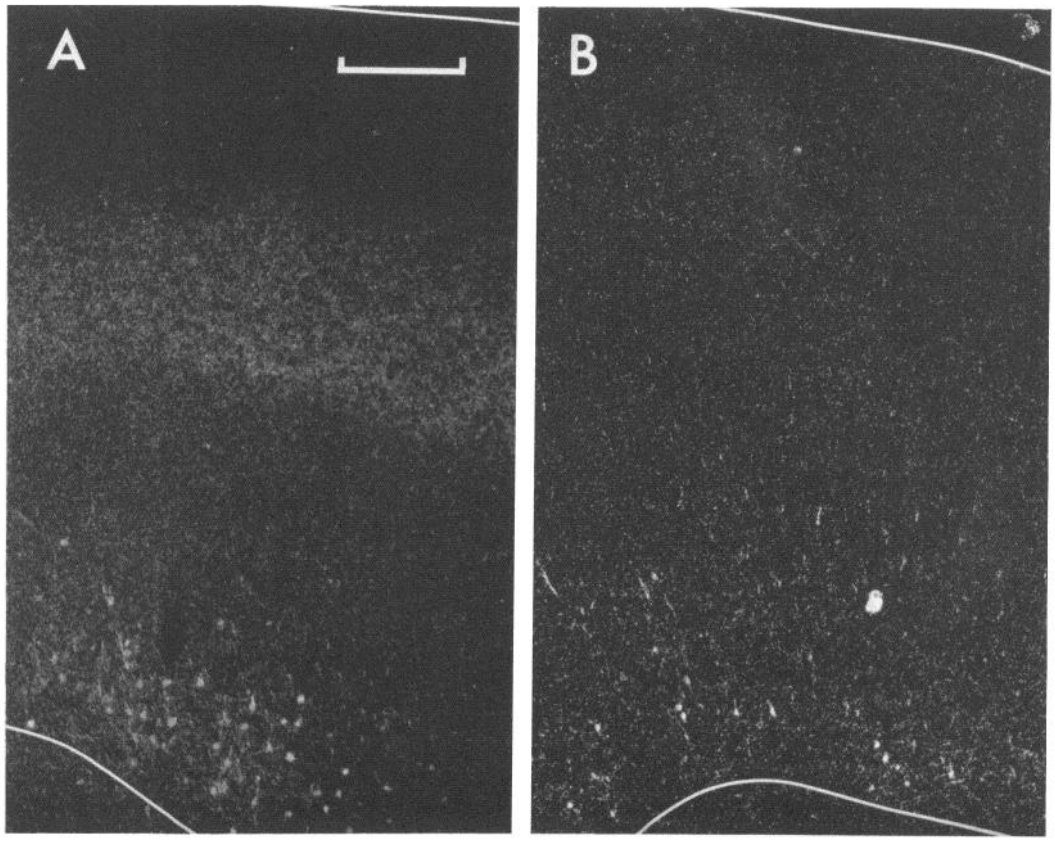

Figure 8. Comparison of autoradiographic labeling patterns in area 17 following injections of D-[^{3}H]aspartate into the LGN (*A*) and the claustrum (*B*). In addition to the cellular labeling in layer VI, there are diffuse bands of label that are thought to represent the terminals of axon collaterals belonging to the labeled cell bodies (see text). After the LGN injection (*A*), there is one such band in layer IV and a less distinct band in layer VI. After the claustral injection (*B*), there is a weak band in layer VI but none in layer IV, even after an exposure of 9 months.

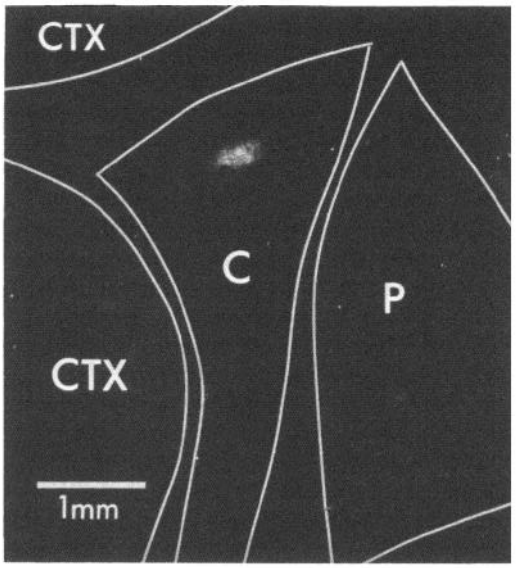

Figure 9. Dark-field autoradiograph showing a zone of terminal labeling in the left claustrum after a [^{3}H]proline injection in ipsilateral area 17 at the representation of the area centralis. As will be shown in the following paper (LeVay and Sherk, 1981), this zone is at the ventral limit of the visual area.

vesicles and made asymmetrical contacts (type 1 of Gray, 1959; see also Colonnier, 1968). Smaller numbers of synapses were found on cell bodies and proximal dendritic shafts, and of these, the majority contained flattened vesicles and formed symmetrical contacts (type 2 synapses).

In one cat, most of areas 17 and 18 was removed by aspiration on one side (with sparing of the underlying splenial gyrus). After 4 days' survival, electron microscopic sections of dorsocaudal claustrum showed large numbers of boutons undergoing electron-dense degeneration (Fig. 11). The degenerating boutons were invariably of Gray's type 1 as indicated by the presence of a prominent postsynaptic density (Gray, 1959). About 10% of all the boutons in the region were in a state of degeneration. They ended preferentially on dendritic spines (Fig. 11*A*), but smaller numbers ended directly on the shafts of beaded dendrites (Fig. 11*B*). These findings (which are in agreement with those of Juraniec et al., 1971) suggest that the bulk of the corticoclaustral projection terminates on the principal cells of the nucleus (the spiny dendrite cells, see above), while a subsidiary component contacts the beaded dendrite interneurons.

We wondered whether the remaining normal type 1 terminals differed in their distribution from the degen-

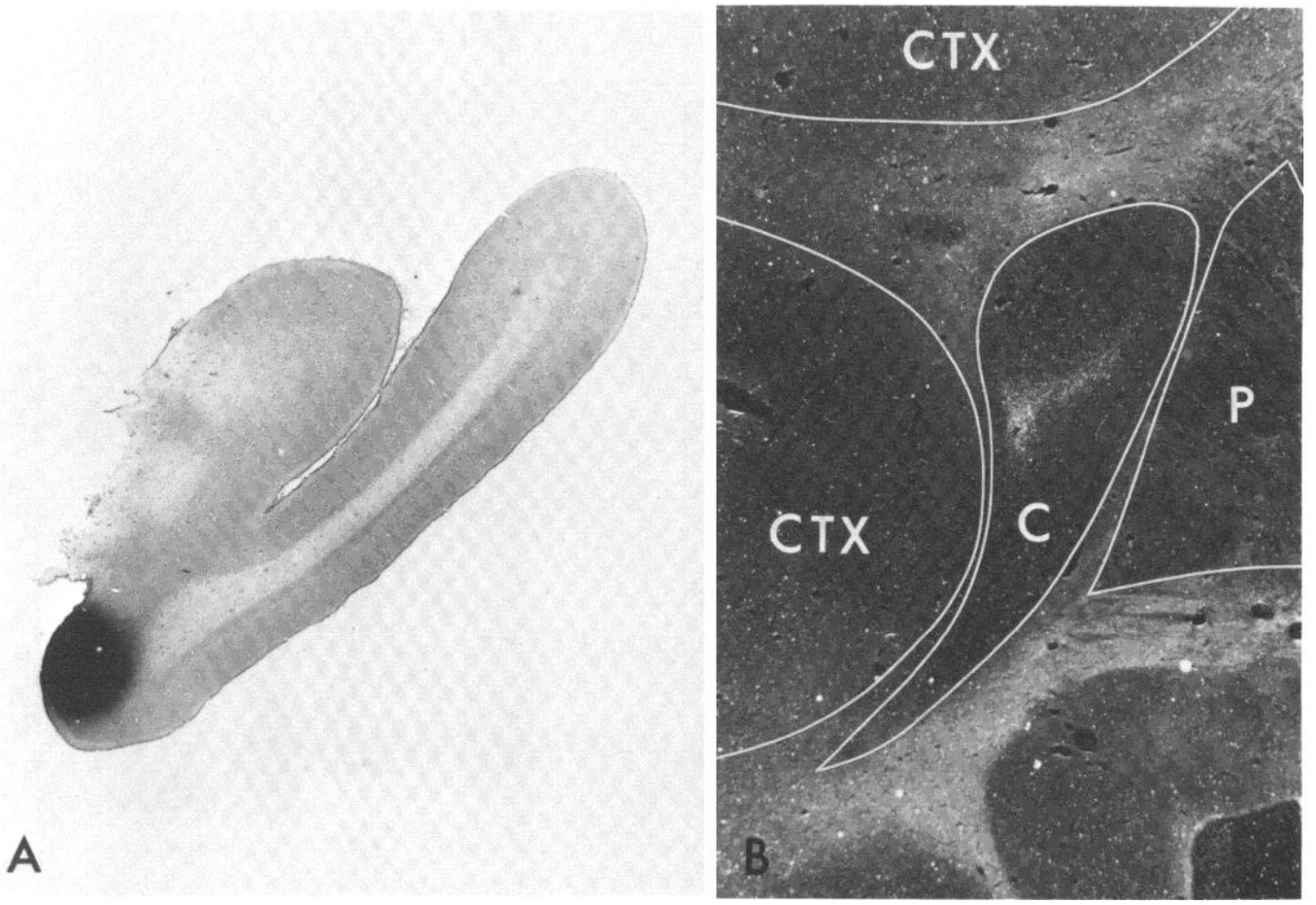

Figure 10. A, [^{3}H]Proline injection site in area 20a. *B*, Resulting labeling in the ipsilateral claustrum. The label appears to lie immediately beneath the visual region.

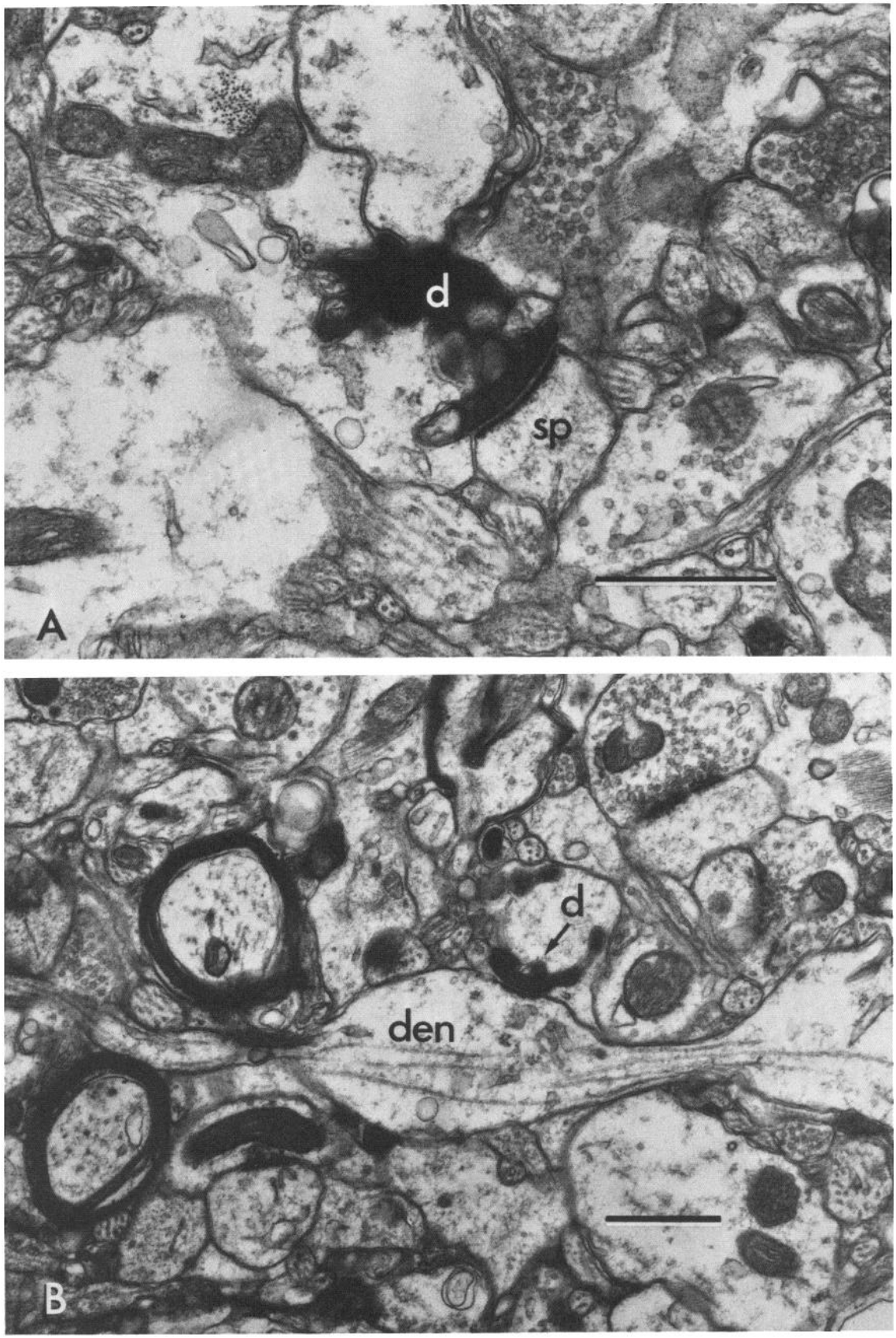

Figure 11. Electron micrographs showing degenerating axon terminals in the visual claustrum 4 days after ablation of visual cortex. In *A*, a large degenerating terminal (*d*) contacts a dendritic spine (*sp*). The prominent postsynaptic density shows this to be an asymmetric synapse. This is the commonest type of contact formed by cortical afferents. In *B*, a degenerating terminal contacts the expanded portion of a beaded dendritic shaft (*den*). The synapse is again asymmetric. The dendrite probably belongs to a type 2 cell (see Fig. 2). *Scale* = 1 μm.

erating ones, as this would suggest that they arose from a different, possibly non-cortical source. We therefore examined the contacts of 100 degenerating and 100 normal type 1 boutons selected at random from the same section. Of the degenerating terminals, 82 contacted spines, 14 contacted shafts, and 4 contacts were unidentified. Of the normal type 1 boutons, 76 contacted spines, 21 contacted shafts, and 3 were unidentified. Thus, the normal boutons did not differ appreciably from the degenerating ones in terms of the structures that they contacted, and we cannot say what fraction of them were afferents from areas 17 and 18 that had not yet degenerated, and how many were afferents from other visual cortical areas, from subcortical regions, or from the axon collaterals of claustral cells.

As mentioned earlier, peroxidase injections into visual claustrum labeled cells not only in visual cortex but also in the adjacent splenial gyrus, a component of the limbic system. (This must be distinguished from the so-called splenial visual area of Kalia and Whitteridge (1973) which lies more laterally in the fundus of the splenial sulcus and which also contained labeled cells.) An injection of [^{14}C]proline was therefore made into the splenial gyrus, and the resulting labeling patterns in the ipsilateral and contralateral claustra are shown in Figure 12. The splenial gyrus projected heavily to the *ventral* part of the caudal claustrum on both sides and did not invade the dorsal, visual area to any appreciable extent.

Over the caudalmost 2 mm of the claustrum, the splenial projection zone lay close to the visual zone. We are uncertain as to whether these two regions directly abutted each other here, an arrangement that would imply overlap between the splenial projection and that from area 20a (see above). Because of the variation from animal to animal, it would require double label experiments to answer this kind of question. More rostrally, however, the splenial projection slid ventrally, leaving a clear gap between it and the visual region. It is possible that the projection from area PLLS (see above) occupies this gap or part of it. In addition, we suspected from HRP experiments (described below) that the caudal portion of an auditory zone might extend into this space. We hoped to be able to delineate this area by making an injection of [^{3}H]proline into primary auditory cortex. To our surprise, such an injection did not result in any labeling of the claustrum (Fig. 13), although there were heavy projections to cortical areas near the claustrum and to the putamen, as well as to the medial geniculate nucleus and inferior colliculus.

Subcortical afferents to claustrum. The subcortical distribution of labeled cells after a peroxidase injection centered in the visual claustrum (but which spread beyond the limits of the nucleus) is charted in Figure 14. The principal zones were as follows: the lateral hypothalamus, the nucleus centralis thalami (both its medial and lateral subdivisions), the medial geniculate nucleus, the lateral posterior-pulvinar complex, the central grey matter of the midbrain, and the locus coeruleus. In addition, occasional labeled cells were found in the dorsomedial thalamic nucleus, the substantia nigra, and the mesencephalic reticular formation. In the thalamus and hypothalamus, the labeling was strictly ipsilateral, but in the brainstem, there were also a few cells on the contralateral side. The lateral geniculate nucleus was inspected carefully for labeling, in view of the claim by Rapisarda et al. (1969), based on physiological evidence, that the visual input to the claustrum is directly from the LGN. No labeled cells were found there.

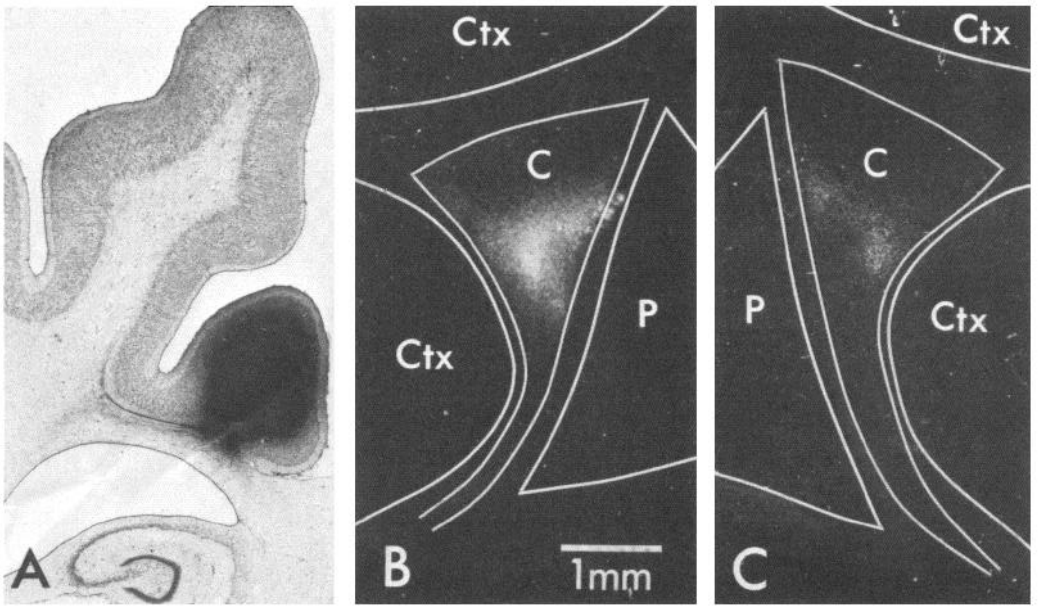

Figure 12. *A*, [^{14}C]Proline injection site in the left splenial (cingulate) gyrus. *B*, The projection zone in the left claustrum lies ventral to the visual area. *C*, The contralateral projection is lighter but terminates in the same region of the claustrum.

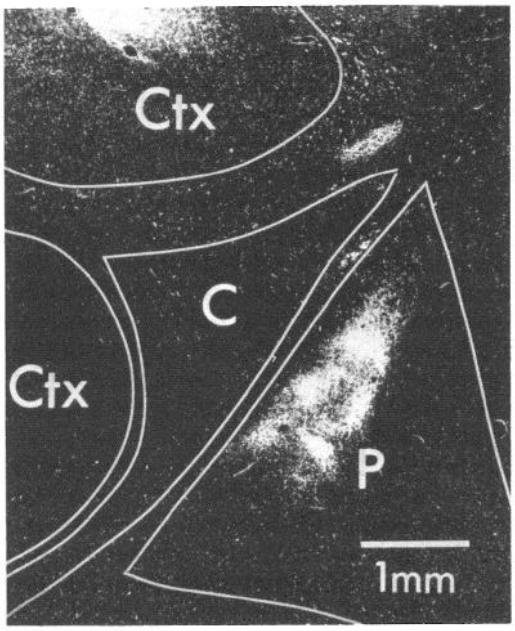

Figure 13. Autoradiograph of the left claustrum and neighboring structures after a [^{3}H]proline injection of primary auditory cortex. The injection was made at the crest of the middle ectosylvian gyrus, at AP +7.5, lateral 14.8, a site that is probably within A1 (see Merzenich et al., 1975). The claustrum is not labeled (at either this or other coronal levels), but there is a dense, patchy projection to the putamen and also a projection to the overlying cortex of the anterior ectosylvian sulcus. The *bright patch* above the dorsomedial corner of the claustrum is an artifact.

As mentioned earlier, we suspected that at least some of these zones of labeling were caused by spread of peroxidase into non-visual claustrum or into neighboring fiber tracts or adjacent nuclei. We therefore examined most of these candidate sources of afferents by [^{3}H]-proline autoradiography.

A [^{3}H]proline injection site in the lateral hypothalamus (at Horsley-Clarke AP +8, lateral 3, vertical 6.5) and the resulting labeling pattern in the ipsilateral claustrum are shown in Figure 15, *A* and *B*. Both the visual and non-visual portions of the nucleus were well labeled. The contralateral claustrum was unlabeled, confirming that the hypothalamoclaustral pathway is strictly ipsilateral.

An injection site in the nucleus centralis thalami, pars medialis, and the resulting claustral labeling are shown in Figure 15, *C* and *D*. Three injections were made into the same nucleus, all in the midline at vertical 12 and at AP +9, +10, and +11. Since the left and right nuclei abut each other at the midline, both were involved in the injection. Both claustra were labeled unambiguously, though more weakly than after the hypothalamic injection. The label tended to be heavier near the edges of the claustrum than in its core. There also was labeling of the putamen (heavier, in fact, than that seen in the claustrum) and of the nearby cortex. Thus, some of the HRP labeling of the nucleus centralis may have been caused by spread to adjacent structures, but the existence of a direct projection from the nucleus centralis to the claustrum was confirmed.

Quite different results were obtained after proline injection into the lateral posterior-pulvinar complex. Claustral HRP injections had resulted in a prominent cluster of labeled cells in the lateral posterior nucleus just ventromedial to the posterior nucleus; therefore, we made one proline injection here, at AP +6 and lateral 7 (Fig. 16*A*). The resulting labeling pattern in the claustrum is shown in Figure 16*B*. There was heavy labeling of the overlying cortex of the anterior ectosylvian sulcus and of the lateral part of the putamen adjacent to the claustrum, but there was no label within the claustrum itself. An injection made more medially in the lateral posterior-pulvinar complex, at AP +6, lateral 3, and 0.5 mm deep to the surface of the thalamus (another site where a prominent group of retrogradely labeled cells was seen in the HRP experiments) yielded similar results: the cortex of the pseudosylvian sulcus, as well as the putamen, were labeled, but the claustrum was spared (not illustrated). We conclude that the lateral posterior-pulvinar complex does not project to the claustrum, and that the peroxidase labeling of cells there had been caused entirely by spread of the enzyme out of the claustrum.

A similar result was obtained after a [^{3}H]proline injection into the medial geniculate nucleus (Fig. 16, *C* and *D*). Labeled fibers streamed past the dorsal surface of the claustrum on their way to terminate in adjacent cortex, but the claustrum itself was unlabeled.

In summary, the claustrum receives subcortical projections from the lateral hypothalamus and the nucleus centralis thalami, but not from the sensory relay nuclei of the thalamus.

Claustral efferents. To study the claustral projection back to the cortex, we made injections of [^{3}H]proline or [^{14}C]proline into the visual claustra of four cats. Survival times ranged from 1 to 7 days. In all four cases, there was some spread of the injected tracer into the deep layers of the overlying cortex of the anterior ectosylvian sulcus (Fig. 17). From the claustrum, a band of labeled fibers ran caudally and dorsally, joined the optic radiation, and broke up into bundles that innervated a number of visual cortical areas. A small contingent of fibers crossed the midline in the splenium of the corpus callosum to innervate contralateral visual areas.

The heaviest projections were to ipsilateral areas 17, 18, and 19. There were weaker projections to areas PMLS and PLLS. All five of these projections were confirmed by the results of HRP injections restricted to these areas. In the case of PLLS, however, labeled cells were found, not in the visual claustrum, but underlying its rostral end, and they were most numerous laterally. This position corresponds with the region shown to receive afferents from PLLS by autoradiography (see above). Some other visual cortical areas were labeled also (areas 20a, 20b, 21a, DLS (dorsal lateral suprasylvian area), and VLS (ventral lateral suprasylvian area) of Palmer et al. (1978) and Tusa and Palmer (1980)).

On the contralateral side, the main projection was to a restricted strip along the area 17–18 border. There did not appear to be any crossed innervation of the more peripheral parts of areas 17 and 18. Other weak projection zones were scattered elsewhere in the cortex but were not studied in detail. The existence of a crossed claustrocortical pathway confirms the work of Norita (1977).

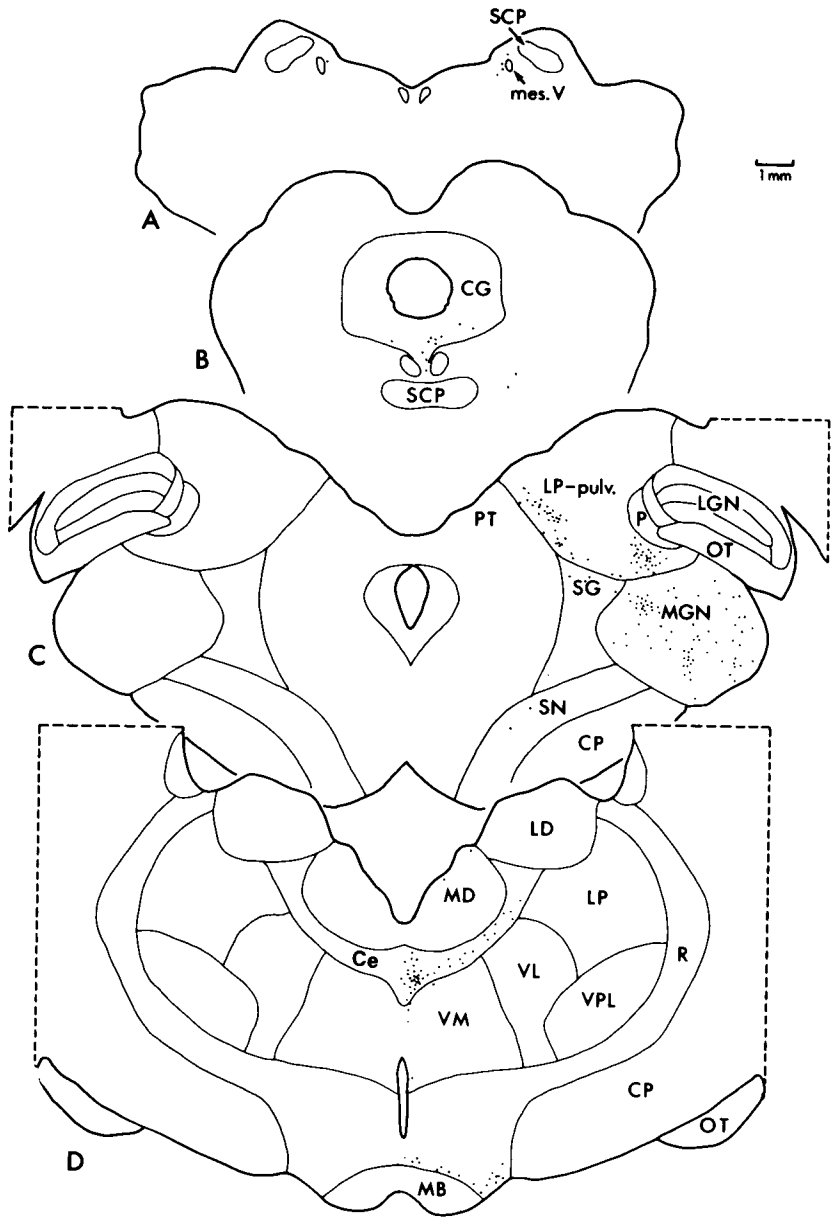

Figure 14. Distribution of retrogradely labeled cells in the thalamus and brainstem after a large HRP injection in the right claustrum. Note that, as shown by [^{3}H]proline autoradiography (see subsequent figures), not all of the HRP-labeled cell groups project to the claustrum itself. *A*, Level of the upper pons. A cluster of labeled cells is found in the region of the locus coeruleus just medial to the mesencephalic tract of the trigeminal nerve. *B*, Level of the superior colliculus. A few labeled cells lie in the central grey matter and the mesencephalic reticular formation. *C*, Level of the middle of the LGN (about AP +5.0). Labeled cells occupy two zones in the lateral posterior-pulvinar complex, one far medially and the other deep to the posterior nucleus. The medial geniculate nucleus contains many labeled cells throughout its extent. *D*, Level of the mammillary bodies (about AP +9.0), showing labeled cells in the nucleus centralis and in the lateral hypothalamus between the mammillary body and the cerebral peduncle.

The autoradiographic labeling pattern in areas 17 and 18, ipsilateral and contralateral to a claustral [^{3}H]proline injection, is illustrated in Figure 18. This particular case showed a fairly even distribution of label across area 17, but, in three other cats, labeling was heaviest in the representation of the periphery of the visual field and fell off toward more central locations, even though the injection sites were close to the representation of the vertical meridian. Even the area 17–18 border was labeled well above background, however. This distribution was similar to that of HRP-labeled cells in the cortex after claustral injections. We cannot rule out unequal spread of the tracers within the claustrum as an explanation for this pattern. We believe it more likely, however, that the claustrum is indeed connected more strongly with the peripheral parts of the visual field representation in the

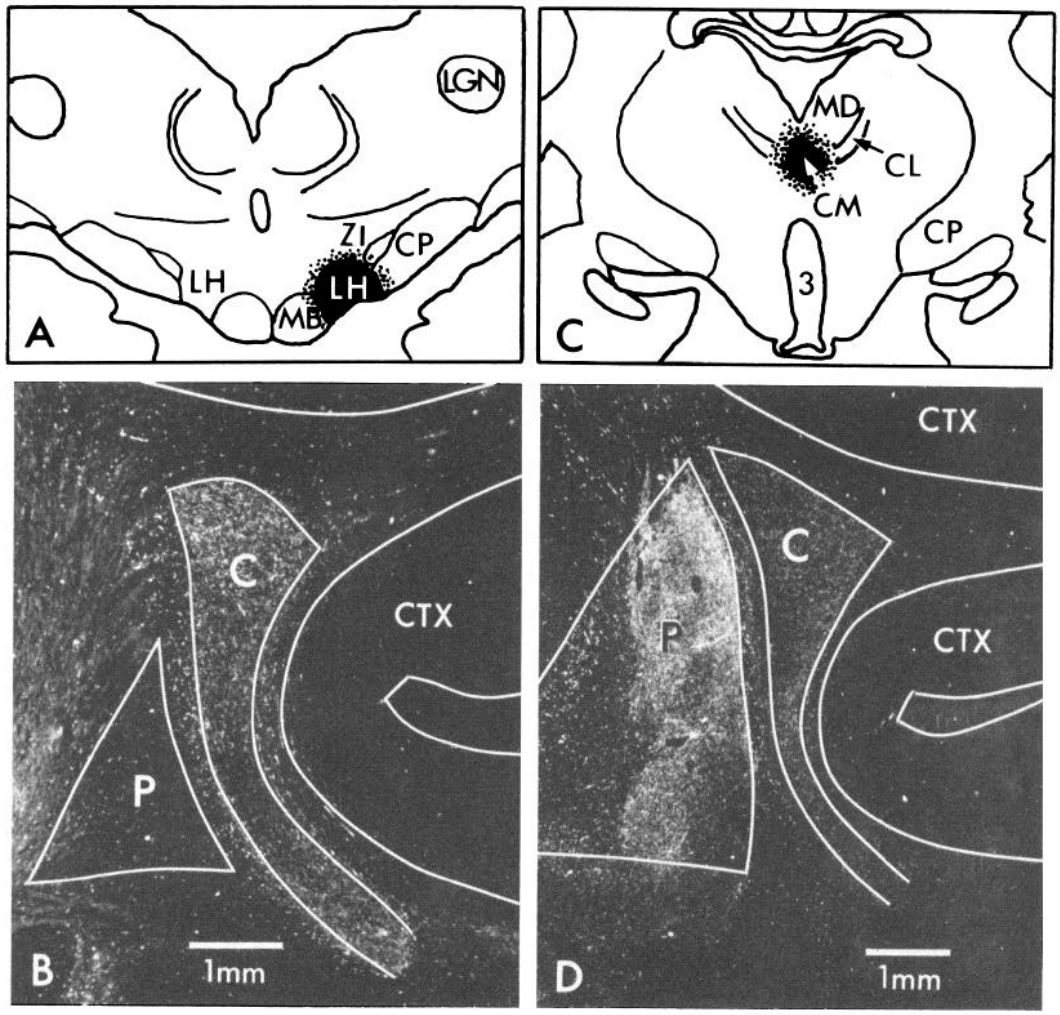

Figure 15. Projections to the claustrum from the lateral hypothalamus and the thalamic nucleus centralis. *A*, Injection site in right lateral hypothalamus (AP +8.0). *B*, Ipsilateral claustrum showing terminal labeling concentrated in the dorsal (visual) region and far ventrally in the stem of the nucleus. *C*, Injection site in the nucleus centralis, pars medialis (AP +10.0). Both the right and left nuclei are involved by the midline injection. *D*, Projection to the right claustrum. Although overshadowed by a heavier projection to the neighboring putamen, the claustrum is labeled well above background, especially toward its edges.

cortex. Further support for this is provided by the results of physiological mapping in the claustrum (LeVay and Sherk, 1981).

As may be seen in Figure 18, the autoradiographic label was distributed throughout the thickness of the cortex with some layer-to-layer variations in density. The laminar distribution was studied in more detail by making grain counts across the layers in cresyl violet-stained sections, and such counts for two animals are shown in Figure 19. Grain density was highest in layer IV, and layer VI was only slightly less strongly labeled. Layer V and the supragranular layers were labeled more weakly, but even layer I, where the label was weakest, had a grain density well above background.

Only one subcortical region, the lateral posterior nucleus, was labeled after [^{3}H]proline injections into the visual claustrum, and this labeling was light. Nevertheless, we attempted to verify the projection by making an injection of peroxidase into this area. No claustral cells were labeled in this experiment, although there was labeling of large pyramidal cells in the deep layers of the nearby cortex (anterior ectosylvian and pseudosylvian sulci). We conclude that the claustrum does not project to the lateral posterior-pulvinar complex, and hence, that the cortex is the sole target of visual claustral axons.

We also studied the claustrocortical projection by retrograde transport methods. The aim of these experiments was not only to verify the targets of the claustral efferents, as mentioned above, but also to delineate the extent of the visual claustrum, to test for a retinotopic organization in the claustrocortical pathway, and to determine which cell types in the claustrum projected to cortex.

A very large injection of HRP made in area 17 and spreading widely into area 18, but not other areas, allowed us to estimate the full extent of the visual claustrum and to judge approximately the fraction of claustral cells projecting to these two areas. Over a period of 6.5 hr, 3.6 μl of 25% HRP, in combination with 3% lysoleci-

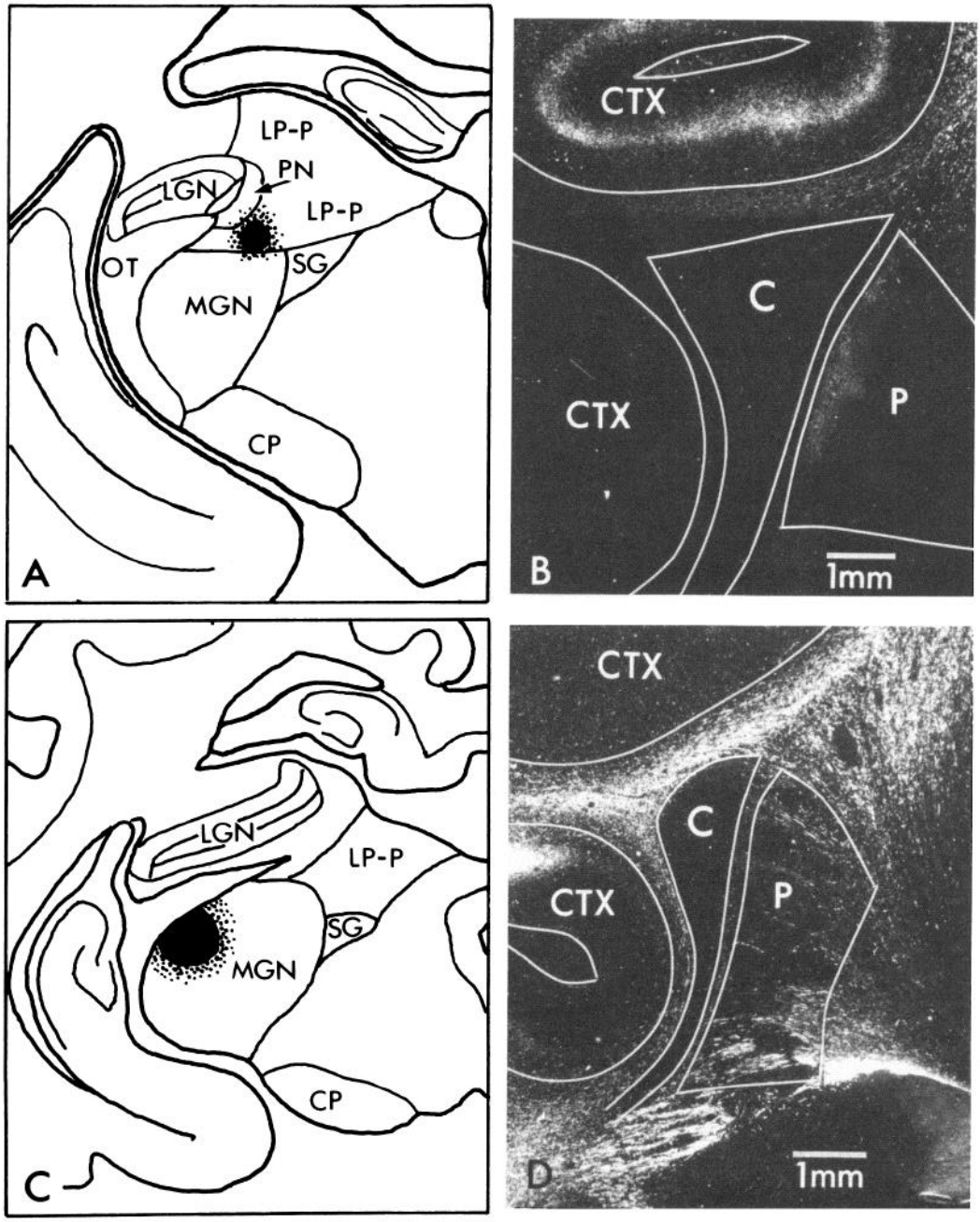

Figure 16. Evidence against projections to the claustrum from the lateral posterior-pulvinar complex and the medial geniculate nucleus. *A*, Injection site in the left lateral posterior-pulvinar complex at AP +6.0. *B*, The claustrum is unlabeled, but there are projections to layer IV of the overlying cortex and to the lateral edge of the putamen. (Incidentally, comparison with Fig. 13 suggests that the auditory and visual inputs to the putamen form two adjacent, non-overlapping zones with an irregular mutual border.) *C*, Injection site in the left medial geniculate nucleus. Only a small part of the nucleus, where it underlies the LGN, is involved. *D*, Again, the claustrum is unlabeled, but it is encapsulated by labeled fibers, some of which terminate in the cortex of the pseudosylvian sulcus.

thin, was injected. In the LGN, retrogradely labeled cells occupied all but the anterior pole of the nucleus, indicating the extensive spread of the injected enzyme. The claustral labeling (Fig. 20) was very heavy on both sides of the brain. Ipsilaterally (Fig. 20*A*), labeled cells extended from the dorsal margin of the nucleus to a depth that varied with anteroposterior position: at the caudal pole, the entire dorsoventral extent of the claustrum contained labeled cells; 2 mm further forward, the labeling reached a depth of about 1 mm below the dorsal margin of the claustrum; and further rostrally, the labeled cells became progressively more confined to the dorsolateral corner. The extent of the visual claustrum, as defined by these experiments, agrees well with the results of physiological mapping (LeVay and Sherk, 1981).

On the contralateral side (Fig. 20*B*), the zone of labeled

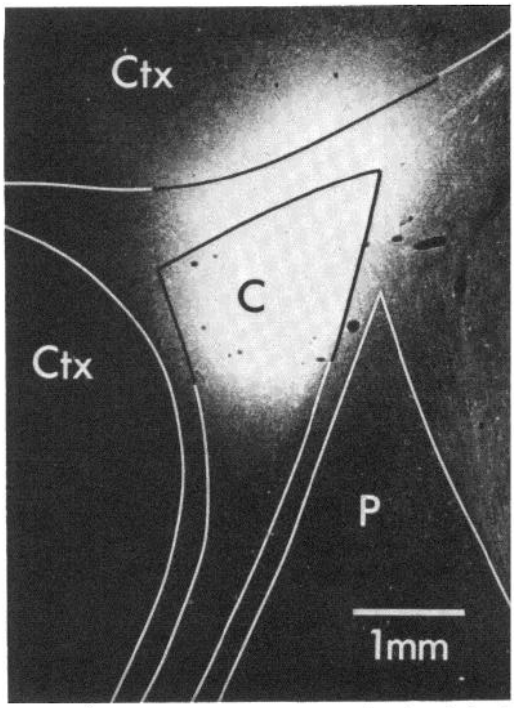

Figure 17. [^{3}H]Proline injection site in the left claustrum. Although the injection was made near the ventral border of the visual region, some of the injected label has spread back dorsally to involve the deep layers of the overlying cortex.

cells was not at the dorsal margin of the claustrum but was displaced ventrally. It was centered at a level corresponding to the lower limit of the labeling on the ipsilateral side. There was considerable overlap of the claustral regions projecting ipsilaterally and contralaterally.

Cell counts made in the ipsilateral claustrum showed that 87% of cells in the visual area were labeled. These were predominantly medium to large cells, with a median diameter of 17.5 μm. The unlabeled cells spanned a wide range of diameters. However, a large majority of cells with diameters of 13 μm or less were unlabeled. We suspect that these small unlabeled cells correspond to the type 2 local axon cells seen in Golgi preparations (see Fig. 2). Evidently, only a small fraction of the principal cells do not project to either area 17 or 18.

The retinotopic organization of the claustrocortical projection was demonstrated in a number of experiments in which smaller injections of HRP were made at physiologically defined sites in the various visual cortical areas. In each case, the zone of retrogradely labeled cells in the claustrum occupied the location predicted from physiological mapping (LeVay and Sherk, 1981). This suggests that cells in a particular claustral location send their axons to the same site in area 17 (and other areas) from which they receive their input. An experiment was done to test this prediction directly: a combined injection of HRP and [^{3}H]proline was made into area 17 at a site whose receptive fields were 17° out in the contralateral

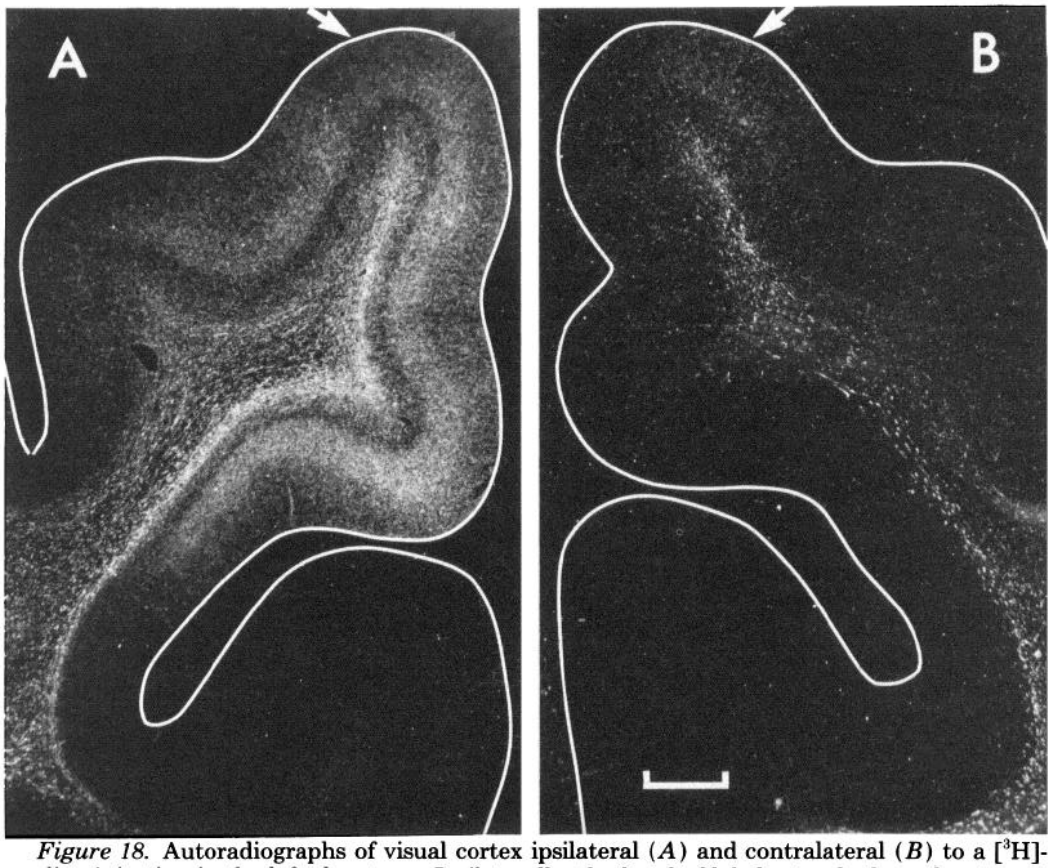

Figure 18. Autoradiographs of visual cortex ipsilateral (*A*) and contralateral (*B*) to a [^{3}H]-proline injection in the left claustrum. Ipsilaterally, the band of label extends throughout areas 17 and 18, although it is somewhat weaker at the area 17–18 border (*arrow*). The major terminal band is in layer IV. A subsidiary band in layer VI is masked partially by heavy labeling of fibers in the white matter that are passing to more caudal regions of cortex. Contralaterally, labeled fibers stream up from the corpus callosum and terminate in a restricted zone near the area 17–18 border (*arrow*).

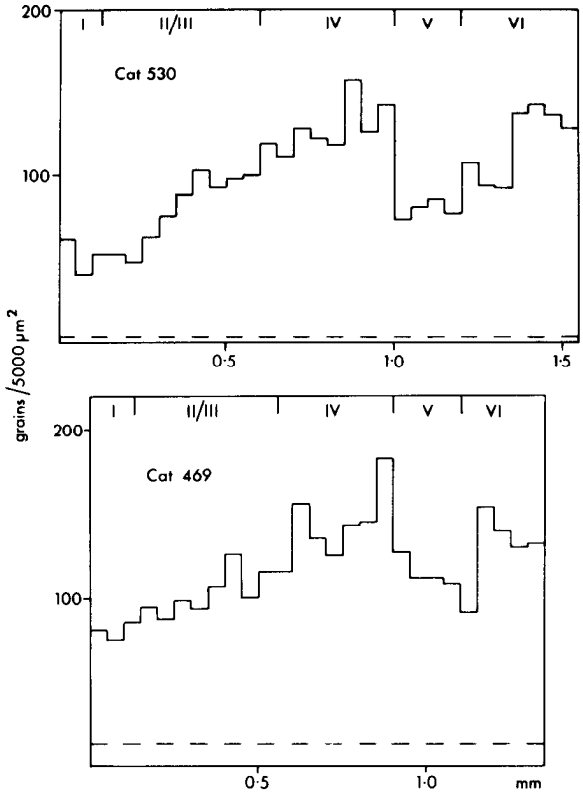

Figure 19. Grain counts made across the cortical layers in two cats that had received [^{3}H]proline injections in the claustrum. Cat 530 (*upper*) survived 7 days, and cat 469 (*lower*) survived 2 days. The labeling pattern is similar in the two cases, with peaks in layers IV and VI and weaker labeling of layer V and the superficial layers. It is important to note that labeling in all layers in both animals is well above background (*dashed line*), which was measured in the cortex of the adjacent splenial gyrus on the same slides

field and 2.5° below the horizontal meridian. The autoradiographic label in the claustrum took the form of a curved band, of which only the two ends are seen in the section illustrated (Fig. 21*A*), since the upper, central portion of the band was tilted back into more posterior sections. The zones of HRP-labeled cells in the adjacent section (Fig. 21*B*) were superimposed exactly on the autoradiographic label. Labeled cells were not seen in the contralateral claustrum in this experiment. It is evident, from these and other experiments, that retinotopic fidelity is preserved over the entire corticoclaustral loop.

An injection of HRP into area 19, within the representation of the area centralis (1° out on the horizontal meridian) produced the claustral labeling shown in Figure 22. Now, labeled cells were found in both ipsilateral and contralateral claustra, and on both sides, they lay at the ventral limit of the visual area, where the vertical meridian is represented (see LeVay and Sherk, 1981). It was true not only of area 19 but also of 17, 18, and PMLS that crossed claustrocortical projections were demonstrated only following HRP injections at or near the cortical representations of the vertical meridian.

Further evidence for a precise reciprocity in the corticoclaustral loop was provided by four experiments in which ^{125}I-WGA, a tracer that is transported both anterogradely and retrogradely, was injected into various visual cortical areas. The resulting cellular (retrograde) and neuropil (anterograde) labeling always occupied the same region of the claustrum.

Peroxidase was also injected into somatosensory and auditory cortex. The injections in the primary somatosensory cortex (two cats) were made at the representation of the top of the head. They gave rise to retrograde labeling in a region of dorsal claustrum anterior to the visual area, extending forward to the rostral pole of the nucleus. Two injections into auditory cortex, that involved both primary and association areas, gave weak labeling of claustral cells in the middle of the anteroposterior extent of the nucleus. These cells were situated more ventrally and appeared to underlie the front end of the visual region as well as the caudal part of the somatosensory area. In none of these experiments were labeled cells found in the visual claustrum.

The claustrum parvum. A description of the visual claustrum would not be complete without mention of the curious structures collectively termed the *claustrum parvum* (Landau, 1936; Druga, 1966a). These are rod-shaped formations, about 50 to 100 μm in diameter and 0.5 to 1.0 mm long, situated near the dorsolateral corner of the claustrum. At the level of the visual region, there are one or two of them embedded within the white matter of the anterior sylvian gyrus (Fig. 23*A*). Every cat that we examined had such structures, but there was considerable variability in their exact position. In some animals, one end was attached to the corner of the main nucleus, while in others, no such continuity could be established. In cresyl violet-stained sections (Fig. 23*B*), the claustrum parvum was seen to contain cells similar in size and staining properties to those of the main nucleus, but each cell body was highly elongated along the main axis of the rod. It is likely that their dendritic trees also are elongated and tightly bundled together.

The connections of the claustrum parvum are the same as those of the main nucleus. Injections of [^{3}H]proline into visual cortex that gave rise to a focus of terminal labeling within the claustrum proper also often produced a small projection zone within part of the claustrum parvum (Fig. 23*C*). Projections from the lateral hypothalamus and the nucleus centralis thalami were identified also. Injections of HRP into visual cortex labeled cells in part of the claustrum parvum (Fig. 23*D*) as well as in the main nucleus. Although we have not worked out the details, it is likely that the claustrum parvum harbors at least a partial map of the visual field.

Discussion

This study has shown that the cat's dorsocaudal claustrum receives a convergent input from several visual cortical areas and that it, in turn, projects back to these same areas. The corticoclaustral loop, which is organized retinotopically in both of its limbs, is subject to nonvisual influences via ascending pathways from the hypothalamus, intralaminar thalamic nuclei, and probably also the locus coeruleus.

The autoradiographic results showed that the visual claustrum receives an input from at least five areas of visual cortex: 17, 18, 19, 21a, and PMLS, while two visual areas, PLLS and 20a, project to regions outside of the zone that we have defined as visual. We do not know

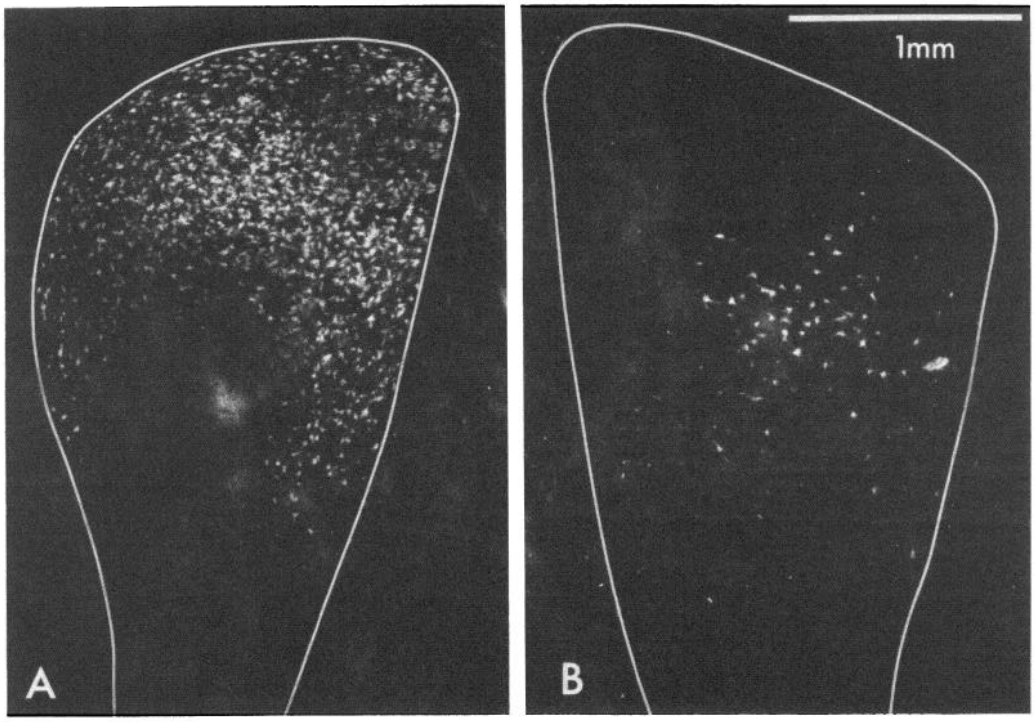

Figure 20. Cells of origin of the claustrocortical pathway. These are dark-field micrographs of the claustrum ipsilateral (*A*) and contralateral (*B*) to a very large HRP injection in the left visual cortex. Ipsilaterally, cells throughout the visual claustrum are labeled, although they are fewer and more weakly labeled in a ventral core-like region. Contralaterally, the labeled cells tend to lie near the ventral border of the visual region. There is clearly considerable overlap between the two populations, however.

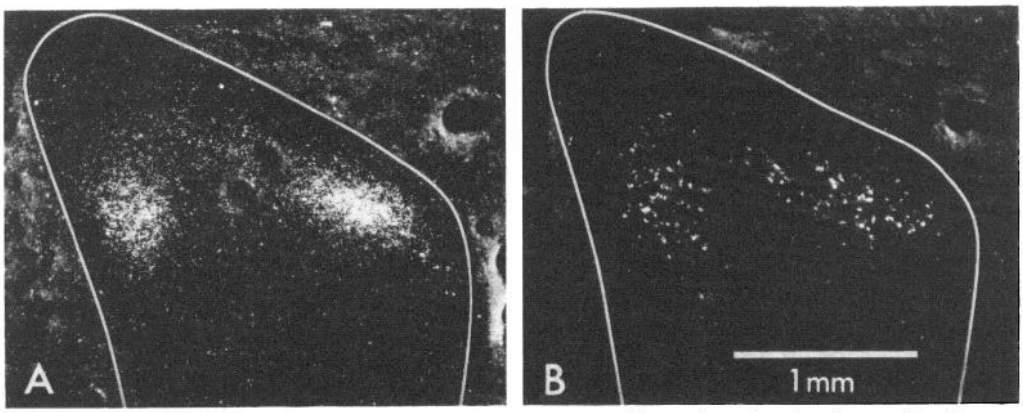

Figure 21. Double label experiment to show topographic reciprocity in the corticoclaustral loop. A mixture of [^{3}H]proline and HRP was injected into the periphery of area 17 on the left side (see text). *A*, Autoradiograph of the left claustrum, showing two patches of terminal labeling. These are actually the two ends of a curved band of label, the central sector of which lies in more caudal sections. *B*, Adjacent section reacted for HRP, showing labeled cells in the same two regions.

whether the remaining visual areas project to the claustrum.

The finding that the bulk of corticoclaustral afferents arise from pyramidal cells in layer VI of the cortex confirms work by Carey et al. (1980) in the tree shrew. In the cat, these form a tier of cells roughly in the middle of the thickness of the layer and, as shown by a double label experiment, are a separate population from those projecting to the LGN. (This latter conclusion was also reached by Olson and Graybiel, 1980.) There appears to be at least one anatomical difference between these two populations; the D-[^{3}H]aspartate transport experiments suggest that the LGN-projecting neurons have axon collaterals ascending to layer IV, while those projecting to the claustrum do not. Whether the corticogeniculate and corticoclaustral neurons also have distinct functional properties remains to be investigated, but it is hard to think what purpose could be served by the existence of anatomically separate populations unless it is to permit functional specialization.

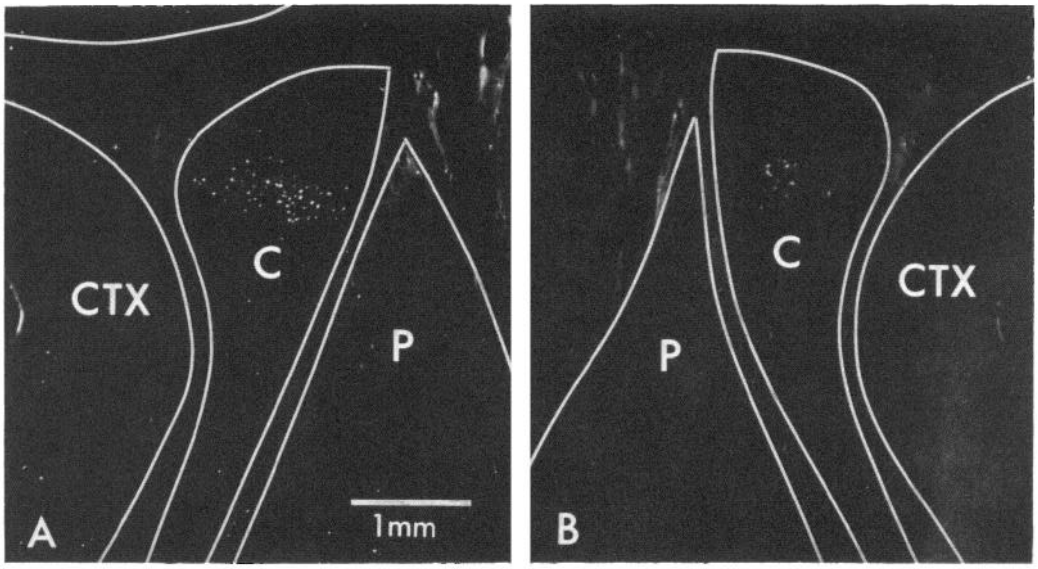

Figure 22. HRP-labeled cells in the claustrum ipsilateral (*A*) and contralateral (*B*) to a peroxidase injection in left area 19 at the representation of the area centralis. The labeled cells are at the ventral border of the visual region on both sides.

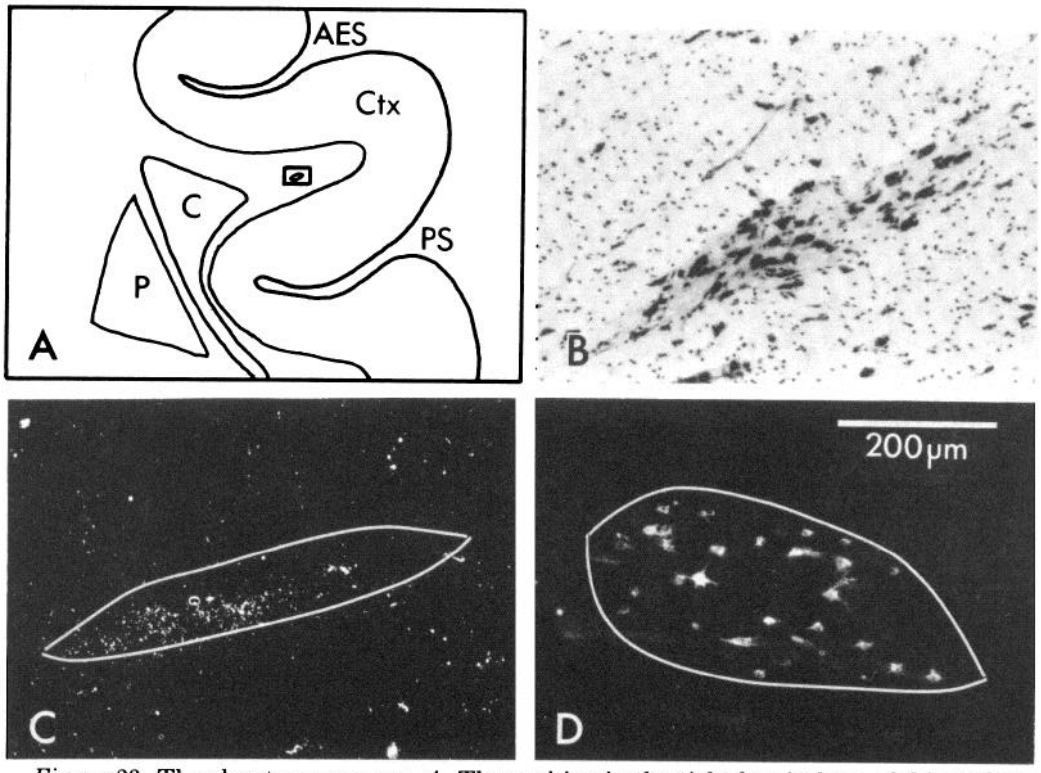

Figure 23. The claustrum parvum. *A*, The position in the right hemisphere of this outlying fragment of claustral tissue (*boxed area*) within the white matter dorsolateral to the main nucleus. *B*, Cresyl violet-stained section of the *boxed region* in *A*. The claustrum parvum is an extended rod, only part of which lies in the plane of this section. Note the fusiform shape of most of its neurons. *C*, Autoradiograph showing terminal labeling in a restricted part of the claustrum parvum after a [^{3}H]proline injection into area 17. *D*, HRP-labeled cells in the claustrum parvum after a large injection into visual cortex (same experiment as illustrated in Fig. 19). The claustrum parvum is unusually fat in this animal. The *scale* applies to *B*, *C*, and *D*.

In addition to the cells in layer VI, smaller numbers of labeled cells were found in other layers, especially at the layer III-IV border. These cells were particularly common in area PMLS. Since this area projects to the putamen and to the cortex overlying the claustrum, it seems possible that their labeling was an artifact caused by spread of the injected peroxidase out of the claustrum. This is an unlikely explanation for the labeling of supra-

granular cells in areas 17, 18, or 19, however, since these areas have no demonstrable projections to targets near the claustrum. The presence of occasional labeled cells in layer V, on the other hand, might be due to spread of peroxidase from the claustrum to the corticopontine tract, which arises from layer V pyramidal cells (Gibson et al., 1976) and, as described above, passes close by the claustrum.

Non-visual cortical areas do not project to the visual claustrum, but at least some of them have their own target zones within the nucleus. We have shown, for example, that the splenial gyrus, a neocortical component of the limbic system, projects to a region of the claustrum ventral to the visual area. Primary auditory cortex appears not to project to the claustrum at all and, according to a recent report by Olson and Graybiel (1980), does not receive a return projection from the claustrum either. Olson and Graybiel do describe, however, connections between the claustrum and the auditory association area Ep. The auditory region in the claustrum, as described by Olson and Graybiel, lies ventral and anterior to the visual region. Consistent with this, we found labeled cells in this zone after HRP injections of auditory cortex that probably included Ep. Olson and Graybiel have shown further that a region of dorsal claustrum anterior to the visual region is interconnected with the somatosensory cortex, and we have confirmed the general location of this region. The segregation of different sensory modalities into different regions of the claustrum appears to be a consistent feature of claustral organization.

Only two sources of subcortical afferents to the claustrum were confirmed by both anterograde and retrograde transport methods, the nucleus centralis thalami and the lateral hypothalamus. The nucleus centralis is a major intralaminar nucleus in the cat, and it contains neurons whose firing is associated with eye movements (Schlag et al., 1974). The same [^{3}H]proline injection that demonstrated the pathway from the nucleus centralis to the claustrum also gave rise to labeling in area 17 as well as other cortical areas. This finding tends to undermine any theory of claustral function in which its intralaminar afferents are the key feature, since they might just as well, one imagines, exert their influence directly on visual cortex.

Little can be said about the hypothalamic projection to the claustrum, since the zone of retrogradely labeled cells forms neither an anatomically nor physiologically defined subregion of the hypothalamus. The [^{3}H]proline injection into this region labeled not only the claustrum but also the dentate gyrus and various thalamic targets; undoubtedly, this part of the hypothalamus contains an assortment of cell types of differing connections and functions.

The visual claustrum receives no direct projection from the lateral geniculate nucleus, the lateral posterior-pulvinar complex or the medial geniculate nucleus, although the latter two nuclei do send axons that pass close by the claustrum on their way to other targets. Rapisarda et al. (1969) reported that visual evoked potentials in the dorsocaudal claustrum survived the destruction of the visual cortex but were eliminated by ablation of the LGN and hence postulated a direct geniculoclaustral pathway. Given their method of lesioning the cortex (application of silver nitrate), it seems likely that the periphery of area 17, deeply buried on the medial surface of the hemisphere, escaped destruction and that the surviving responses were due to corticoclaustral input from this region.

We did not make [^{3}H]proline injections into the mesencephalic central grey matter or into the locus coeruleus and hence cannot say, on the basis of our results, whether they in fact project to the claustrum as suggested by the HRP experiments. Pickel et al. (1974) have however demonstrated a coeruleoclaustral projection in the rat by autoradiography.

The internal structure of the claustrum is relatively simple. The most common cell type is the large, spiny dendrite neuron. As the electron microscopic degeneration study showed that the majority of corticoclaustral terminals contacted spines, it is clear that this cell type receives the bulk of the cortical input. Furthermore, since the great majority of cells in the claustrum project back to visual cortex, as shown by peroxidase experiments, it seems likely that the basic corticoclaustral loop involves only one synapse in the claustrum. The second cell type, a small spine-free stellate cell, receives a lesser portion of the cortical input onto its beaded dendrites. Cell size comparisons suggest that this cell type does not project back to visual cortex (and hence probably has no extraclaustral projection). This fits well with the Golgi observation that this cell has a locally arborizing axon which presumably contacts principal cells in the same region of the claustrum. The Golgi and electron microscopic appearance of the claustrum resembles a highly simplified version of the cerebral cortex without the lamination or the specialization of cell shape that are associated with lamination. By analogy with the cortex (LeVay, 1973), one might speculate that the principal cells' axons form Gray's type 1 synapses both in their distant arborizations in the cortex and in their local collateral systems, while the spine-free interneurons would form Gray's type 2 synapses.

Neither the Golgi study nor the transport experiments produced any evidence for connections between different regions of the claustrum. None of these methods, however, is ideally suited for the study of short connections, and thus we cannot rule out the possibility that some interconnection of the visual with the adjacent non-visual portions of the claustrum may exist.

The finding that the dorsocaudal claustrum projects back to the cortex confirms the retrograde degeneration study of Narkiewicz (1964), and the retinotopic nature of this projection also has been reported by other investigators (tree shrew, Carey et al., 1979; cat, Olson and Graybiel, 1980). The combined injection of [^{3}H]proline and HRP at a physiologically defined site in visual cortex showed particularly clearly that a part of the cortex dealing with a certain region of visual space receives information from the claustrum concerning the same region.

One of the most intriguing aspects of the corticoclaustral circuit is the laminar pattern of termination of the claustrocortical axons. All four animals studied showed the same pattern: labeling was heaviest in layer IV with a subsidiary band in layer VI. Layer V and the supragranular layers were labeled more lightly. Our findings

are not in entire agreement with two other reports (Carey et al., 1979; Olson and Graybiel, 1980) which have reported a major claustral input to layer I. In our experiments, this was the most weakly labeled layer, although it certainly was labeled above background. The conclusions of Carey et al. (1979) were based on HRP injections (in the tree shrew and galago) that were thought to be restricted to layer I. A later autoradiographic study from the same laboratory (Carey et al., 1980) showed the major input (in the tree shrew) to be to layer IV, as we find in the cat. As to the autoradiographic study by Olson and Graybiel, we do not think that the difference in the results can be explained by differences in survival time, since we obtained similar patterns with survivals ranging from 1 to 7 days. We can suggest only that the heavy layer I projection reported by Olson and Graybiel may have been caused by spread of the injected label to some nearby cortical area.

Thus the same layers (IV and VI) that are innervated most densely from the claustrum also receive the bulk of the geniculocortical projection (LeVay and Gilbert, 1976). This is in contrast to other afferent systems, which tend to avoid layer IV. The laminar arrangement of the corticoclaustral loop bears a striking resemblance to that of the loop between the cortex and the LGN.

We could not demonstrate any subcortical projections from the claustrum. Only the lateral posterior nucleus was labeled after [^{3}H]proline injections into the claustrum, and the reciprocal HRP experiment showed that this projection actually arose from deep layers of nearby cortex rather than from the claustrum itself. Trojanowski and Jacobson (1975) have reported finding peroxidase-labeled cells in the claustrum after injections into the monkey's pulvinar complex, but the injections in that study were so large and poorly localized that one may suspect that there was involvement of the optic radiation. As to the other reported targets of claustral axons, the putamen (Druga, 1972; Flindt-Egebak and Olsen, 1979) and the dorsomedial and suprageniculate nuclei of the thalamus (Flindt-Egebak and Olsen, 1979), we could find no evidence for transported label in these structures. Either these projections arise from non-visual areas of the claustrum or the previous results were due to damage to fibers of passage or to spread of injected label.

The present study has described the anatomy of a simple, probably monosynaptic loop between the visual cortex and the visual claustrum. What functional significance could such a loop have? First, the information passing through this loop might be modified by the non-cortical afferents to the claustrum. Second, because the claustrum receives a convergent projection from several visual cortical areas, it forms one route by which these areas may influence each other. Finally, although the corticoclaustral projection originates in layer VI, the return pathway terminates most heavily in layer IV, so that the entire loop may be considered a kind of interlaminar pathway. This forms an alternative to the direct projection from layer VI to layer IV (Gilbert and Wiesel, 1979). In this connection, it is interesting that the corticoclaustral neurons themselves appear not to possess collateral projections to layer IV.

The neurons of the dorsocaudal claustrum might be thought of as a group of cortical interneurons displaced to a distant site. Although the reason for this displacement remains obscure, it does offer the opportunity to study in isolation one synaptic link in the cortical processing of visual information.

References

Baughman, R. W., and C. D. Gilbert (1980) Aspartate and glutamate as possible neurotransmitters of cells in layer VI of the visual cortex. Nature *287:* 848–850.

Carey, R. G., D. Fitzpatrick, and I. T. Diamond (1979) Layer I of striate cortex of *Tupaia glis* and *Galago senegalensis*: Projections from thalamus and claustrum revealed by retrograde transport of horseradish peroxidase. J. Comp. Neurol. *186:* 393–438.

Carey, R. G., M. F. Bear, and I. T. Diamond (1980) The laminar organization of the reciprocal projections between the claustrum and striate cortex in the tree shrew, *Tupaia glis*. Brain Res. *184:* 193–198.

Carman, J. B., W. M. Cowan, and T. P S. Powell (1964) The cortical projection upon the claustrum. J. Neurol. Neurosurg. Psychiatry *27:* 46–51.

Colonnier, M. (1964) The tangential organization of the visual cortex. J. Anat. *98:* 327–344.

Colonnier, M. (1968) Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscope study. Brain Res. *9:* 268–287.

Druga, R. (1966a) The claustrum of the cat (*Felis domestica*). Folia Morphol. (Praha) *14:* 7–16.

Druga, R. (1966b) Cortico-claustral connections. I. Fronto-claustral connections. Folia Morphol. (Praha) *14:* 391–399.

Druga, R. (1968) Cortico-claustral connections. II. Connections from the parietal, temporal and occipital cortex to the claustrum. Folia Morphol. (Praha) *16:* 142–149.

Druga, R. (1972) Efferent projections from the claustrum (an experimental study using Nauta's method). Folia Morphol. (Praha) *20:* 163–165.

Flindt-Egebak, P., and R. B. Olsen (1979) Some efferent connections of the feline claustrum. Neurosci. Lett. (Suppl.) *1:* 159

Frank, E., W. A. Harris, and M. B. Kennedy (1980) Lysophosphatidyl choline facilitates labeling of CNS projections with horseradish peroxidase. J. Neurosci. Methods *2:* 183–189.

Gibson, A., G. Mower, J. Baker, and M. Glickstein (1976) Corticopontine visual cells in the cat. Soc. Neurosci. Abstr. *2:* 1112.

Gilbert, C. D., and J. P. Kelly (1975) The projections of cells in different layers of the cat's visual cortex. J. Comp. Neurol. *163:* 81–106.

Gilbert, C. D., and T. N. Wiesel (1979) Morphology and intracortical projections of functionally characterized neurons in the cat visual cortex. Nature *280:* 120–125.

Gray, E. G. (1959) Axosomatic and axodendritic synapses of the cerebral cortex: An electron microscope study. J. Anat. *93:* 420–434.

Hirasawa, K., S. Okano, and S. Kamio (1939) Beitrag zur Kenntnis über die corticalen extrapyramidalen Fasern der Area temporalis superior (area 22) beim Affen. Z. Mikrosk. Anat. Forsch. *44:* 74–84.

Jayaraman, A., and B. V. Updyke (1979) Organization of visual cortical projections to the claustrum in the cat. Brain Res. *178:* 107–115.

Juraniec, J., O. Narkiewicz, and T. Wrzolkowa (1971) Axon terminals in the claustrum of the cat—an electron microscope study. Brain Res. *35:* 277–282.

Kalia, M., and D. Whitteridge (1973) The visual areas in the splenial sulcus of the cat. J. Physiol. (Lond.) *232:* 275–283.

Kievit, J., and H. G. J. M. Kuypers (1975) Subcortical afferents to the frontal lobe in the rhesus monkey studied by means of

retrograde horseradish peroxidase transport. Brain Res. *85:* 261–266.

Landau, E. (1936) Quelques nouvelles considerations sur l'avant-mur. Arch. Anat. Histol. Embryol. (Strasb.) *23:* 165–181.

LeVay, S. (1973) Synaptic patterns in the visual cortex of the cat and monkey. Electron microscopy of Golgi preparations. J. Comp. Neurol. *155:* 15–42.

LeVay, S., and C. D. Gilbert (1976) Laminar patterns of geniculocortical projection in the cat. Brain Res. *113:* 1–19.

LeVay, S., and H. Sherk (1980a) The cortico-claustral loop. Exp. Brain Res. *41:* A8–A9.

LeVay, S., and H. Sherk (1980b) Visual area in cat claustrum. 2: Structure, receptive fields and outputs. Soc. Neurosci. Abstr. *6:* 482.

LeVay, S., and H. Sherk (1981) The visual claustrum of the cat. II. The visual field map. J. Neurosci. *1:* 981–992.

Lund, J. S. (1973) Organization of neurons in the visual cortex, area 17, of the monkey (*Macaca mulatta*). J. Comp. Neurol. *147:* 455–495.

Malmgren, L., and Y. Olsson (1978) A sensitive method for histochemical demonstration of horseradish peroxidase following retrograde axonal transport. Brain Res. *148:* 279–294.

Merzenich, M. M., P. L. Knight, and G. L. Roth (1975) Representation of cochlea within primary auditory cortex in the cat. J. Neurophysiol. *38:* 231–249.

Mettler, F. A. (1935) Corticofugal fiber connections of the cortex of *Macaca mullatta*. The frontal region. J. Comp. Neurol. *61:* 509–542.

Narkiewicz, O. (1964) Degenerations in the claustrum after regional neocortical ablations in the cat. J. Comp. Neurol. *123:* 335–356.

Nevin, T. T., D. Tanaka, and W. L. R. Cruce (1978) A modified Epon embedding and counterstaining procedure for light microscopic examination of Golgi impregnated neurons in vertebrates. Neurosci. Lett. *7:* 307–312.

Norita, M. (1977) Demonstration of bilateral claustro-cortical connections in the cat with the method of retrograde axonal transport of horseradish peroxidase. Arch. Histol. Jap. *40:* 1–10.

O'Leary, J. L. (1941) Structure of the area striata of the cat. J. Comp. Neurol. *75:* 131–164.

Olson, C. R., and A. M. Graybiel (1980) Sensory maps in the claustrum of the cat. Nature *288:* 479–481.

Palmer, L. A., A. C. Rosenquist, and R. J. Tusa (1978) The retinotopic organization of the lateral suprasylvian areas in the cat. J. Comp. Neurol. *177:* 237–256.

Pettigrew, J. D., M. L. Cooper, and G. G. Blasdel (1979) Improved use of tapetal reflection for eye-position monitoring. Invest. Ophthalmol. Vis. Sci. *18:* 490–495.

Pickel, V. M., M. Segal, and F. E. Bloom (1974) A radioautographic study of the efferent pathways of the nucleus locus coeruleus. J. Comp. Neurol. *155:* 15–42.

Rapisarda, C., A. Azzaroni, and F. Infantellina (1969) An electrophysiological analysis of the visual projections of the claustrum in unanaesthetized cats. Arch. Sci. Biol. (Bologna) *53:* 130–148.

Reinoso-Suárez, F. (1961) *Topographischer Hirnatlas der Katze*, Merck, Darmstadt, West Germany.

Riche, D., and J. Lanoir (1978) Some claustro-cortical connections in the cat and baboon as studied by retrograde horseradish peroxidase transport. J. Comp. Neurol. *177:* 435–444.

Rogers, A. W. (1973) *Techniques of Autoradiography*, Elsevier, Amsterdam.

Sanderson, K. J. (1971) The projection of the visual field to the lateral geniculate and medial interlaminar nuclei in the cat. J. Comp. Neurol. *143:* 101–118.

Sanides, D., and C. S. Buchholtz (1979) Identification of the projection from the visual cortex to the claustrum by anterograde axonal transport in the cat. Exp. Brain Res. *34:* 197–200.

Schlag, J., I. Lehtinen, and M. Schlag-Rey (1974) Neuronal activity before and during eye movements in thalamic internal medullary lamina of the cat. J. Neurophysiol. *37:* 982–995.

Sherk, H., and S. LeVay (1980) Visual area in cat claustrum. 1. Inputs and topography. Soc. Neurosci. Abstr. *6:* 482.

Sherk, H., and S. LeVay (1981a) Visual claustrum: Topography and receptive field properties in the cat. Science *212:* 87–89.

Sherk, H., and S. LeVay (1981b) The visual claustrum of the cat. III. Receptive field properties. J. Neurosci. *1:* 993–1002.

Sims, K. B., D. L. Hoffman, S. I. Said, and E. Z. Zimmerman (1980) Vasoactive intestinal polypeptide (VIP) in mouse and rat brain: An immunocytochemical study. Brain Res. *186:* 165–183.

Spatz, W. B. (1975) Thalamic and other subcortical projections to area MT (visual area of superior temporal sulcus) in the marmoset *Callithrix jacchus*. Brain Res. *99:* 127–134.

Squatrito, S., P. P. Battaglini, C. Galletti, and E. Riva Sansaverino (1980) Autoradiographic evidence for projections from cortical visual areas 17, 18, 19 and the Clare-Bishop area to the ipsilateral claustrum in the cat. Neurosci. Lett. *19:* 265–269.

Streit, P. (1980) Selective retrograde labeling indicating the transmitter of neuronal pathways. J. Comp. Neurol. *191:* 429–463.

Trojanowski, J. Q., and S. Jacobson (1975) Peroxidase labeled subcortical afferents to pulvinar in rhesus monkey. Brain Res. *97:* 144–150.

Tusa, R. J., and L. A. Palmer (1980) Retinotopic organization of areas 20 and 21 in the cat. J. Comp. Neurol. *193:* 147–164.

Whitlock, D. G., and W. J. H. Nauta (1956) Subcortical projections from the temporal neocortex in *Macaca mulatta*. J. Comp. Neurol. *106:* 183–212.