The Journal of Neuroscience
May 1986, 6(5): 1452-1462

An Experimental Analysis of the Origins of Somatostatin-like Immunoreactivity in the Dentate Gyrus of the Rat

I. Bakst,* C. Avendano,† J. H. Morrison,‡ and D. G. Amaral*

*The Salk Institute for Biological Studies and The Clayton Foundation for Research—California Division, San Diego, California 92138, †Departmento de Morphologia, Universidad Autonoma de Madrid, Madrid, Spain, and ‡Division of Preclinical Neuroscience and Endocrinology, Research Institute of Scripps Clinic, La Jolla, California 92037

In previous studies, fibers demonstrating somatostatin-like immunoreactivity were observed in the outer half of the molecular layer of the dentate gyrus in the rat and monkey. They occupy the same region as those of the perforant pathway that originates in the entorhinal cortex. Numerous somatostatin immunoreactive neuronal cell bodies were also observed in the hilar region, though stained axonal profiles could not be followed from these cells into the molecular layer. In the present study, several experimental procedures were employed to determine the origin of the somatostatin-positive fibers in the molecular layer.

Transection of the perforant path fibers resulted in such characteristic changes as shrinkage of the molecular layer and sprouting of AChE-positive fibers. There was no apparent decrease, however, in the density of somatostatin-positive fibers. In fact, since the stained fibers occupied a narrower band in the shrunken molecular layer, their density appeared greater. Injections of kainic acid into the hilar region produced a lesion of hilar neurons, including those positive for somatostatin. In the region of cell loss, there was a marked reduction of somatostatin-immunoreactive fibers in the ipsilateral molecular layer, with no detectable changes in the homotopic contralateral molecular layer. The distribution of AChE fibers, which presumably have an extrinsic origin, was not altered by the treatment. In a final series of experiments, the retrograde tracer wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was injected into the hilar region and sections were prepared for the simultaneous demonstration of the tracer and of somatostatin-like immunoreactivity. Somatostatin-positive neurons demonstrating WGA-HRP reaction product were observed primarily in the ipsilateral hilar region, but a few double-labeled cells were also seen in the same area of the contralateral side.

These studies indicate that a population of intrinsic neurons located in the polymorphic layer of the dentate gyrus projects to the outer half of the ipsilateral molecular layer. A similar, but very much smaller, projection also extends to the contralateral dentate gyrus. Taken together, these projections appear to account for much of the somatostatin-like immunoreactivity in the molecular layer of the dentate gyrus.

In recent studies (Bakst et al., 1985; Morrison et al., 1982), an antiserum directed against somatostatin $28_{(1-12)}$($SS28_{1-12}$) has revealed an extensive immunoreactive fiber system in the hippocampal formation of the rat and monkey. While the fibers are observed in all hippocampal fields, the most prominent plexus occupies the outer two-thirds of the molecular layer of the dentate gyrus. This zone is the major site of termination of the perforant path that originates in the entorhinal cortex. Preliminary attempts with the combination of the retrograde tracer wheat germ agglutinin–horseradish peroxidase (WGA-HRP) and somatostatin immunohistochemistry, however, failed to demonstrate that the entorhinal cortex was the origin for the somatostatin-positive fibers in the molecular layer (Bakst et al., 1985).

Numerous neuronal cell bodies demonstrating somatostatin-like immunoreactivity have also been observed in the hippocampal formation (Bakst et al., 1985; Feldman and Lichtenstein, 1982; Kohler and Chan-Palay, 1982; Morrison et al., 1982; Vincent et al., 1985; Zimmer et al., 1983). In our previous studies, an antiserum directed against somatostatin 28 (SS28) revealed a much higher density of somatostatin immunoreactive cell bodies than antisera directed against somatostatin 14 (SS14) or $SS28_{1-12}$, and the highest density of the immunoreactive cell bodies occurred in the polymorphic layer or hilar region of the dentate gyrus. Since Golgi studies have shown that many of the cells in the hilar region are local circuit neurons, and at least some have axons or axon collaterals that reach the molecular layer, it is possible that they are the origin of the somatostatin innervation (Amaral, 1978; Ramon y Cajal, 1911). However, it has not been possible in our preparations to follow stained axons from the somatostatin immunoreactive cells of the hilar region into the molecular layer. Since normal immunohistochemical preparations do not give a clear indication of the origin of the somatostatin fibers, in the present series of studies we have employed several experimental approaches to determine whether they originate intrinsically, from the cells of the hilar region, or from some extrinsic afferent source.

Materials and Methods

Transections of the perforant pathway

In these experiments, 10 female Sprague-Dawley rats weighing 180–200 gm were used. The animals were anesthetized with chloral hydrate (0.35 gm/kg) and positioned in a Kopf stereotaxic apparatus. The perforant pathway was transected by lowering a 3-mm-wide blade (oriented mediolaterally and angled posteriorly 20° relative to the coronal plane) through a burr hole to the ventral surface of the brain (Amaral et al., 1980). The entrance point of the middle of the blade was 0.8 mm anterior

Received Aug. 12, 1985; revised Oct. 9, 1985; accepted Oct. 15, 1985.

This work was supported in part by NIH Grant NS-20004 to D.G.A., NIA Grant AG-0513 to H.J.M., and by a grant from the Sloan Foundation. The work was conducted in part by the Clayton Foundation for Research—California Division. D.G.A. is a Clayton Foundation Investigator.

We would like to thank Ms. Janet Kurz for histological assistance, Mr. Kris Trulock for photographic processing, and Ms. Pat Thomas for typing the manuscript. We would also like to express our appreciation to Dr. Robert Benoit, who generously supplied the antisera used in these studies.

Correspondence should be addressed to Dr. Bakst, Developmental Neurobiology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

to the transverse sinus and 4.8 mm lateral to the midline. The animals were then allowed a 10–14 d survival period.

Kainic acid injections

Sixteen female Sprague-Dawley rats weighing 200–300 gm were used for these studies. The animals were anesthetized and placed in the stereotaxic apparatus, and a burr hole was drilled above the intended injection site. Injections were aimed at the hilar region of the dentate gyrus at septal and temporal levels using coordinates from the atlas of Paxinos and Watson (1982). A volume of 0.5 μl of freshly prepared kainic acid solution (0.5, 1, or 1.5 mg/ml in 0.1 M phosphate buffer) was injected through a glass micropipette connected to an air-pressure delivery system (Amaral and Price, 1983). Animals in these experiments survived for 2 weeks after injection.

Double-labeling studies (WGA-HRP and somatostatin immunohistochemistry)

Six additional female rats were injected with the retrograde tracer wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). These animals were surgically prepared as above, and using coordinates from the atlas of Paxinos and Watson (1982), 50 nl injections of a 1% WGA-HRP solution (in distilled water) were placed through glass micropipettes into the dentate gyrus at different septotemporal levels. Animals in these experiments were sacrificed 24 hr after injection.

Perfusion

At the time of sacrifice, animals were anesthetized with an overdose of Nembutal and transcardially perfused. After a brief rinse with either a cold (4°C) saline (0.9% NaCl) solution or 1% paraformaldehyde solution in 0.1 M phosphate buffer, the animals were perfused with cold (4°C) 4% paraformaldehyde in 0.1 M phosphate buffer for either 8 min or 1 hr. Some of the animals were then perfused for 15 min with a 5% sucrose solution (in 0.1 M phosphate buffer) to remove excess fixative. The brains were then removed from the skull and placed in a solution of 20% glycerol (in 0.1 M phosphate buffer) for 24 hr prior to sectioning. In the cases with shorter perfusions of the fixative, the brains were not perfused with the sucrose solution but removed from the skull and placed in the same fixative for an additional 6 hr prior to being placed in the 20% glycerol solution.

Histological processing

Coronal sections through the entire hippocampal formation were cut at 50 μm (40 μm for the WGA-HRP experiments) with a freezing microtome. For the perforant path transection experiments, adjacent one-in-five series of sections were either processed immunohistochemically for the demonstration of somatostatin-like immunoreactivity, processed histochemically for the demonstration of AChE activity, or stained for Nissl substance. In the kainic acid experiments, the material was processed in a similar manner, though two antisera (one directed at SS28 to label neuronal cell bodies and one directed at $SS28_{1-12}$ to label fibers—see below) were used to stain separate series of sections.

Immunohistochemistry

Two primary antisera (S309, S320—kindly donated by Dr. Robert Benoit) were used in these studies. Details of the immunohistochemical procedures and characteristics and specificity of the antisera have previously been described in detail (Bakst et al., 1985; Benoit et al., 1982; Morrison et al., 1983; and references therein). In short, free-floating tissue sections were processed for the demonstration of somatostatin-like immunoreactivity by the unlabeled second antiserum method (PAP) of Sternberger et al. (1970) or by the avidin–biotin–peroxidase method using a Vectatstain kit (Vector Labs, Burlingame, CA). Antiserum S320 has an antigenic determinant that corresponds to the last 8 amino acids of $SS28_{1-12}$, reacts with $SS28_{1-12}$ exclusively, and preferentially demonstrates immunoreactive fibers, whereas antiserum S309 is directed against the first 14 amino acids of SS28, reacts predominantly with SS28 but has about 10% cross-reactivity with $SS28_{1-12}$, and demonstrates fibers and numerous neuronal cell bodies.

AChE histochemistry

The AChE preparations were processed according to the method described by Hedreen et al. (1985). A detailed description of this procedure is also presented in a previous paper (Bakst and Amaral, 1984).

Double-labeling procedures

The procedure for the simultaneous visualization of retrogradely transported WGA-HRP reaction product and somatostatin-like immunoreactivity closely followed the method outlined by Rye et al. (1984). Technical aspects and limitations of this procedure are discussed in Amaral and Kurz (1985). In brief, free-floating sections are first reacted with the chromogen tetramethyl benzidine to demonstrate the WGA-HRP. The reaction product is then stabilized and blackened by placing the sections in a solution containing diaminobenzidine, cobalt chloride, and hydrogen peroxide. The tissue is then thoroughly washed and processed for the localization of somatostatin-like immunoreactivity. The resulting preparations demonstrate fine, black reaction product indicative of retrogradely transported WGA-HRP and light brown cytoplasmic and vacuolar staining indicative of somatostatin immunoreactivity.

Analysis

In the perforant path transection experiments, the placement of the lesion was studied in the Nissl sections, and the completeness of the deafferentation was judged by the extent of such characteristic changes as shrinkage of the molecular layer and sprouting of AChE-positive fibers. Measurements of the width of the molecular layer, the width of the region containing somatostatin immunoreactive fibers and the width of the AChE-rich band were taken from the supra- and infrapyramidal blades of the dentate gyrus at a mid-septotemporal level in five operated rats both ipsi- and contralateral to the lesion.

The extents of the kainic acid lesions were determined by examining hilar cell loss in the Nissl-stained material. The loss of somatostatin-positive cell bodies in the same region was also assessed in adjacent sections processed with antiserum S309. In sections processed with antiserum S320, the density of fibers demonstrating somatostatin-like immunoreactivity was compared with levels distant to the lesion site on the ipsilateral side and with the homotopic region of the contralateral dentate gyrus.

The distribution of hilar cells containing WGA-HRP reaction product was examined in the dentate gyrus ipsi- and contralateral to the injection site. The locations of the double-labeled cells in the contralateral hilar region were plotted using a computer-linked X–Y plotter.

Results

Normal distribution of somatostatin-like immunoreactivity

The normal distribution of somatostatin-like immunoreactivity in the rat dentate gyrus is shown in Figure 1. In sections processed with antiserum S320, the outer two-thirds of the molecular layer contains a plexus of immunoreactive fibers (Fig. 1*A*), with the superficial half of this layer somewhat denser than the deep half (Figs. 1*A*, 2*C*). Stained fibers are scarce in the inner third of the molecular layer and in the granule cell layer, and only scattered fibers are observed in the hilar region. Sections processed with antiserum S309 (Fig. 1*B*) demonstrate numerous immunoreactive cell bodies scattered throughout the hilar region of the dentate gyrus. There is a tendency for greater fiber density and more immunoreactive cell bodies at temporal levels of the dentate gyrus.

Perforant pathway transections

Transection of the perforant pathway does not reduce the somatostatin-like immunoreactivity in the molecular layer. Of the 10 animals prepared with lesions, 8 had appropriately placed lesions, and all histological procedures were successfully carried out. In each of these cases, the thickness of the molecular layer decreased by about 30% (based on 45 measurements from the somatostatin preparations and 36 measurements from the AChE preparations—Figs. 2 and 3). The outer two-thirds of the molecular layer showed the greatest shrinkage; the somatostatin-positive region decreased in width by approximately 42% (40 measurements) and the AChE-positive outer band decreased by approximately 45% (35 measurements). The width of the inner, unstained portion of the molecular layer did not change appreciably.

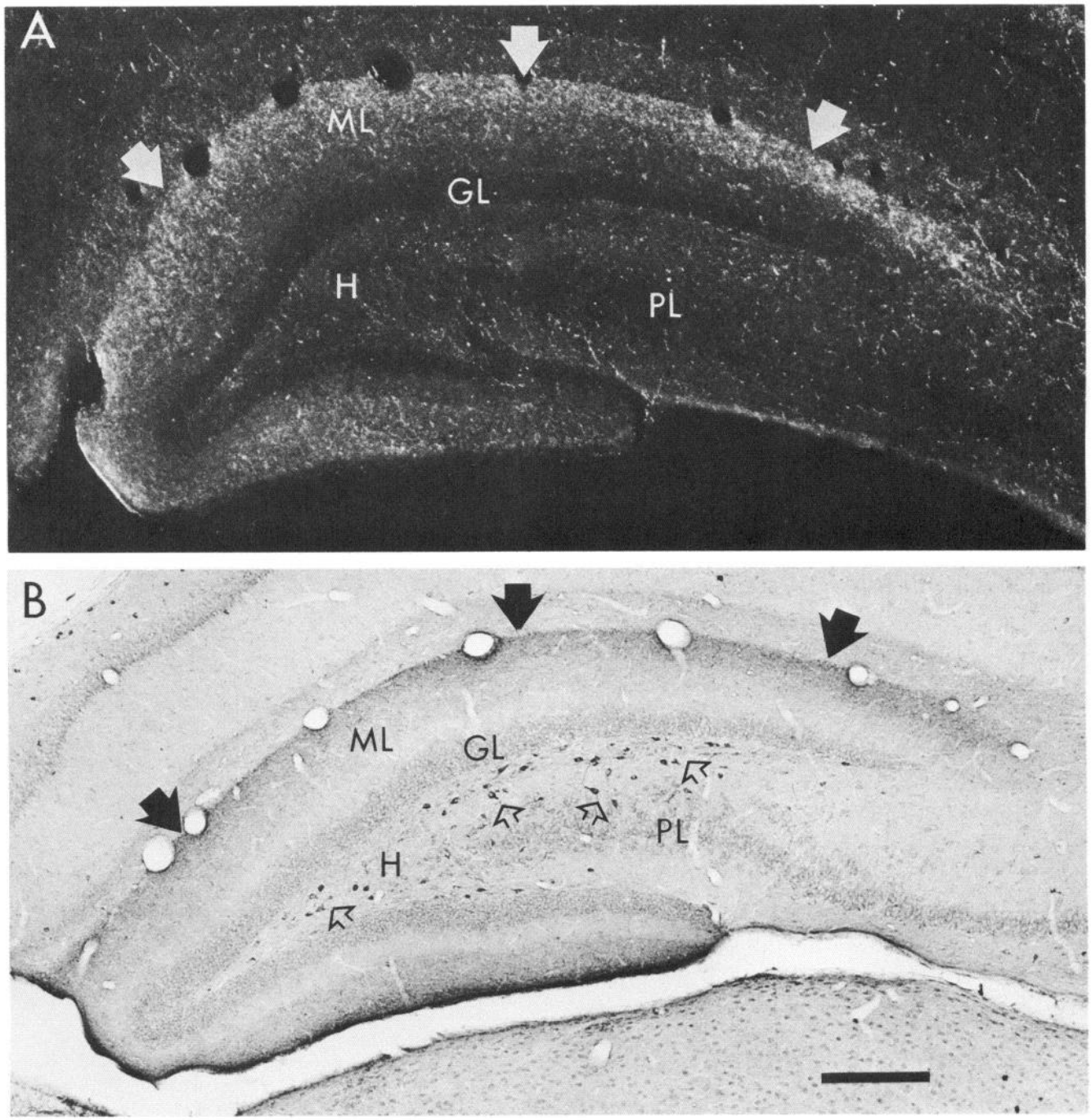

Figure 1. Normal distribution of somatostatin-like immunoreactivity in the dentate gyrus of the rat. *A,* Dark-field photomicrograph of a coronal section through the rostral dentate gyrus that has been processed with antiserum S320. Fiber and terminal staining are present in the outer two-thirds of the molecular layer. The hippocampal fissure is indicated by *bold white arrows. B,* Section processed with a cocktail of antisera S320 and S309. In this case, the bright-field photomicrograph demonstrates the distribution of somatostatin-positive neuronal cell bodies in the hilar region (*open arrows* indicate labeled cells). Abbreviations (which apply to all other illustrations): *GL,* granule cell layer; *H,* hilus or polymorphic layer of the dentate gyrus; *ML,* molecular layer of the dentate gyrus; *PL,* pyramidal cell layer of the hippocampus. Calibration marker, 250 μm.

It is evident from Figures 2 and 3 that the distribution of somatostatin-positive fibers undergoes changes that parallel those affecting the AChE-positive fibers; in each case, the band of stained fibers is substantially thinner, but the density appears greater. This characteristic response to deafferentation of the dentate gyrus (Lynch et al., 1972) is presumably due to the same number of fibers being more closely packed in the shrunken molecular layer. Wagner et al. (1983) have demonstrated, in fact, that while the shrinkage of the molecular layer also appears to produce a higher density of glutamate decarboxylase and cytochrome oxidase, there is no net increase in the levels of these enzymes.

Kainic acid injections

The hilar cells of the dentate gyrus are highly sensitive to the neurotoxic effects of kainic acid (Fonnum and Walaas, 1978; Kohler and Schwarcz, 1983; Nadler and Cuthbertson, 1980; Nadler et al., 1980a, b; Sloviter and Damiano, 1981). In the

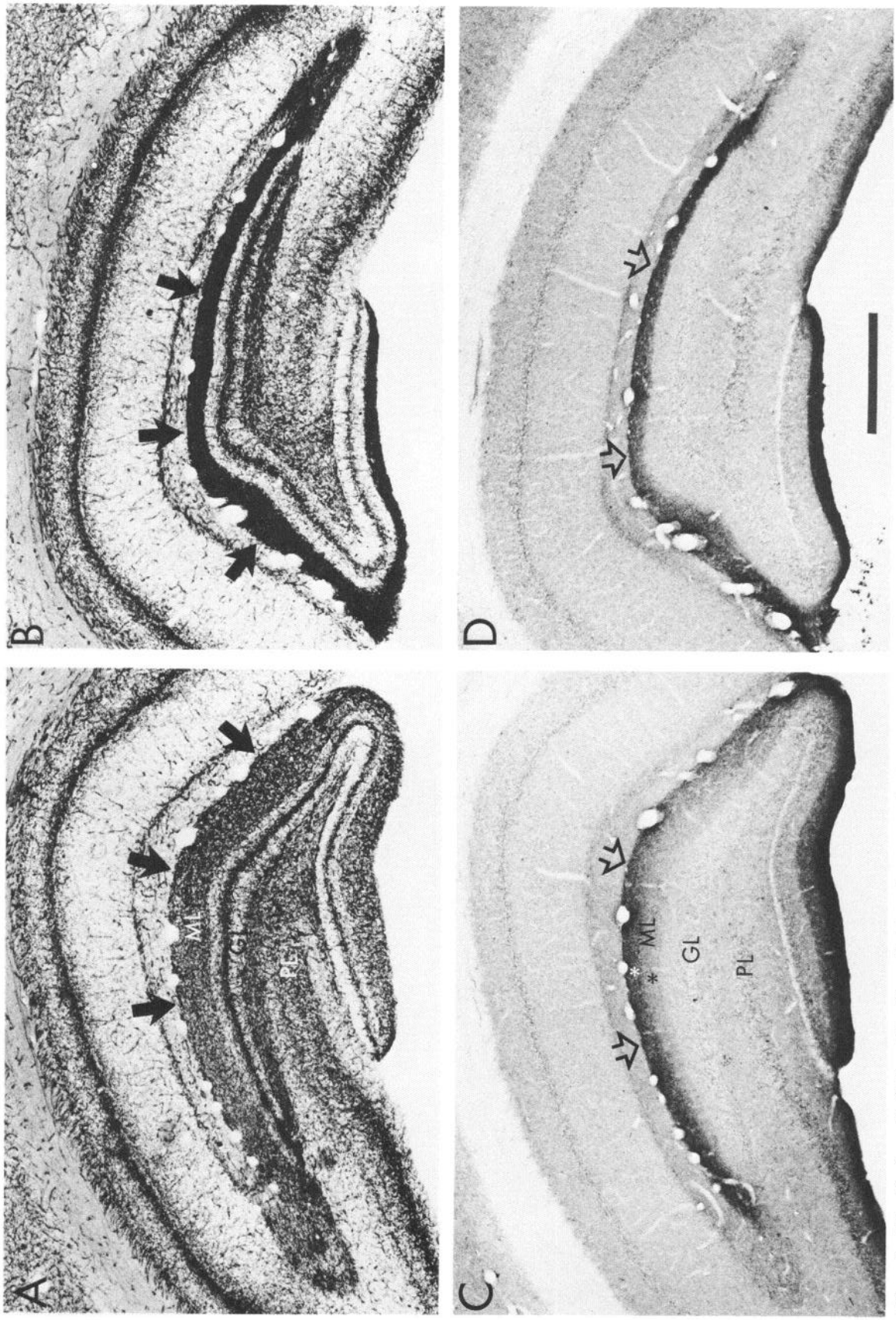

Figure 2. Distribution of AChE-positive fibers (*A, B*) and somatostatin-immunoreactive fibers (*C, D*) following a unilateral transection of the perforant path. *B* and *C*, Ipsilateral to the transection; *A* and *C*, contralateral side of the same section. The hippocampal fissure is marked by *bold arrows*. It is clear that the molecular layer has undergone substantial shrinkage, which has resulted in an intensification of staining of the outer AChE- and somatostatin-positive bands. The two sublayers evident in the somatostatin staining in *C* are not differentiable in *D*. Calibration marker, 500 μm.

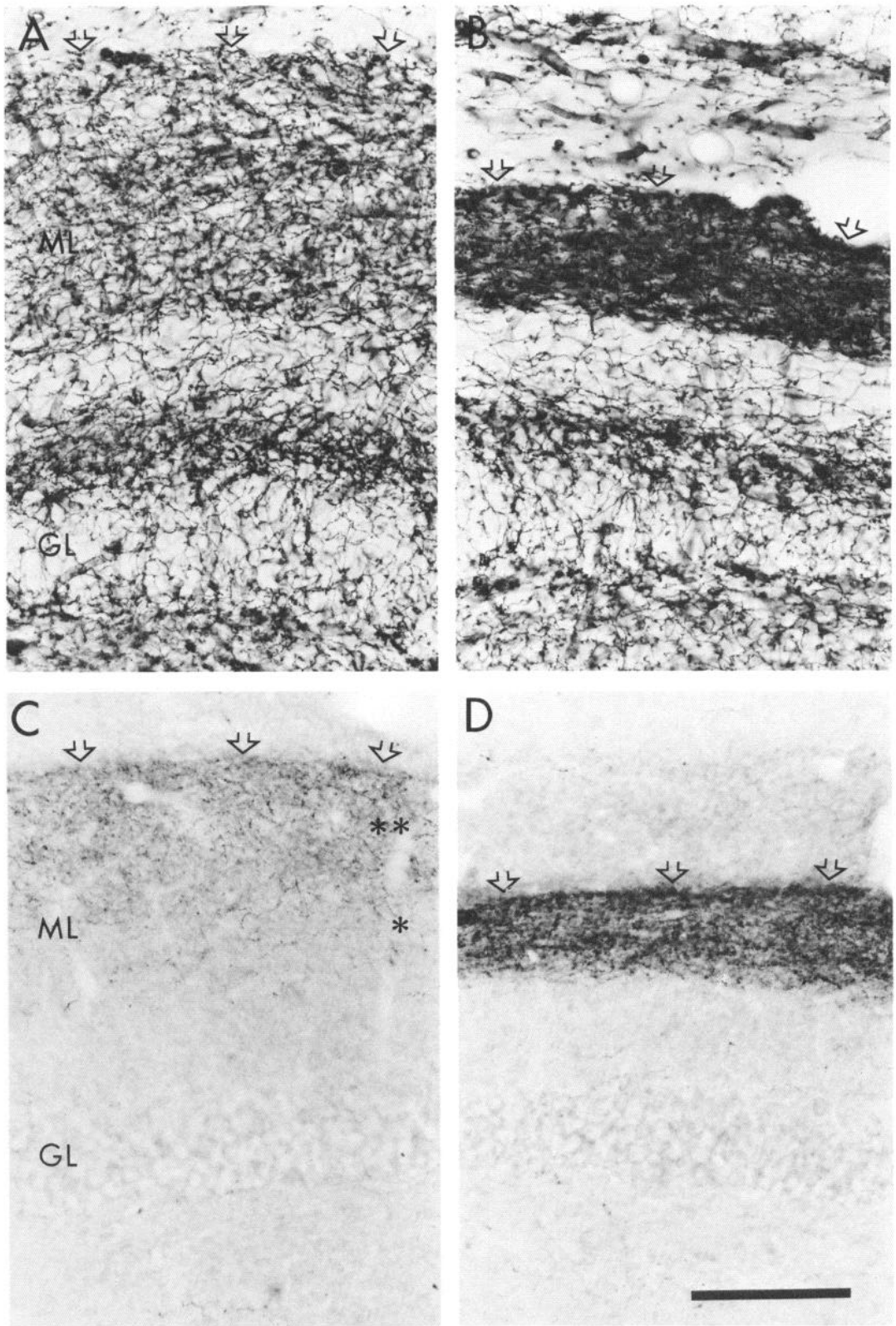

Figure 3. Higher magnification photomicrographs of the suprapyramidal blades of the sections shown in Figure 2. *A* and *B*, AChE preparation; *C* and *D*, somatostatin preparation. The right side is taken ipsilateral to the perforant path transection. The hippocampal fissure is marked with *open arrows*. *Asterisks* in *C* mark the sublayers of somatostatin staining. The shrinkage of the molecular layer is clear, as is the intensification of the staining of the outer portion of the molecular layer. Calibration marker, 100 μm.

present studies, injection of kainic acid into the dentate gyrus created a focal lesion of the hilar cells and, in some cases, of the cells of the adjacent pyramidal cell layer of the hippocampus. There is also an apparent loss of volume of the hilar region resulting in the closer apposition of the blades of the granule cell layer. There was little or no damage, however, to the granule cell layer itself. The extent of the damage resulting from the injection was quite variable and, in part, could be related to the volume and concentration of the kainic acid injected and the location of the focus of the injection. As noted by others (Schwob et al., 1980), we also observed lesions in distant structures such as the piriform cortex, entorhinal cortex, and the amygdaloid complex. It is unlikely, however, that these lesions contributed to changes in the distribution of somatostatin in the dentate gyrus, since our perforant path transections ruled out the entorhinal cortex as a source of the fibers and neither the piriform cortex nor the amygdaloid complex projects to the dentate gyrus. Of the injected animals, 10 had appropriately placed lesions and adequate histological processing.

In each of these cases, tissue processed with antiserum S320 demonstrated a substantial loss of somatostatin-like immunoreactivity in the molecular layer of the damaged region (Figs. 4*B*, 5*B*). Somatostatin-positive fibers were never completely eliminated, however, and there appeared to be a gradient of decreasing fiber loss both rostral and caudal to the kainic acid injection. The extent of decreased somatostatin-like immunoreactivity was spatially correlated with the loss of somatostatin-positive hilar cells as determined from adjacent sections processed with antiserum S309. Rostral and caudal to the focus of cell and fiber loss, there is a gradual return of somatostatin-positive cell bodies that parallels the graded return of immunoreactive fibers in the molecular layer. The labeled fibers closest to the lesion site are located along the hippocampal fissure and invade deeper regions at levels more distant from the lesion. There was a tendency for immunoreactive fibers to appear closer to the caudal end of the lesion than to the rostral end, indicating that the somatostatin-positive fibers tend to project rostrally. There are also indications of a transverse topography to this fiber system; in cases where the kainic acid injection was focused in the lateral aspect of the hilar region, somatostatin-immunoreactive cells were often still observed in the medial or deep aspect of the hilar region and immunoreactive fibers remained in the molecular layer above this area. Since the distribution of AChE-stained fibers (which are largely of extrinsic origin) was not altered by the kainic acid injection (Fig. 5, *C* and *D*), it is unlikely that the loss of somatostatin staining is due to a nonspecific toxic effect of the treatment. It should be noted that the distribution of AChE-positive fibers is maintained in the molecular layer despite the loss of AChE-positive hilar cells (Fig. 5, *C* and *D*).

WGA-HRP injections

In three of the six animals injected with WGA-HRP, the placement of the injections was appropriate and the histological processing was adequate to demonstrate double-labeled cells. The analysis of these preparations was complicated somewhat by the fact that the somatostatin-like immunoreactivity is not diffuse in the cytoplasm, but rather has a particulate appearance. In general, the retrogradely transported WGA-HRP was seen as fine, intensely black particles, whereas the somatostatin immunoreactivity was seen as larger brown particles (Fig. 6) but this distinction was often difficult to make.

In experiment HS4A, only the molecular layer of the medial aspect (crest) of the rostral dentate gyrus was involved by the injection. Double-labeled cells were observed in the hilar region of the dentate gyrus but not in other fields of the hippocampal formation, including the entorhinal cortex. In this case, a few double-labeled cells were also observed in the contralateral hilar region. We did not observe double-labeled cells in subcortical afferent structures such as the septal nuclei or the supramamillary area in any of these double-labeling experiments. In experiment HS1A, the injection involved all three layers of the dentate gyrus. Numerous double-labeled cells were seen in the hilar region ipsilateral to the injection. A number of double-labeled cells were also seen in the contralateral hilar region (Fig. 6). While it would be of value to determine the percentage of commissurally projecting neurons that also demonstrate somatostatin-like immunoreactivity, difficulties in discriminating single- from double-labeled cells in the present experiments preclude a quantitative analysis. Zimmer et al. (1983) have also demonstrated that some somatostatin-positive cells contribute to the commissural projection and estimate that the number is approximately 1% of the total hilar cells.

In the third case, experiment HS3A, the injection was placed at a caudal level of the dentate gyrus. Most of the double-labeled cells were observed caudal to the level of the injection, and we detected no double-labeled cells on the contralateral side. Taken together, these double-labeling studies support the findings from the kainic acid experiments that somatostatin-positive cells of the hilar region project to the molecular layer and further indicate that at least a few of these cells project commissurally as well.

Discussion

These studies were designed to determine the origin of the somatostatin-positive fibers in the outer two-thirds of the molecular layer of the dentate gyrus. They have indicated that the major portion of this projection arises intrinsically from local circuit neurons located in the polymorphic cell layer. A very meager projection also arises from the same region of the contralateral dentate gyrus.

Since the somatostatin-positive fibers are situated in the terminal region of the perforant path fibers from the entorhinal cortex, it was a reasonable possibility that the somatostatin fibers originated in this area as well. Transection of the perforant path fibers, however, led to no noticeable decline in the somatostatin staining in the molecular layer. This is consistent with our inability to double-label somatostatin-positive cells in the monkey entorhinal cortex following injections of WGA-HRP into the dentate gyrus (Bakst et al., 1985).

The only other major extrinsic afferent known to terminate in the outer two-thirds of the molecular layer is the septal projection. Our own observations, and those of Kohler and Eriksson (1984) and Vincent et al. (1985), indicate, however, that there are few, if any, somatostatin-positive cells in the portions of the medial septum and nucleus of the diagonal band that project to the dentate gyrus. Furthermore, we were not able to demonstrate double-labeled cells in the septal complex in the WGA-HRP experiments. Nor were we able to confirm the hypothalamic origin of hippocampal somatostatin immunoreactivity suggested by the lesion studies of Palkovits et al. (1982). In their study, parasagittal knife cuts in the lateral hypothalamus reduced somatostatin levels in the dorsal hippocampus by 46%, whereas bilateral fornix transections resulted in a 62% reduction. Kohler et al. (1984) have recently demonstrated a diffuse projection from the lateral hypothalamic area to several fields of the hippocampal formation. Most (95%) of the cells contributing to this projection were immunoreactive for alpha-melanocyte stimulating hormone, but a few cells (5%) that projected to the entorhinal cortex demonstrated somatostatin-like immunoreactivity. While we did not observe double-labeled somatostatin-positive cells in the lateral hypothalamus following injection of WGA-HRP into the dentate gyrus, it is possible that at least some of the remaining fibers observed in the kainic acid experiments have a hypothalamic origin. The major hypothalamic projection to the dentate gyrus, however, arises in the supra-

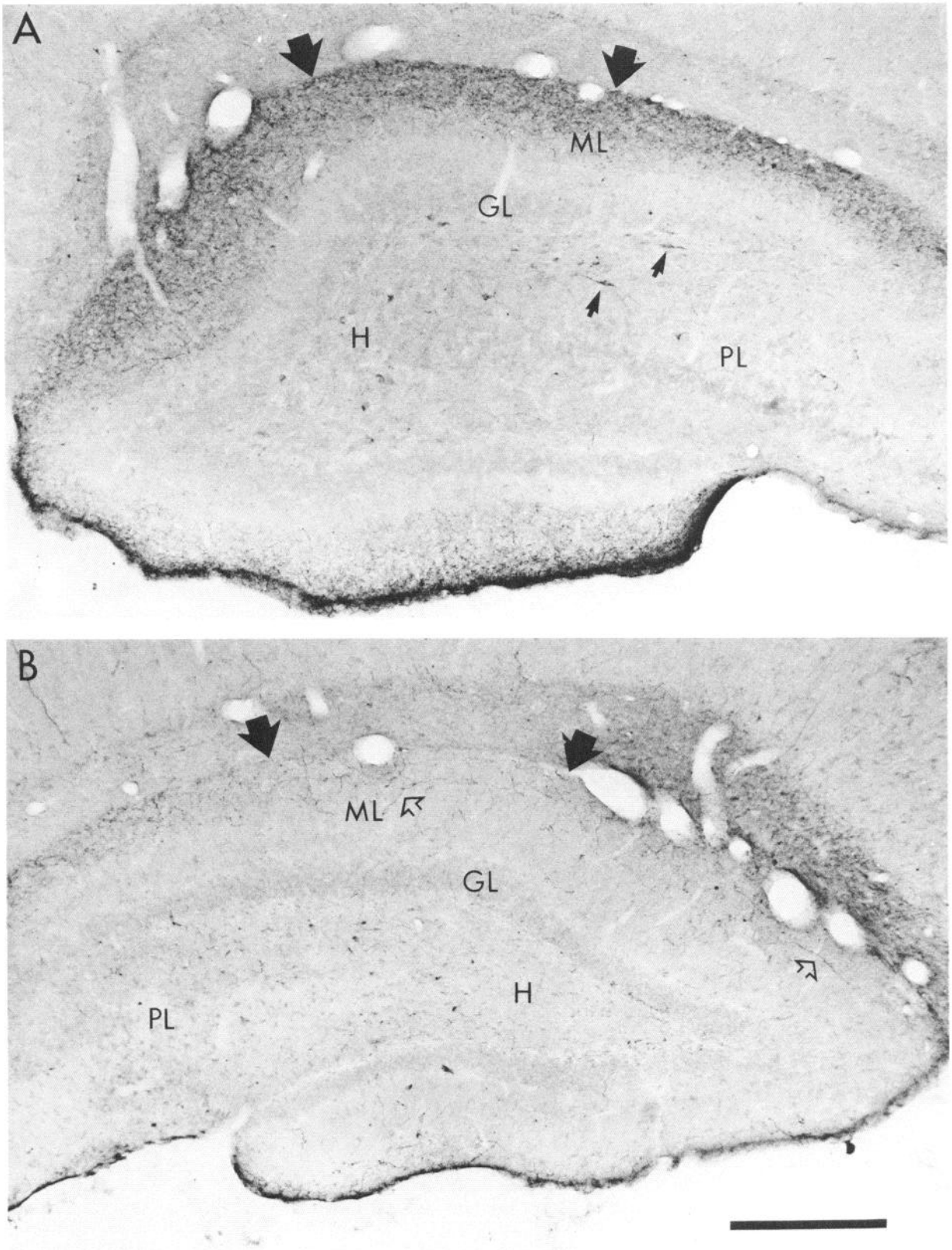

Figure 4. Coronal sections through the rostral dentate gyrus contralateral (*A*) and ipsilateral (*B*) to an intrahilar injection of kainic acid. In this case, 0.5 μl of kainic acid solution (0.5 mg/ml) was injected into the dentate gyrus and the tissue was processed with antiserum S320. *A,* Normal distribution of fiber and terminal staining is apparent in the outer portion of the molecular layer, and a few positive neuronal cell bodies (*arrows*) are seen in the hilar region. *B,* Fiber and terminal plexus are markedly decreased, though a few positive fibers remain (*open arrows*). Calibration marker, 250 μm.

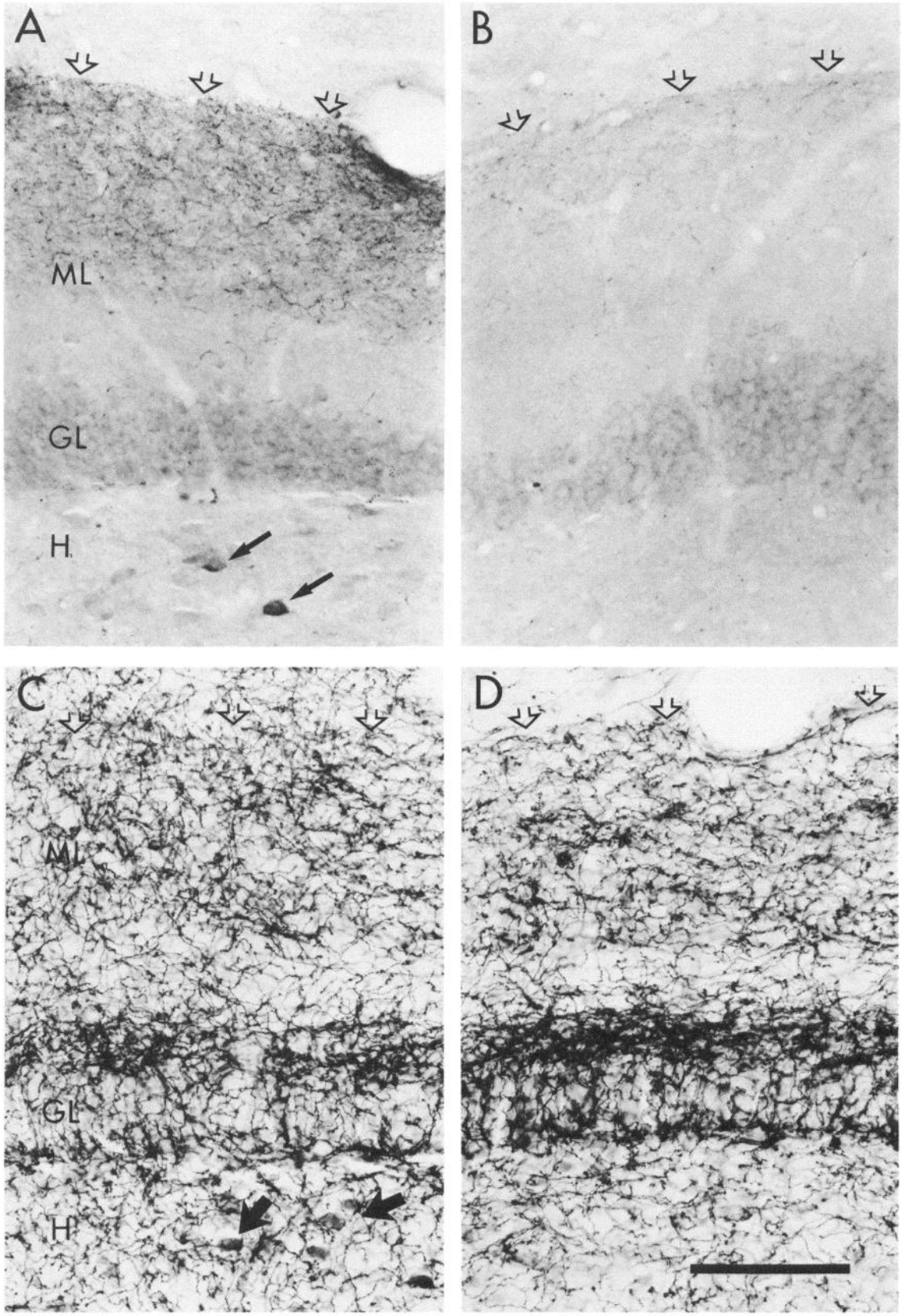

Figure 5. Higher magnification photomicrographs through the suprapyramidal blade of the dentate gyrus from the same experiment shown in Figure 4 processed either for the demonstration of somatostatin (*A, B*) or AChE (*C, D*). Panels *B* and *D* are ipsilateral to the injection site. A comparison of panels *A* and *B* demonstrates the loss of stomatostatin immunoreactivity resulting from the kainic acid injection. Positive cell bodies (*arrows* in *A*) in the contralateral hilar region are not seen on the side of the lesion. In panels *C* and *D*, no obvious differences are seen in the distribution of AChE-positive fibers, despite the loss of hilar cells that demonstrate AChE (*arrows* in *C*). Calibration marker, 100 μm.

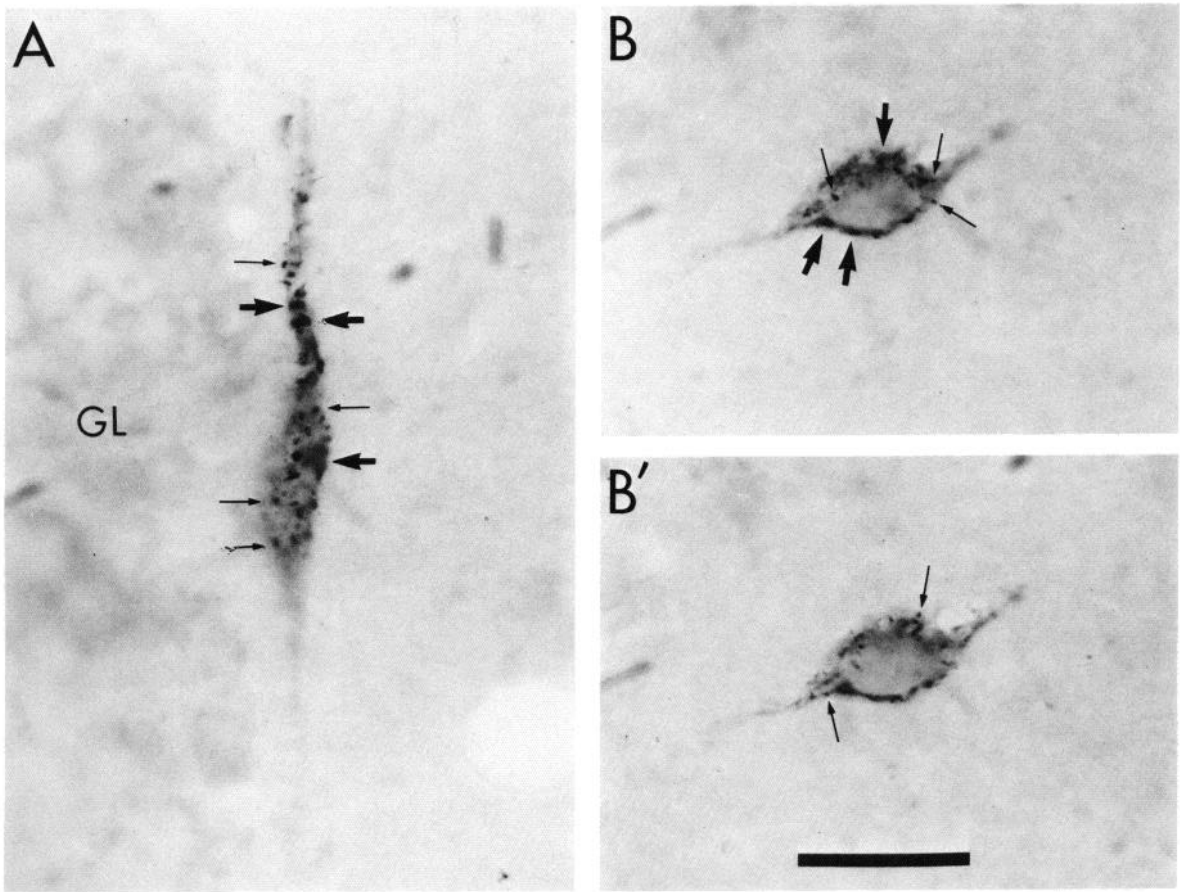

Figure 6. Examples of WGA-HRP and somatostatin double-labeled cells observed in the hilar region contralateral to the injection site. *Fine arrows* point to particles indicative of WGA-HRP reaction product, and *bold arrows* point to larger patches of somatostatin-like immunoreactivity. Panels *B* and *B'* show two planes of focus through the same double-labeled cell. Calibration marker, 20 μm.

mamillary region (Wyss et al., 1979), and cells in this area have not been shown to demonstrate somatostatin immunoreactivity (Vincent et al., 1985).

The only remaining source of the somatostatin fibers is the intrinsic somatostatin-positive cells of the polymorphic layer. Ramon y Cajal (1911) had noted from his Golgi studies that several of the polymorphic cells sent axons into the molecular layer, and this has been confirmed in more recent studies (Amaral, 1978). However, the magnitude of this projection was difficult to appreciate from Golgi studies alone. In the somatostatin immunohistochemical preparations, it is possible to follow labeled fibers from the hilar region through the granule cell layer and into the outer portion of the molecular layer, where they generally divide and ramify profusely (Fig. 7). This is a rare occurrence, however, and the fibers have not been followed back to their cells of origin. As we have noted previously (Bakst et al., 1985), the antiserum directed against $SS28_{1-12}$ demonstrates few neuronal cell bodies in the hilar region. While it seems likely that the somatostatin-positive cells demonstrated in the hilar region with the antiserum directed against SS28 give rise to the $SS28_{1-12}$-containing fibers in the molecular layer, this has not been experimentally confirmed. One possible explanation for the discrepancy in staining patterns with the two antisera is that SS28, which is produced in the cell bodies, is cleaved to $SS28_{1-12}$ and is rapidly transported into axons and terminals (Morrison et al., 1983).

In the present study, injections of kainic acid produced a focal loss of hilar cells, including those positive for somatostatin. Correlated with the area of cell damage was a nearly complete loss of somatostatin-positive fibers in the molecular layer. To control for nonspecific toxic effects of the kainic acid, the distribution of AChE-positive fibers, which originate extrinsically, was also studied, and the drug treatment appeared to have little or no effect on their organization.

Thus, each of these experimental approaches has provided data consistent with the conclusion that an intrinsic somatostatin projection originates from cells in the hilar region and terminates in the outer two-thirds of the molecular layer. It will be of interest to determine the synaptic organization of this projection. It is well established that fibers of the perforant path account for approximately 85% of the synapses in this region (Hoff et al., 1982; Matthews et al., 1976a, b). Aside from diffuse noradrenergic (Koda et al., 1978) and cholinergic terminals (Houser et al., 1983), only GABAergic fibers and terminals have been reported there (Ribak et al., 1978). The GABAergic fibers, though, may be of particular relevance to the somatostatin projection. It appears that the major portion of this GABAergic projection also originates intrinsically from cells located in the hilar region and from the dentate basket cells that lie at the border of the hilus and the granule cell layer (Ribak and Seress, 1983; Seress and Ribak, 1983). It is of interest, therefore, that Schmechel et al. (1984) have demonstrated that many of these GAD-positive cells are also immunoreactive for somatostatin. It is conceivable, therefore, that some of the somatostatin-positive fibers in the molecular layer are also GABAergic. It is equally clear, however, that not all GAD-immunoreactive cells and fibers are somatostatin-positive. The GABAergic dentate basket cells, for example, were only rarely somatostatin-positive in our preparations, and the dense GABAergic basket plexus that innervates the granule cell layer was not stained in the somatostatin preparations.

While the major somatostatin projection appears to arise from

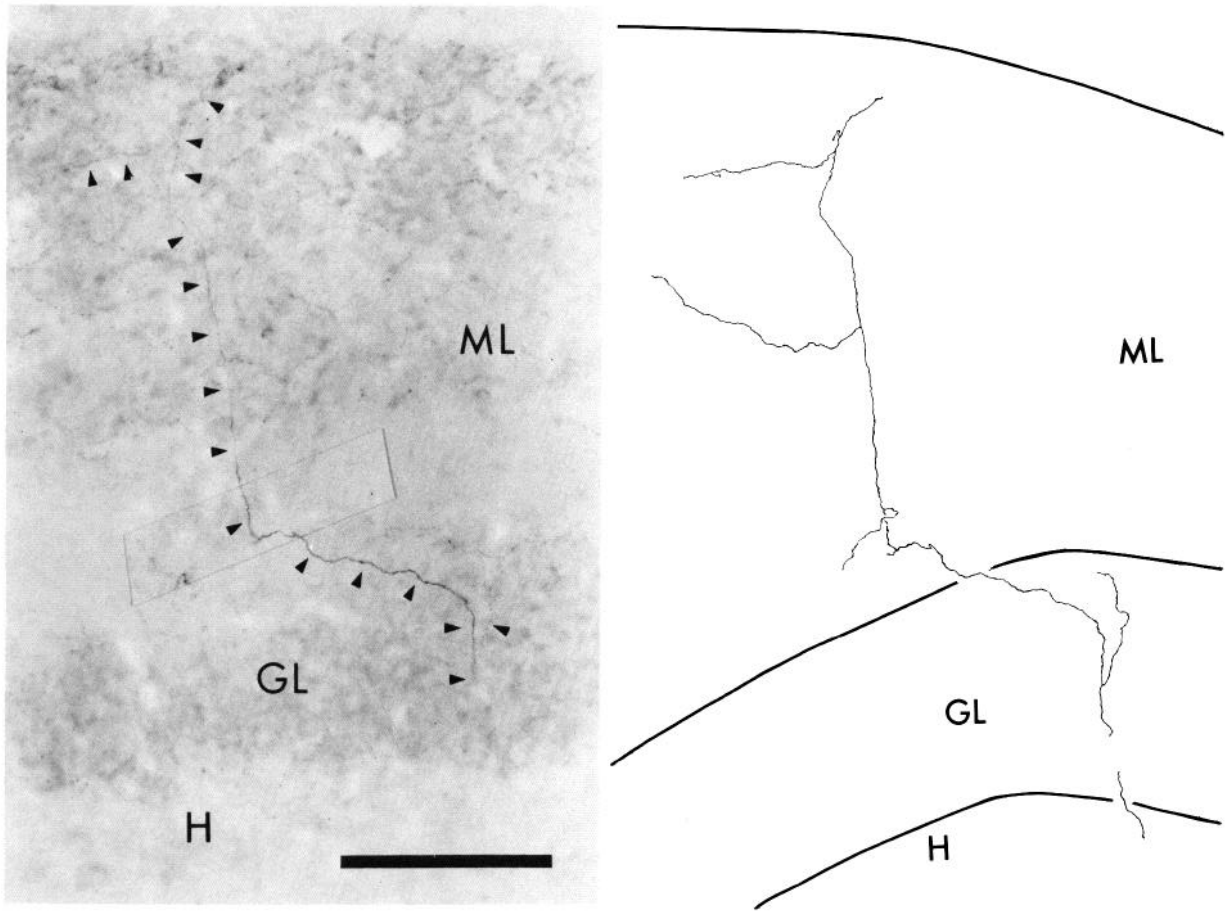

Figure 7. Photomicrograph (*left*) and camera lucida drawing (*right*) show a somatostatin-positive fiber (processed with antiserum S320) that is first seen in the hilus, travels through the granule cell layer, and bifurcates in the superficial portion of the molecular layer. Calibration marker, 50 μm.

the ipsilateral hilar region, a small component originates in the contralateral dentate gyrus as well. This is not surprising, since most associational connections in the hippocampal formation have a commissural counterpart. It is known, for example, that the commissural and associational projections to the dentate gyrus and to the hippocampus arise, in each case, from the same neurons (Laurberg and Sorensen, 1981; Swanson et al., 1980, 1981). What is not clear is the proportion of somatostatin-positive fibers in the commissural projection. As we noted above, since the major terminal field of the commissural connection to the dentate gyrus (the inner one-third of the molecular layer) is essentially free of somatostatin-positive fibers and our kainic acid injections caused little change in the contralateral distribution of somatostatin immunoreactivity, it appears that the commissural somatostatin projection is minor. Swanson et al. (1981) have estimated that 80% of the hilar cells give rise to a commissural projection and Zimmer et al. (1983) have estimated that 20–25% of the hilar cells are somatostatin-positive. Thus, if all somatostatin-positive cells projected commissurally, they could account for no more than 25% of the projection. Zimmer et al. (1983) estimated, however, that only 1% of the hilar cells are both somatostatin-positive and project commissurally.

We should also comment on why this projection has not previously been demonstrated, especially given the extensive anatomical analysis of the rodent hippocampal formation. Since the projection from the hilus to the outer portion of the molecular layer is relatively local, it would seem that in previous studies of the dentate gyrus it was simply obscured by the background of the anterograde tracers. One advantage of the immunohistochemical methods is that they offer the possibility of studying chemically defined intrinsic circuits that would be obscured by current tract-tracing methods. An appropriate adjunct to immunohistochemistry is the recently developed method employing the lectin *Phaseolus vulgaris* leucoagglutinin (PHA-L) as an anterogradely transported marker (Gerfen and Sawchenko, 1984). Using this highly localized tracing method, we have been able to demonstrate a projection from the hilar region to the outer half of the molecular layer both ipsilaterally and, albeit very lightly, contralaterally (Krzemieniewska and Amaral, unpublished observations).

Finally, the loss of cortical and hippocampal somatostatin has been correlated with the pathology of Alzheimer's disease (Davies and Terry, 1981; Davies et al., 1980; Rossor et al., 1980). The decrease in somatostatin levels is often as dramatic as the loss of cholinergic markers such as choline acetyltransferase. However, whereas the cholinergic innervation of the hippocampus is almost exclusively of extrinsic origin (Amaral and Kurz, 1985; Houser et al., 1983), the somatostatin innervation appears to be of local origin. It may be profitable, therefore, to consider the loss of local circuit connections as contributing to the behavioral and cognitive defects associated with Alzheimer's disease.

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