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Auditory Connections and Neurochemistry of the Sagulum

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ABSTRACT

We studied the cytoarchitecture, neurochemical organization, and connections of the sagulum. The goal was to clarify its role in midbrain, lateral tegmental, and thalamic auditory processing. On cytoarchitectonic grounds, ventrolateral (parvocellular) and dorsomedial (magnocellular) subdivisions were recognized. The patterns of immunostaining for γ-aminobutyric acid (GABA) and glycine were distinct. Approximately 5–10% of the neurons were GABAergic, and more than one type was identified; GABAergic axon terminals were abundant in number and varied in form. Glycinergic neurons were much rarer, <1% of the population, and glycinergic axon terminals were correspondingly sparse. Wheat germ agglutinin conjugated to horseradish peroxidase was used for purposes of connectional mapping, and biotinylated dextran amines revealed the structure of corticosagular axons. All nine cortical areas injected project to the ipsilateral sagulum. Five (areas AI, AII, SF, EPD, and Te) had heavier projections than the others. Areas AI and AII projected throughout the rostrocaudal sagulum. Labeling from AI was moderate in density and concentrated in the central sagulum, whereas the input from AII was heavier and ended more laterally. Suprasylvian fringe input was light, especially caudally, and was chiefly in the central sagulum. The projection from the dorsal region of the posterior ectosylvian gyrus was comparatively stronger and was in the dorsolateral sagulum. Finally, the temporal cortex sent axons to the most lateral sagulum, spanning the dorsoventral extent, whereas insular cortex axons ended diffusely in the dorsolateral sagulum. Corticofugal axons ranged from fine boutons en passant to larger globular terminals. The sagulum may represent the earliest significant opportunity in the ascending auditory pathway for corticofugal modulation. The most extensive input arises from the polymodal association areas. The sagulum then projects divergently to the dorsal cortex of the inferior colliculus and the dorsal division of the medial geniculate body. The projection from the dorsal division of the auditory thalamus to nonprimary auditory cortex completes this circuit between the forebrain and the midbrain and represents a nexus in the ascending and descending auditory systems. Such circuits could play a critical role in auditory-motor adjustments to sound. J. Comp. Neurol. 401:329–351, 1998.

Indexing terms: cerebral cortex; medial geniculate body; inferior colliculus; thalamus; midbrain; sensorimotor integration

This study examines the connections of a small midbrain tegmental nucleus, the sagulum, with the inferior colliculus, medial geniculate body, and cerebral cortex, and it describes the neurochemical organization of this obscure nucleus. The sagulum is of interest because its role in the ascending auditory system is not well understood, and because its influence on descending auditory processing is unknown. Its strategic position in the midbrain, adjoining the lateral lemniscal nuclei and abutting the subcollicular

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region near the inferior colliculus, suggests that it could influence many facets of acoustic processing. Our first objective was to characterize the types of sagulum neurons based on normal architectonic preparations and on the dendritic and somatic configuration of well labeled sagulum projection neurons that terminate in the inferior colliculus or medial geniculate body. We next examined the chemical organization of the sagulum to determine the neurotransmitter identity of cells contributing to local processing and the identity and distribution of chemically labeled axon terminals. A third goal was to chart the projections of the sagulum to the inferior colliculus and the medial geniculate body because these are the principal conduits by which auditory brainstem information ascends to the cortex. A final question was whether corticofugal projections reach the sagulum. If they do not, then the sagulum may operate largely independent of the cortex, like the dorsal nucleus of the lateral lemniscus, and its role may be limited essentially to the ascending system. If the cortex does project to the sagulum, then it might modify the output of nuclei whose information reaches the forebrain, and the sagulum could have both an ascending feedforward role and serve as a target for descending signals.

For a nucleus of comparatively modest size relative to the lateral lemniscal nuclei, the sagulum has a surprisingly large set of connections. It receives input from the contralateral sagulum (Henkel and Shneiderman, 1988; Shneiderman et al., 1988; Hutson et al., 1991), the dorsal nucleus of the lateral lemniscus (Shneiderman et al., 1988), and the inferior colliculus (Henkel and Shneiderman, 1988). This convergence suggests that the sagulum could receive different information from these structures or that it is a common target of neurons in these several nuclei with some shared physiologic properties. The sagulum in turn has diverse projections to the contralateral sagulum (Henkel and Shneiderman, 1988), the dorsal nucleus of the lateral lemniscus (Shneiderman et al., 1988; Hutson et al., 1991), the inferior colliculus (Brunso-Bechtold et al., 1981; Hutson et al., 1991), and the medial geniculate body (Morest, 1965; Whitley and Henkel, 1984; Hutson et al., 1991). This finding suggests that the sagulum has a variety of postsynaptic targets in both the midbrain and thalamic auditory systems that set it apart from the inferior colliculus or lateral lemniscal nuclei (Calford and Aitkin, 1983).

The sagulum is a part of the lateral tegmental system and a participant in the extralemniscal auditory pathway (Morest, 1965; Morest and Oliver, 1984; Henkel and Shneiderman, 1988). There is little data on sagulum connections with more distant auditory centers. Although it receives a projection from the auditory cortex in rats (Feliciano et al., 1995), degeneration studies in the cat provided marginal or equivocal results even after massive cortical ablations. Thus, only a few corticofugal fibers appeared to terminate in the sagulum; smaller lesions restricted to the primary (AI) or secondary (AII) auditory cortex, or to polysensory insulotemporal or posterior ectosylvian association areas failed to produce anterograde degeneration in the sagulum (Diamond et al., 1969). This finding may reflect the relative sensitivity of the methods used, and it has led us to reexamine this question with the most sensitive anterograde tracers available.

MATERIALS AND METHODS

Surgical procedures

All experimental procedures followed approved institutional animal care and use protocols. Adult cats (2.5–4 kg) of either sex, free from middle ear infection and with a normal Preyer's reflex, were used. Either isofluorane

Abbreviations

AAF	anterior auditory field
AI	primary auditory cortex
AII	second auditory cortical area
BIC	brachium of the inferior colliculus
CC	caudal cortex of the inferior colliculus
CG	central grey
CIC	commissure of the inferior colliculus
CN	central nucleus of the inferior colliculus
Cu	cuneiform nucleus
CTF	central tegmental field
D	dorsal nucleus of the medial geniculate body
DC	dorsal cortex of the inferior colliculus
DD	deep dorsal nucleus of the medial geniculate body
DNLL	dorsal nucleus of the lateral lemniscus
DS	dorsal superficial nucleus of the medial geniculate body
EPD	dorsal portion of the posterior ectosylvian gyrus
EPI	intermediate portion of the posterior ectosylvian gyrus
EPV	ventral portion of the posterior ectosylvian gyrus
GABA	γ-aminobutyric acid
IC	inferior colliculus
IcT	intercollicular tegmentum
INLL	intermediate nucleus of the lateral lemniscus
Ins	insular cortex
LdTN	laterodorsal tegmental nucleus
LGB	lateral geniculate body
LL	lateral lemniscus
LN	lateral nucleus of the inferior colliculus
LP	lateral posterior nucleus
M	medial division of the medial geniculate body
MCP	middle cerebellar peduncle
MRF	mesencephalic reticular formation
NBIC	nucleus of the brachium of the inferior colliculus
OR	optic radiation
OT	optic tract
Ov	ovoid part of the ventral division of the medial geniculate body
P	posterior auditory field *or* posterior thalamic nucleus
PN	pontine nuclei
Pul	pulvinar nucleus
PZ	paralemniscal zone
RN	red nucleus
RP	rostral pole nucleus of the inferior colliculus
Sa	sagulum
Sal	lateral part of the sagulum
Sam	medial part of the sagulum
SC	superior colliculus
SCP	superior cerebellar peduncle
SF, SF/daz	suprasylvian fringe/dorsal auditory zone
SGI	stratum griseum intermediale
Sgl	suprageniculate nucleus, lateral part
Sgm	suprageniculate nucleus, medial part
SGP	stratum griseum profundum
SGS	stratum griseum superficiale
SN	substantia nigra
Spf	subparafascicular nucleus
SpN	suprapeduncular nucleus
Su	subcollicular zone
Te	temporal cortex
V	main laminated part of the ventral division of the medial geniculate body
VL	ventrolateral nucleus of the medial geniculate body
VN	trigeminal nerve
VNLL	ventral nucleus of the lateral lemniscus
VP	ventral posterior auditory field

(inhalant, 1–3%) or sodium pentobarbital (i.p., 40 mg/kg) was used for anesthesia. Electrocardiograph readings, respiration rate, temperature, and O_2 saturation were monitored continuously and maintained at physiologically appropriate levels. When corneal and pedal reflexes were absent and the temporal muscles were relaxed, the animal was placed in a stereotaxic head holder and a midsagittal incision was made. A craniotomy was performed to expose the auditory cortex. The dura was cut and retracted and a small window prepared to reveal the region of interest. Sterile silicon oil was applied to the brain to prevent desiccation.

We analyzed the projections from nine auditory cortical areas, including AI (see list of abbreviations), AII, AAF, P, EP (dorsal and intermediate), Ins, Te, and SF. Areal and sulcal patterns and architectonic features were derived from physiological (Woolsey, 1960; Imig and Reale, 1980) and connectional (Bowman and Olson, 1988a,b) studies. A total of 42 hemispheres with injections confined to one architectonic field or a related group of areas were available.

Cortical deposits of wheat germ agglutinin conjugated to horseradish peroxidase (5% WGA-HRP; Sigma Chemical Co., St. Louis, MO) were made by pressure through a glass pipet (tip diameter, 30 μm) by using an electronically controlled hydraulic injector (Nanoliter Injector, World Precision Instruments, Sarasota, FL). The injections were limited to one cortical area and were made at a depth of 1,000–1,800 μm. For the biotinylated dextran amines (BDA) experiments, a glass pipet (30- to 50-μm tip diameter) was used to deliver a 10–20% solution of tracer iontophoretically (Molecular Probes, Eugene, OR; molecular weight of 3K) in normal saline (alternating 5 μA positive current/15–30 minutes) or by pressure, by use of the nanoliter injector and BDA dissolved in distilled water.

The thalamic injections were targeted stereotaxically (Berman and Jones, 1982). Because their purpose was to label many sagulum neurons and to discern any reciprocal connections from the thalamus to this nucleus, injections were bilateral. The tracer was delivered with a 22-gauge microliter syringe; the volume injected was 0.1–0.15 μl. A total of 12 experiments were available, and the range of the deposits included every major architectonic subdivision except the rostral pole.

To examine the connections between the sagulum and the inferior colliculus, caudal visual cortex was aspirated to expose the midbrain. Iontophoretic deposits (1.5 μA for 30 minutes) of WGA-HRP were made through a pipet (tip diameter, 25 μm). A total of four experiments were available. Each experiment reported below was repeated at least once, usually with a different tracer. Only cases that have been replicated are included.

Histological procedures

Postoperative survivals for WGA-HRP studies were 3–4 days for cortical injections, 2 days for thalamic injections, and 1 day for inferior collicular injections. Postoperatively, the animal was reanesthetized and perfused through the heart with 0.1 M phosphate buffer (PB, pH 7.4, the vehicle for all solutions; 36°C) and 0.02% lidocaine hydrochloride (5 minutes; 60 ml/minute). The primary fixative was 0.5% glutaraldehyde/0.5% paraformaldehyde (5 minutes; ~500 ml). The secondary fixative was 1.5% glutaraldehyde/1% paraformaldehyde (20 minutes; ~2,000 ml). After 1–2 hours, the fixative was replaced with postfixation cryoprotectant (10% sucrose).

In the BDA experiments, animals were reanesthetized after survival for 3–28 days and perfused with the same washout as in the WGA-HRP studies, followed by 4% paraformaldehyde. The tissue was cryoprotected in 30% sucrose and 50-μm-thick frozen sections were cut on a sliding microtome. They were processed with the Vector Elite ABC kit (see below) followed by nickel-cobalt intensified diaminobenzidine (Adams, 1981) as the chromogen.

The skull was exposed, the head was placed in a stereotaxic holder, and the brain was blocked in the frontal plane. Tissue was equilibrated in 30% sucrose and frozen, serial sections 60-μm-thick were cut and collected. Anterograde or retrograde labeling was demonstrated by the tetramethylbenzidine method (Mesulam, 1978). These sections were counterstained with neutral red for cytoarchitectonic analysis. Adjacent, unreacted sections were Nissl stained for analysis of thalamic nuclear boundaries. Sections through the injection site were incubated with diaminobenzidine (Adams, 1981), because it yields a more accurate view of the size of the injection than the sections developed for tetramethylbenzidine.

The medial geniculate body was processed to demonstrate the thalamocortical cells of origin. This method served as an independent check on the cortical injection site (Morel and Imig, 1987). Thalamic and cortical architectonic subdivisions were drawn without knowledge of or reference to the locus of midbrain labeling.

Immunocytochemical procedures

A library of material prepared with antibodies for glutamic acid decarboxylase- (GAD), γ-aminobutyric acid (GABA), and glycine-immunoreacted material was available from approximately 20 adult cats. These specimens included 25-μm-thick frozen sections immunostained for GAD (antiserum courtesy of Dr. D.E. Schmechel; Oertel et al., 1981; see Winer and Larue, 1988 for details), and 50-μm-thick sections immunoreacted for GABA and glycine. Fixation for GABA/glycine immunocytochemistry ranged from 4% paraformaldehyde/0.25% glutaraldehyde to 2% paraformaldehyde/3% glutaraldehyde. Postembedded material from 1- to 1.5-μm-thick semithin sections was also prepared; these sections were cut with glass knives from 100- to 200-μm-thick serial slabs through the auditory midbrain, from which the critical levels were selected for further study; reference sections stained with toluidine blue were used for architectonic analyses. Antisera to GABA (Incstar, Stillwater, MN) and four antisera to glycine (from Dr. R.J. Wenthold, Glycine II; Chemicon International, Temecula, CA; Biodesign International, Kennebunk, ME; HTI Bioproducts, Inc., Ramona, CA) were used. Free-floating sections were immunostained by the avidin-biotin peroxidase (ABC) method (Vector Laboratories, Burlingame, CA) at dilutions of 1:2,000/-1:5,000 (rabbit anti-GABA; Incstar or Dr. R.J. Wenthold) and 1:400/-1:1,500 (rabbit anti-glycine from Dr. R.J. Wenthold) after incubation in the appropriate blocking serum. Postembedded material was block stained in osmium tetroxide, dehydrated, embedded, and polymerized. Semithin sections were then cut, etched, deosmicated, rehydrated, and incubated on the slide by using the primary antiserum at concentrations of 1:2,000 (GABA: Incstar) and 1:200– 1:10,000 (Glycine: R.J. Wenthold; Chemicon International; Biodesign International; HTI Bioproducts Inc.); ABC immunoperoxidase detection kits (Vector Laboratories) and streptavidin-biotin kits (Kirkegaard & Perry, Inc., Gaithersburg, MD) were used with similar results. Cobalt-nickel-

intensified diaminobenzidine was the chromogen (Adams, 1981). A more complete account of these methods and procedures is available (Winer et al., 1995; Larue and Winer, 1996). Controls were devoid of specific immunoreactivity.

Analysis and graphics processing

The drawings of Nissl preparations and the connectional observations were made with a drawing tube. Sections with representative patterns of retrograde or anterograde labeling were selected for presentation.

GABAergic and glycinergic neurons were plotted on a Neurolucida system (MicroBrightField, Colchester, VT) from two adjacent plastic embedded sections that included half of the brainstem (Fig. 2). Large sections facilitated comparisons between nuclei, because appreciable expanses of the auditory midbrain were prepared identically.

Figures 11 and 12 were edited on a computer after conventional negatives were made on a light microscope (Leica DMRP) by using black-and-white 35 mm film (Kodak T-Max 100; Rochester, NY). The negatives were scanned (Nikon LS-1000 35-mm scanner) and edited (Adobe Photoshop 3.9) to enhance the images. The plates were composed and labeled by using standard methods (Adobe Illustrator 5.0). Final prints were made on a dye sublimation color printer (Fargo Pictura 310e). The primary data were not altered in either form or content by these procedures.

RESULTS

Cytoarchitecture

The sagulum (La., cloak) is a wedge-shaped nucleus approximately 1,900-μm long, 500-μm wide, and 3,000-μm tall located beneath the lateral nucleus of the inferior colliculus (Fig. 1, LN); it lies lateral to the dorsal and intermediate nuclei of the lateral lemniscus and caudal to the central tegmental field. Its posterior face tapers gradually and disappears as the caudal cortex of the inferior colliculus protrudes freely above the brainstem in frontal sections; rostrally, the sagulum ends more abruptly, behind the parabigeminal nucleus.

Many sagulum neurons were smaller than cells in the intermediate nucleus of the lateral lemniscus and the even larger neurons in the adjoining dorsal nucleus of the lateral lemniscus (Fig. 1, DNLL). The dorsomedial sagulum contained a population of larger neurons that distinguished it from the ventrolateral, parvocellular subdivision (Fig. 1). The affiliation of these large neurons as part of the sagulum was confirmed by their projection to the medial geniculate body (Fig. 4F–J) and the inferior colliculus (Fig. 5C–G), and on the grounds of receiving cortical input (Figs. 9E, 10F,G). Although the magnocellular neurons were comparable in size to those in the intermediate nucleus of the lateral lemniscus, they were appreciably smaller than those in the adjacent dorsal nucleus. In Nissl preparations, many sagulum cells were oval or fusiform, and most had their major axis parallel or slightly oblique to the lateral surface of the brainstem. Dorsomedial magnocellular neurons had more varied shapes and often were triangular or multipolar, with little in the way of a preferred orientation. The rare horizontally oriented neurons were most common in the dorsal half (Fig. 1).

The superior border was formed by the lateral nucleus of the inferior colliculus (Fig. 1, LN), whose neurons were larger and less densely packed than those of the sagulum. Often, this boundary was marked by a blood vessel entering the brainstem perpendicularly. Ventrally, the sagulum extended as a thin sliver of neurons between the middle cerebellar peduncle and the ventral nucleus of the lateral lemniscus.

Immunocytochemistry

The various antisera to GABA labeled the same population and proportion of neurons, and the identical types of axon terminals (puncta). The outcomes of glycine immunostaining were likewise similar irrespective of the antiserum. The account that follows is based on postembedded material because it provides the most direct comparison of the patterns of GABA and glycine.

GABAergic neurons and axon terminals. We estimate that 5–10% of the neurons were GABAergic (Fig. 2A), and this group was varied in size and shape. GABAergic cells were interspersed among clusters of immunonegative neurons. The largest GABAergic neurons were darkly immunostained and lay in the dorsomedial sagulum. Immunopositive cells were scattered and solitary. Many GABAergic neurons had somatic diameters of 10–15 μm. The limited dendritic immunostaining in semithin sections made it difficult to identify further the subtypes of immunopositive neurons on morphologic grounds or to correlate these with the profiles of sagulothalamic (Fig. 4E) or sagulocollicular (Fig. 5B) projection neurons. It seems unlikely that they represent only one class because even cells <10 μm in somatic diameter were often immunopositive and had conspicuous dendritic immunoreactivity.

The axon terminals (puncta) were predominantly fine (~0.5 μm in diameter) and granular, and they were prominent throughout the sagulum (Fig. 3A). A few much larger, globular endings were present; in some instances,

Fig. 1. Midbrain cytoarchitecture in a Nissl preparation from a 30-μm-thick, celloidin-embedded section midway through the caudorostral extent of the sagulum. Sagulum neurons were among the smallest in the auditory midbrain. The borders of the sagulum were formed by the following structures. A thin rim of acellular neuropil on the free margin of the brainstem represented the marginal zone; dorsally, the cells in the lateral nucleus (LN) of the inferior colliculus were larger and more heterogeneous and represented the junction with the cuneiform nucleus (Cu); medially, the larger, horizontally oriented cells of the dorsal nucleus of the lateral lemniscus (DNLL) and the well-developed neuropil of the lateral lemniscus (LL) were present; ventromedially, cells in the lateral lemniscus and its intermediate nucleus (INLL) had a broader range of size than those in the sagulum and a more variable somatic orientation. The paralemniscal zone (PZ), like the sagulum, was dominated by small-to-medium-sized neurons. Two subdivisions within the sagulum were recognized. The lateral region (Sal) contained many small cells that were usually oriented vertically, with their long axis inclined dorsolaterally. These neurons formed clusters whose packing density decreased dorsally. The borders between clusters were marked by blood vessels passing from lateral to medial. In the dorsal half of the lateral territory, descending and ascending fibers dispersed the neurons further. Neurons in the medial part of the sagulum (Sam) were almost twice as large as those in the lateral region and had more heterogeneous orientations. The cells that form a lateral wing on the dorsal nucleus of the lateral lemniscus were still larger than the biggest neurons in the lateral sagulum. Planachromat, N.A. 0.35, ×320. For abbreviations, see the list.


LN
CN
Cu
Su
Cu
DNLL
Sam
CTF
PZ
100 μm
D
M
L
V
Sal
INLL
LL
MCP
IcT
DC
CN
LN
CG
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MCP
VN
1 mm

Figure 1

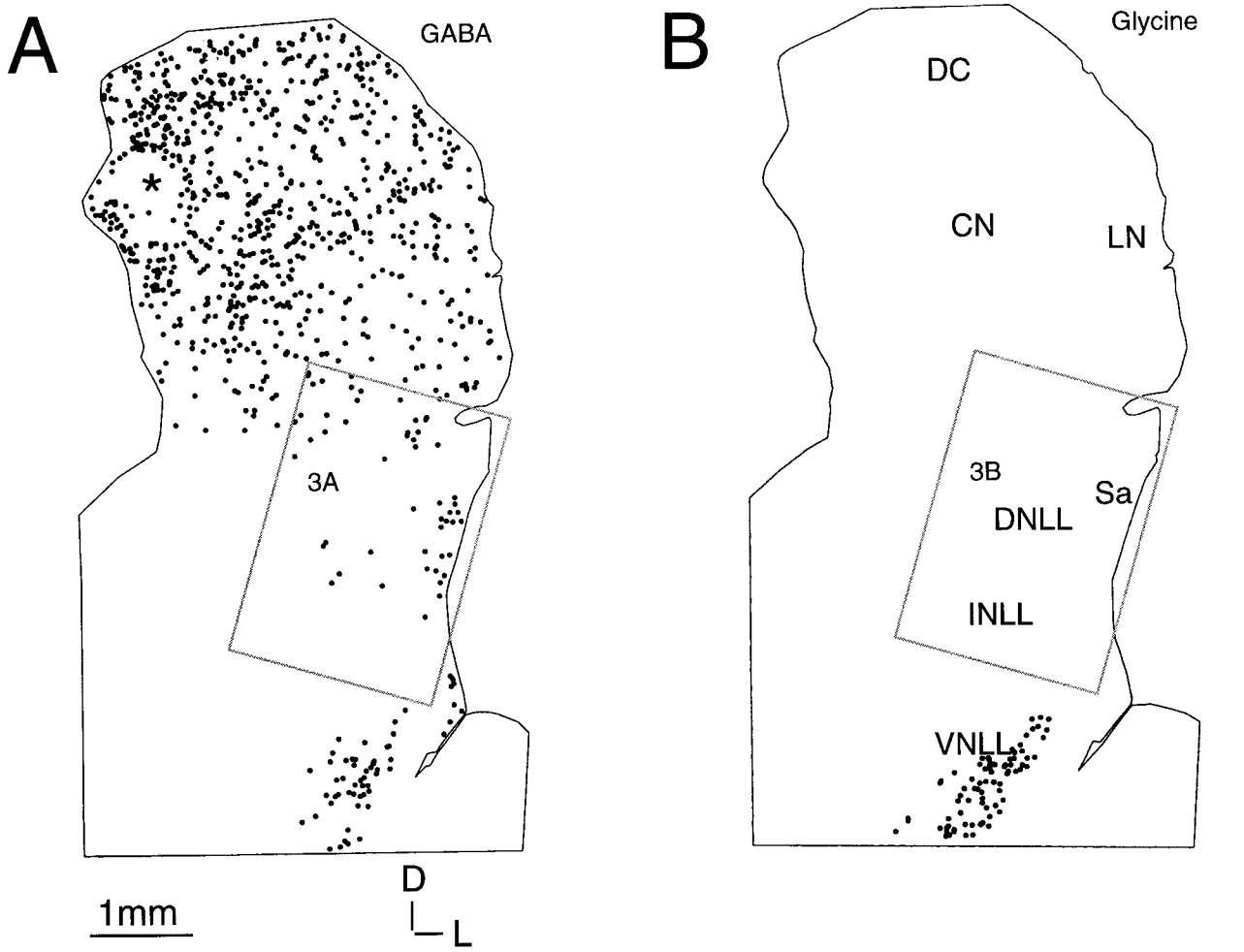


Fig. 2. GABAergic (A) and glycinergic (B) neurons plotted in adjoining pair of semithin sections. For protocol, see Figure 3. **A**: GABAergic cells were present in almost every midbrain auditory nucleus, and they were conspicuous in the inferior colliculus (DC, CN, LN; see Oliver et al., 1994) and in the sagulum (Sa). Asterisk, tissue artifact. Boxed regions mark the locus of photomicrographic observations in Figure 3. Planapochromat, N.A. 0.65, ×312. **B**: The distribution of glycine-immunoreactive somata in the midbrain was in sharp contrast to that for GABA. The only appreciable concentration of such neurons was in the ventral nucleus of the lateral lemniscus (VNLL). In the light of a previous report of a significant population of glycine-positive neurons in the cat sagulum (Stanforth et al., 1995), we tested and compared four different antisera in tissue prepared by using free-floating and postembedding techniques. All antisera stained axons and puncta throughout the central nucleus of the inferior colliculus and the nuclei of the lateral lemniscus. Intense neuronal somatic staining was seen in the ventral nucleus of the lateral lemniscus. In the sagulum, glycine-positive neurons were observed only when nonspecific staining resulted from an excessive concentration of primary antiserum, which produced glycinergic somata throughout the central nucleus of the inferior colliculus as well. For abbreviations, see list.

these were not associated with preterminal fibers and they might represent a second class of terminal. The puncta were uniformly dense along the lateral one third of the sagulum, where the lateral lemniscal axons were rare. More medially, the lemniscal axons, many of which were GABA-negative, dispersed the GABAergic terminals into clusters. Some terminals may arise from extrinsic sources because there were small fascicles of GABAergic axons confined mainly to the medial half of the sagulum and that followed an approximately dorsomedial-to-ventrolateral path. Some fibers were up to 2-μm thick.

Although many puncta were found in the neuropil, others encircled immunonegative neuronal somata. Most GABA-negative cells received axosomatic profiles, and these puncta often extended some distance onto the proximal dendrites. In contrast, few or no puncta occurred on GABAergic somata.

Glycinergic neurons and axon terminals. The pattern of glycinergic immunoreactivity differed markedly from the GABAergic arrangement as seen in adjoining pairs of semithin sections immunostained with different antibodies. The medial border of the sagulum was marked clearly by the virtual absence of glycine immunostaining relative to the moderate amount of immunoreactive material in the neighboring central nucleus and lateral lemniscus (Fig. 3B). Dorsally, however, the glycine immunoreactivity in the lateral nucleus was very similar to that in the sagulum. The principal distinctions were that: 1) only an occasional sagulum neuron was glycine-immunoreactive, and these usually colocalized GABA; 2) the rare (<1%)

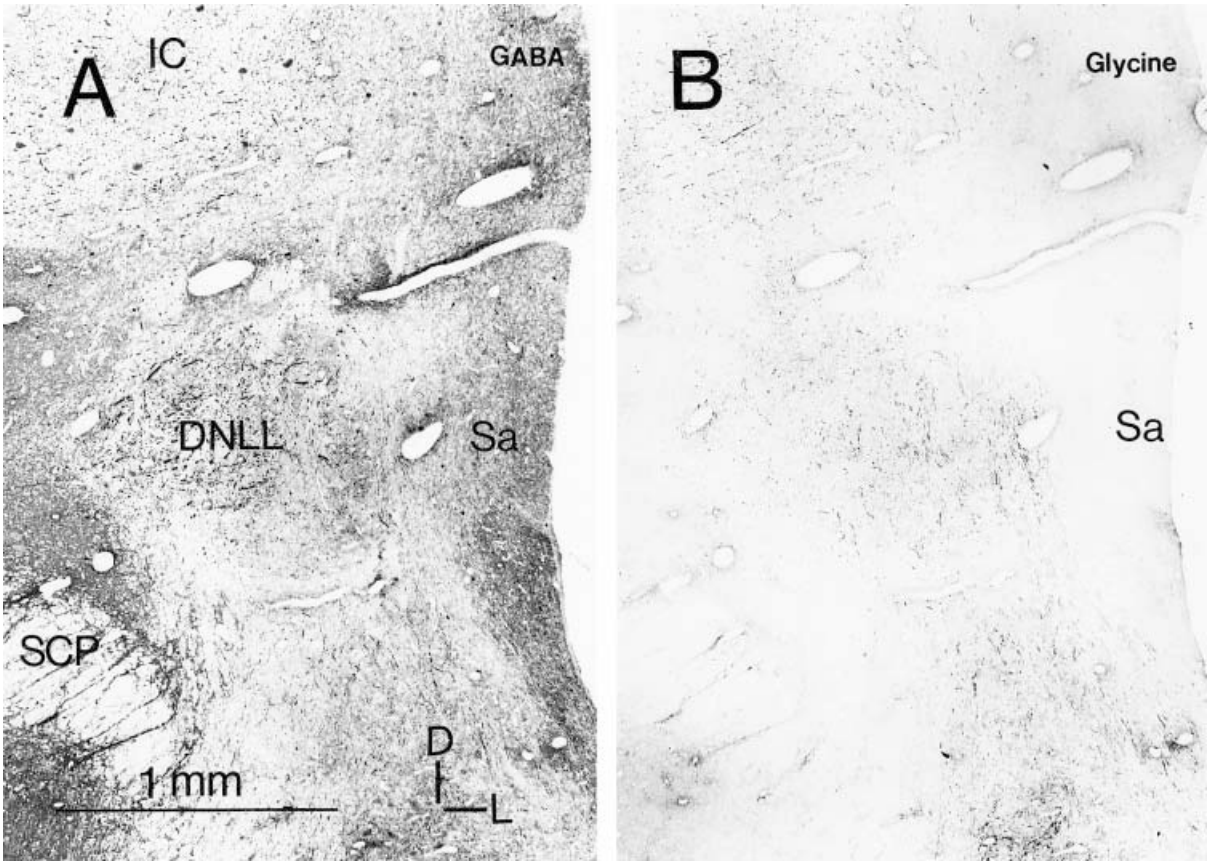


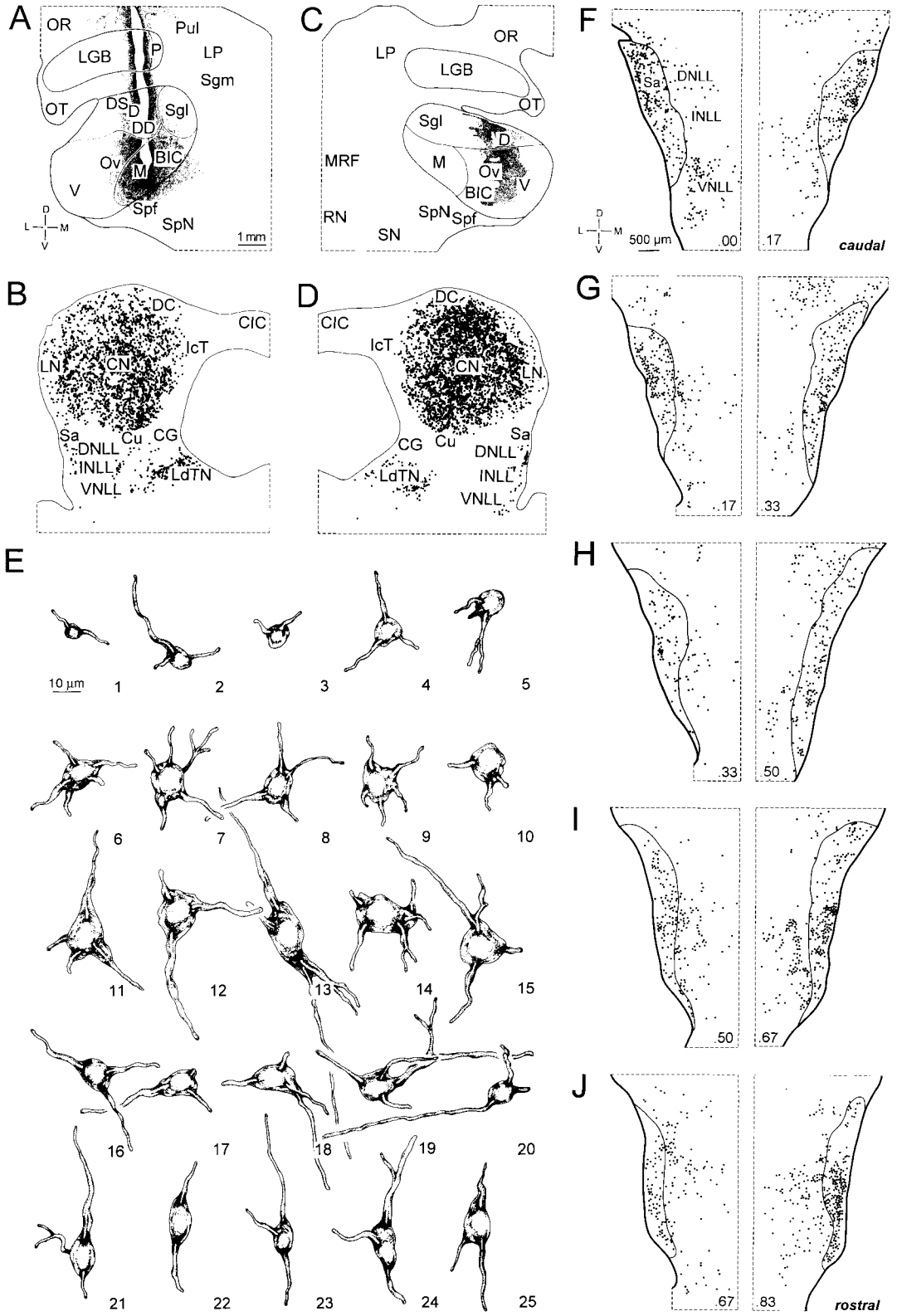
Fig. 3. Photomicrographs of immunocytochemical results. **A**: In a semithin section treated with antiserum for GABA, the sagulum (Sa) was clearly demarcated from the paler lateral lemniscal fibers interposed between it and the dorsal nucleus of the lateral lemniscus (DNLL). There was continuity in GABAergic staining between the sagulum and the overlying lateral nucleus (above the large horizontal blood vessels; not labeled). Protocol: Planapochromat, N.A. 0.16, ×50. Plastic-embedded section, 1-μm-thick; GABA (Incstar), 1:2,000 dilution. **B**: An adjacent semithin section stained for glycine. The sagulum in this representative section contained no glycine-immunoreactive neurons, and the few immunopositive preterminal axons and puncta were not conspicuous at this magnification; in contrast, both the dorsal nucleus of the lateral lemniscus and the inferior colliculus had abundant glycinergic puncta and no glycine-positive neurons. As with GABA, there was a continuity in glycine immunostaining between the sagulum and the lateral nucleus: in both, only a few puncta and no neurons were present. HTI glycine, 1:200 dilution. For abbreviations, see list.

glycine-positive neurons were in the central part of the size range for sagulum neurons, ~10–12 μm in somatic diameter; 3) there were few puncta, and these formed two groups: medium-sized terminals ~1 μm in diameter and small endings less than half that size, but approximately equal in density; 4) both medium-sized (1- to 2-μm in diameter) and very large caliber (>5 μm in diameter) preterminal axons followed the same orientation as the GABAergic fibers believed to be of lateral lemniscal origin; and 5) few axosomatic puncta were present on either immunonegative or glycinergic neurons.

Sagulothalamic projections

Sagulum neurons projecting to the auditory thalamus were identified by injecting WGA-HRP bilaterally in the medial geniculate body to examine the total projection of the sagulum. In prior experiments with unilateral injections, the bulk of the sagulothalamic neurons were ipsilateral to the injection (Winer et al., 1996, Fig. 1C). The first injection involved much of the dorsal and medial divisions (Fig. 4A, D, DD, M), with some encroachment into the suprageniculate (Fig. 4A, Sgl) and ovoid (Fig. 4A, Ov) nuclei. The ipsilateral retrograde labeling involved, to differing degrees, each of the three principal parts of the inferior colliculus as well as the sagulum. Most neurons were concentrated in the ventromedial part of the central nucleus (Fig. 4B, CN), as expected on physiologic grounds (Merzenich and Reid, 1974). There were also many labeled cells in the dorsal cortex (Fig. 4B, DC) and lateral nucleus (Fig. 4B, LN), suggesting that tectothalamic axons passing to more lateral auditory thalamic territories were interrupted. The retrogradely labeled sagulum neurons were concentrated in the caudal two-thirds of the nucleus, preferentially in the dorsal half (Fig. 4F–**J**, left side).

In the same animal, a second, contralateral injection involved the dorsal nuclei proper (Fig. 4C, D) and extensive territories in the mid-to-high frequency range of the ventral division (Fig. 4C, V, Ov; Imig and Morel, 1984). The entire central nucleus of the inferior colliculus contained retrogradely labeled neurons, including the dorsal and lateral, low-frequency region (Merzenich and Reid, 1974). The dorsal cortex (Fig. 4D, DC) had many labeled cells, the lateral nucleus (Fig. 1, LN) far fewer (Fig. 4D, LN), and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rostral

Figure 4

much of the intercollicular tegmentum had none (Fig. 4D, IcT). The ipsilateral projection differed from that of the first injection in three ways. First, almost all of the caudorostral sagulum contained labeled neurons. Second, these cells were more clustered. Finally, most neurons were concentrated in the ventral and anterior sagulum, whereas the first injection marked cells preferentially in either the caudal and dorsal sagulum (Fig. 4F) or the rostroventral part (Fig. 4J). In both experiments there was appreciable involvement of the lateral lemniscal neurons medial to the sagulum, and particularly more rostrally. There may be a topography in sagulogeniculate input, although our injections are bilateral as well as too large to resolve its finer details.

The processes of sagulothalamic cells of origin were filled so extensively with reaction product—for up to 100 μm in some instances—that some inferences can be made about their somatodendritic form (Fig. 4E: 13,18). The dendritic orientations were heterogeneous, and ranged from small multipolar cells with somata 8–10 μm in diameter and two to five primary dendrites with a stellate configuration (Fig. 4E: 1–5), to neurons up to three times as large, with long, sparsely branched processes parallel or orthogonal to the lateral lemniscal axons (Fig. 4E: 11–15). Other multipolar neurons were seen that had either a horizontal (Fig. 4E: 19) or a vertical (Fig. 4E: 22) orientation, in contrast to stellate neurons.

Sagulocollicular projections

Unilateral inferior colliculus injections produced retrogradely labeled neurons bilaterally in the sagulum. An injection centered at the junction of the central nucleus and the dorsal cortex diffused into the intercollicular tegmentum, and the dorsal part of the lateral nucleus was involved slightly (Fig. 5A). There were approximately twice as many neurons labeled ipsilaterally (Fig. 5C–G, large open circles), and these neurons were found throughout the sagulum, whereas the contralateral neurons were caudal and medial. This finding suggests a connectional asymmetry like that in the projection from the lateral lemniscal nuclei (Fig. 5D, INLL). The sagulocollicular connections were often reciprocal, i.e., the ipsilateral sagulum contained dense anterograde labeling throughout (Fig. 5C–G, right side, fine dots). Although the descending projection occupied most of the nucleus in caudal sections, it was concentrated laterally in more rostral sections (Fig. 5G). A much weaker, contralateral anterograde projection was found medially and it was concentrated in the more dorsal parts of the section (Fig. 5C–G, left side, fine dots). In the central sagulum the dorsomedial subnucleus had many labeled neurons contralaterally (Fig. 5E, lateral to DNLL) and none ipsilaterally. The ipsilateral and contralateral projections were not completely symmetrical (see Fig. 5G for further examples).

Golgi-like bulk filling of the sagulocollicular cells of origin revealed that a range of neurons with different somatodendritic profiles were labeled. These neurons included multipolar cells (Fig. 5B: 3,7,11) as well as cells with prominent horizontal (Fig. 5B: 14) or vertical processes (Fig. 5B: 6,17). Some sagulocollicular neurons were slightly larger than sagulogeniculate cells (compare Figs. 4E, 5B). Horizontal and large neurons (especially the latter) were more numerous in the dorsomedial sagulum (Morest and Oliver, 1984; present results); more of these neurons project to the inferior colliculus than to the medial geniculate body (compare Figs. 4F–J and 5C–G). Some neurons (Fig. 4E: 16–20, and 5B: 13–16) resembled in their dendritic configuration principal elongate neurons in the dorsal nucleus of the lateral lemniscus, although care was taken to exclude any lateral lemniscal cells from this study.

Corticosagular projections

All auditory cortical areas studied projected to the sagulum, although not equally. The input was exclusively ipsilateral, and anterograde only (Figs. 6–10, D–G). Nearby structures such as the lateral lemniscal nuclei were traversed by only a few fibers of passage. The injections in each experiment were limited to one area (Figs. 6A–10A) as defined by its cytoarchitecture (Winer, 1992), and they did not invade the white matter (Figs. 6B–10B).

Injections in caudal AI (Fig. 6A,B) labeled axon terminals throughout the rostrocaudal sagulum (Fig. 6D–G). Retrogradely labeled thalamic cells (Fig. 6C) were concentrated in the lateral (low-to-middle frequency) portion of the ventral nucleus (Imig and Morel, 1984) with other neurons clustered in the suprageniculate nucleus and a few cells scattered in the medial division. The sagulum labeling was moderately dense and concentrated in the central region along its medial border (Figs. 6D–G, 11A). The projection consisted of small patches of intense labeling that varied in shape and density (Fig. 6E). Axons above the sagulum often formed thin fascicles of fibers that, on entering its capsule, broke into smaller aggregates of terminal fields in which the linear configuration of the preterminal fibers was lost (Fig. 6D–G). In none of the experiments (Figs. 6–10) was there appreciable labeling in the rostralmost 15–20% of the sagulum.

A slightly larger set of injections in the central part of AII (Fig. 7A,B) retrogradely labeled many cells in the three dorsal division nuclei in the medial geniculate body and in the medial division and the suprageniculate nucleus (Fig. 7C) and virtually none in the ventral division, suggesting little or no involvement of adjoining primary fields. The corticofugal projection was appreciably heavier than that

Fig. 4. Sagulum projections to the medial geniculate body in an experiment with bilateral injections. **A**: The large injection of WGA-HRP was centered in the medial division; the track crossed the dorsal division and the tracer spread into the suprageniculate (Sgl) and ovoid (Ov) nuclei. The center of the track is white and the diffusion is indicated by fine stippling. **B**: Retrogradely labeled cells in the ipsilateral inferior colliculus were concentrated mainly in the central nucleus (CN), less so in the dorsal cortex (DC) and lateral nucleus (LN). Sagulum (Sa) neurons were also labeled. **C**: An injection in the right medial geniculate body of the same cat was restricted largely to the ventral (V) and dorsal (D) divisions. **D**: Many neurons were labeled in the ipsilateral inferior colliculus. Sagulum neurons were also labeled. **E**: Varieties of sagulothalamic neurons with extensively labeled processes. A broad range of cells were identified, including small (1), medium-sized (10), and large multipolar neurons (14), and horizontal (20) and vertical (22) cells. The many neurons with well-filled processes and oriented dendritic fields suggest that there may be a laminar fibrodendritic arrangement within the sagulum. **F–J**: Retrogradely labeled neurons (dots) were present bilaterally throughout the rostrocaudal extent of the sagulum. They were more concentrated in the dorsal part of the nucleus in caudal sections; more rostrally, they were most densely packed in the ventral part. Some perilemniscal neurons were labeled as well as a few cells in the adjacent tegmentum. Numbers represent the caudorostral percentage for each section for Figures 4–10. Two-day survival, 0.15 μl of WGA-HRP. For abbreviations, see list.

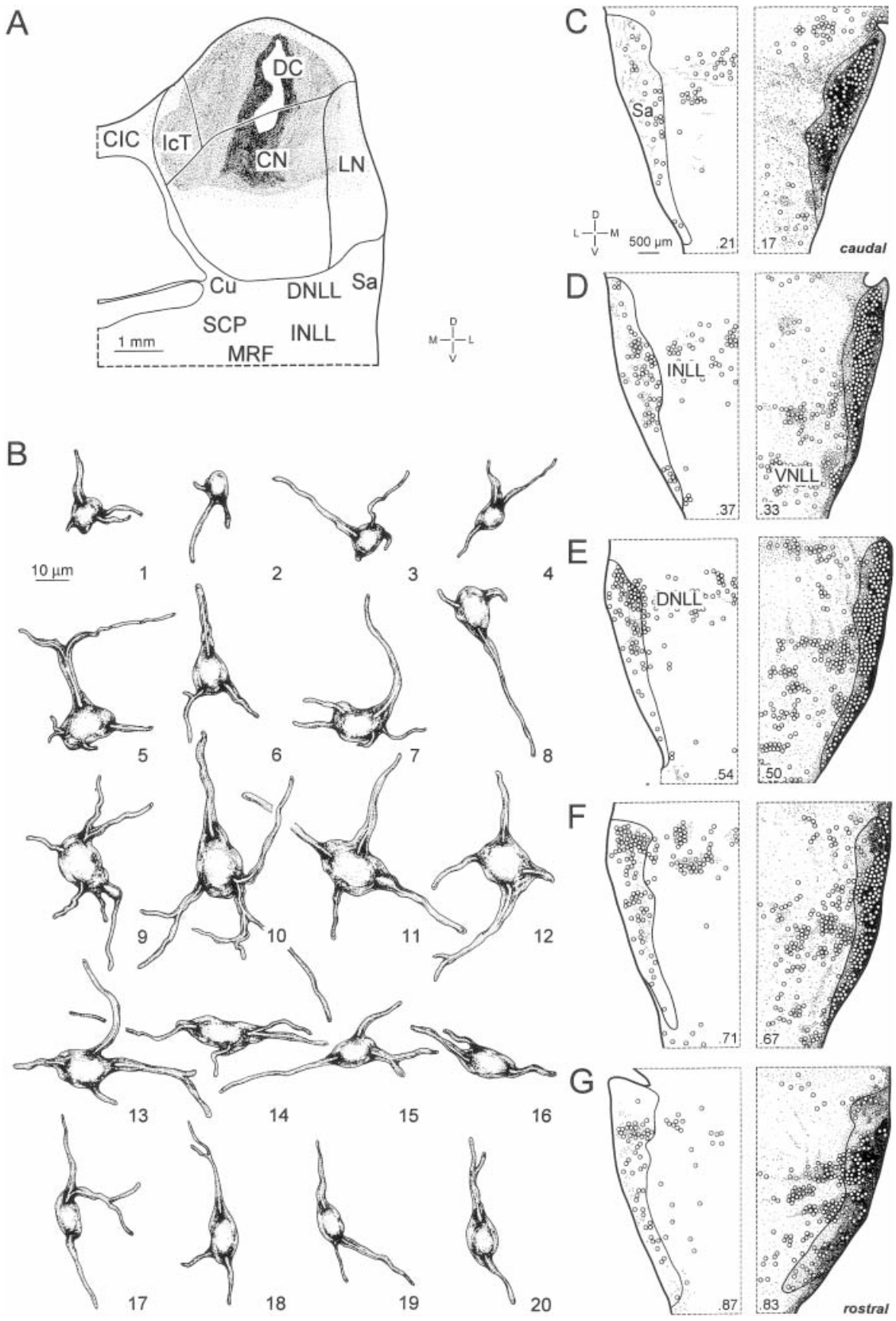

Fig. 5. Reciprocal connections between the sagulum and the inferior colliculus. **A**: The wheat germ agglutinin conjugated to horseradish peroxidase injection site was centered in the right inferior colliculus and involved the dorsal cortex (DC) and the dorsal region of the central nucleus (CN); there was some diffusion into the intercollicular tegmentum (IcT) and the lateral nucleus (LN). **B**: Retrogradely labeled sagulocollicular neurons. Many of the same varieties that projected to the medial geniculate body were labeled (see Fig. 4E for comparison). **C–G**: Plots of sagulum neurons projecting to the inferior colliculus (open profiles) and the colliculosagular anterograde labeling (fine dots). Neurons were labeled bilaterally; those in the ipsilateral sagulum were more numerous. Cells were distributed throughout the nucleus, although the colliculosagular projection was mainly ipsilateral. Labeled cells were more numerous in its dorsomedial region in this experiment than after thalamic injections (compare Figs. 4F–J and 5C–G). The anterograde labeling in the ipsilateral sagulum was massive and concentrated chiefly in the most lateral part of the nucleus, especially rostrally, and extended to the marginal zone. The contralateral anterograde transport was sparse and diffuse, with patches of labeling more medially than in the ipsilateral projection. One-day survival, 0.15 µl. For abbreviations, see list.

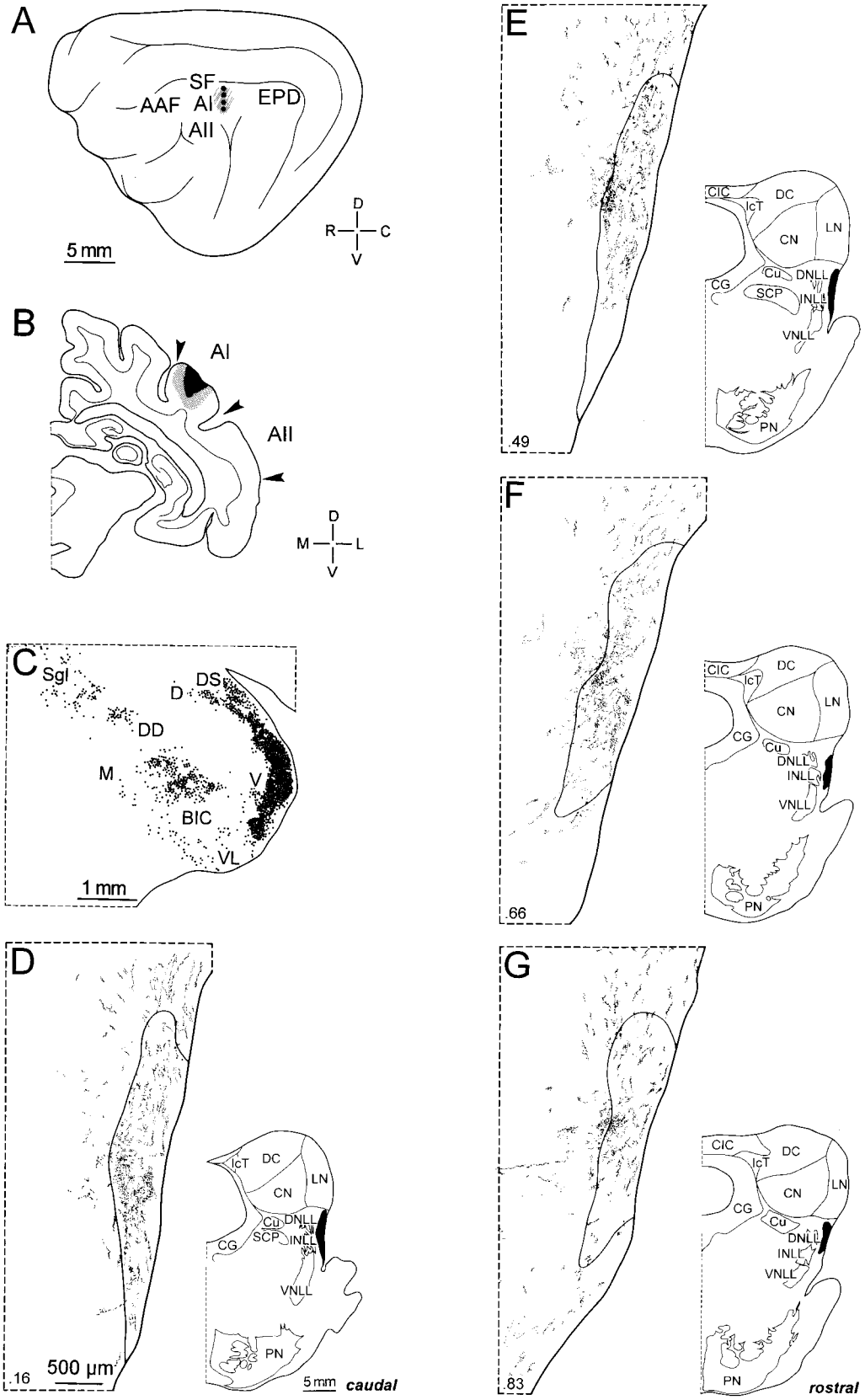

Fig. 6. Projections to the sagulum from area AI. **A**: Four deposits of wheat germ agglutinin conjugated to horseradish peroxidase (black dots) and the ensuing diffusion (stippled area) were limited to caudal AI. **B**: Coronal section through the injection site. The track (black) and diffusion (shaded area) did not invade other cortical areas or the white matter. **C**: Retrogradely labeled medial geniculate body cells (dots) were concentrated in the lateral portion of the ventral nucleus, with a few neurons in the suprageniculate nucleus (Sgl) and medial division (M); this pattern is consistent with an AI injection. **D–G**: In this and subsequent experiments (Figs. 7–10), only anterograde labeling in the ipsilateral sagulum was present (fine dots). The transport was strongest in the central and medial regions. Moderate labeling was found throughout the nucleus except at its extreme lateral margin. Axons were also seen dorsal and lateral to the sagulum, represented by the black area in the inset diagrams on the right. Transport extended through the sagulum caudorostrally. Three-day survival, 0.6 µl. For abbreviations, see list.

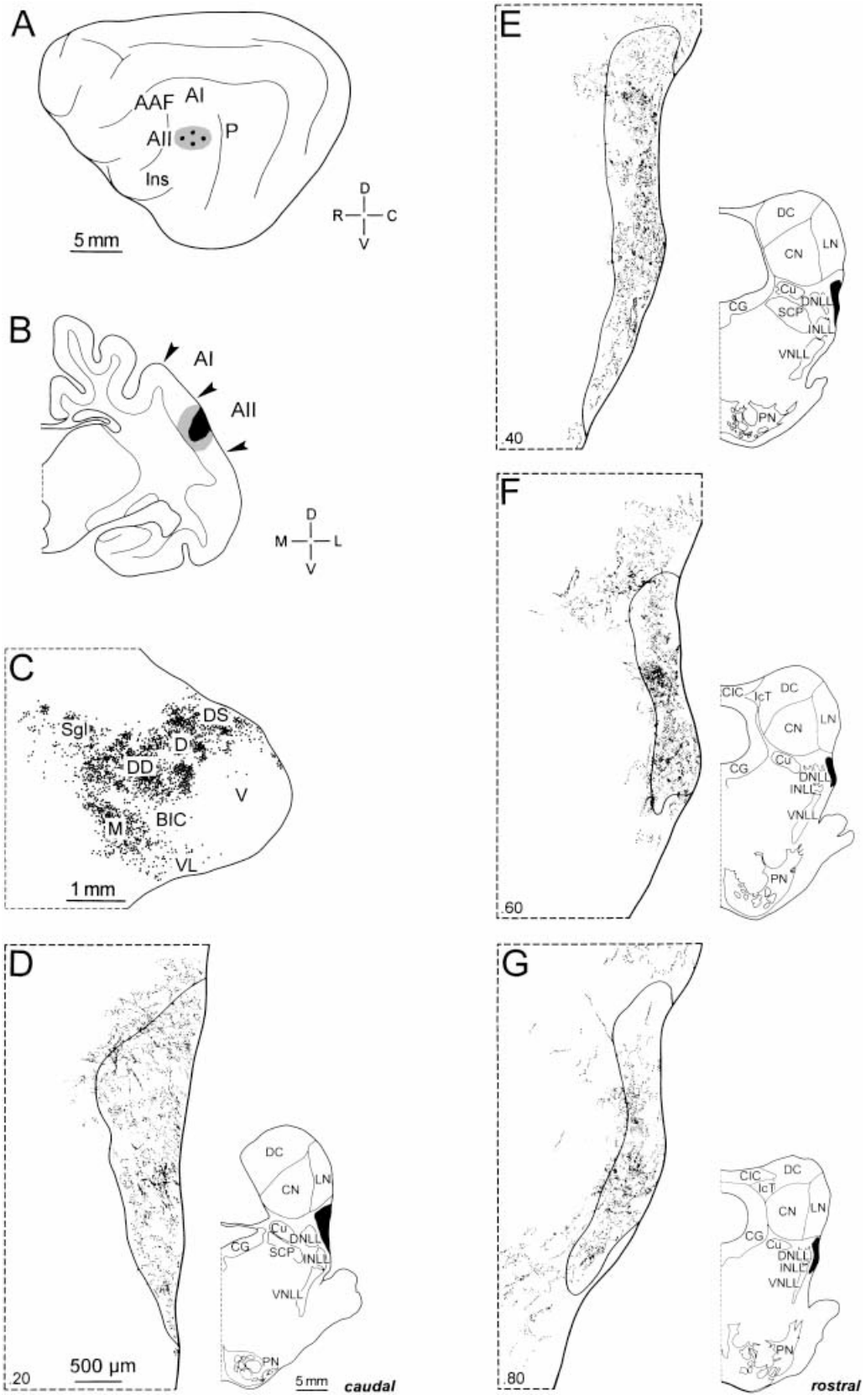

Fig. 7. Corticofugal input to the sagulum from area AII. **A**: Four deposits of wheat germ agglutinin conjugated to horseradish peroxidase were made that filled most of AII along its caudorostral limits. **B**: Coronal section through the center of the injections. The tracer did not enter AI, insular cortex, or the white matter. **C**: Retrogradely labeled medial geniculate body cells were concentrated in three dorsal division nuclei (DS, D, DD), the suprageniculate (Sgl) nucleus, and the medial division (M). This pattern is in accord with an AII injection (Winer et al., 1977; Winer, 1992). **D–G**: Labeling in the ipsilateral sagulum was heavier and more lateral than that from AI. The labeling moved ventrally in rostral sections. As in the AI experiment, foci of transport were surrounded by unlabeled zones (see, for example, F). This finding suggests that the corticosagular topography is specific even for a cortical area without a tonotopic arrangement (Schreiner and Cynader, 1984). There was appreciable labeling in the region bordering the lateral nucleus (D). Four-day survival, 0.6 µl. Conventions as in Figure 6, here and in Figures 8–10. For abbreviations, see list.

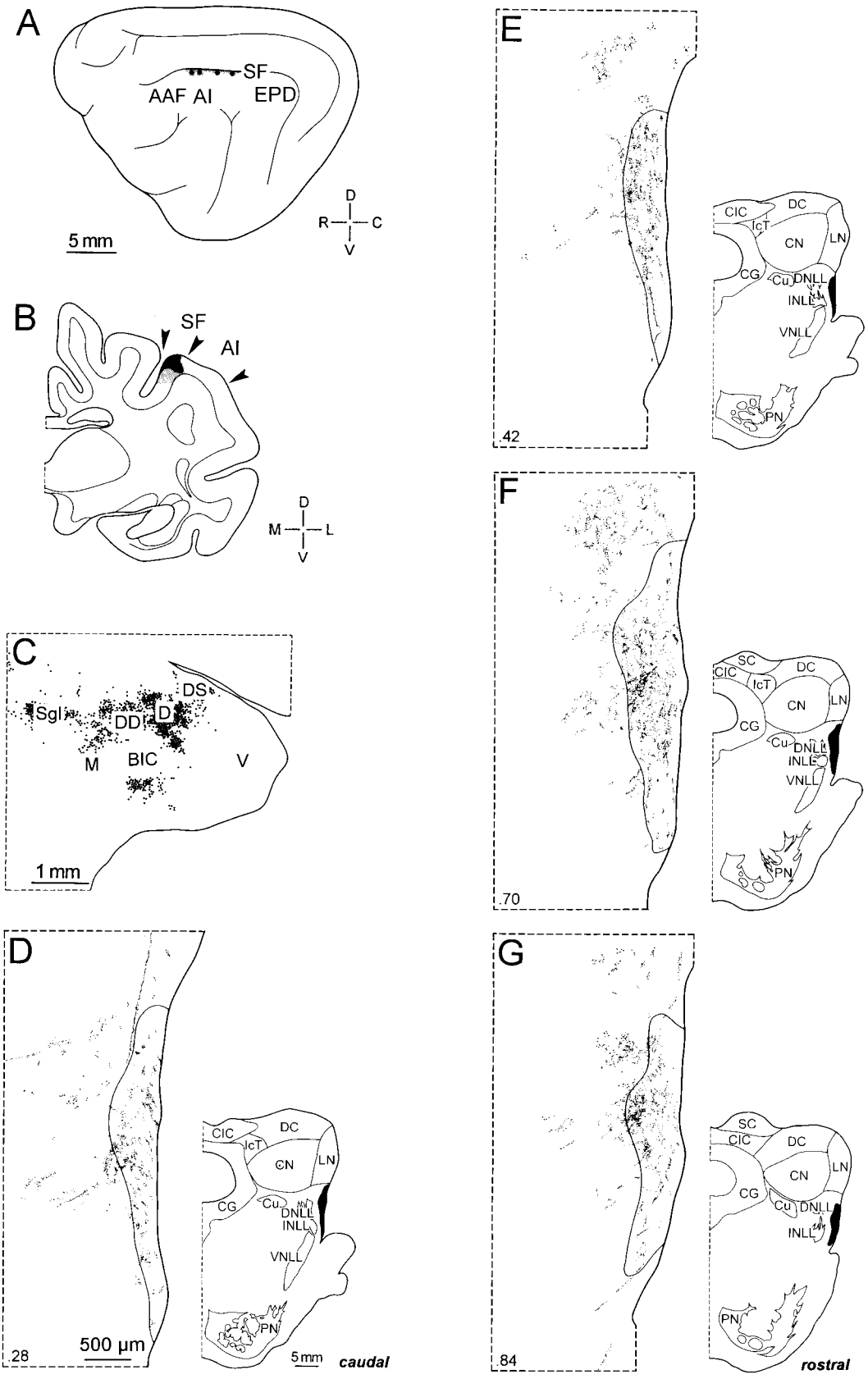

Fig. 8. Projections from the suprasylvian fringe area (SF) to the sagulum. **A**: Four WGA-HRP injections were made along the dorsal bank of the middle suprasylvian gyrus. **B**: The tracer did not invade AI, although there may have been minor encroachment into the visual association areas located deeper in the sulcus. **C**: Retrogradely labeled auditory thalamic cells were concentrated in the medial division (M) and the dorsal (D) nucleus, with others in the deep dorsal (DD) and suprageniculate (Sgl) nuclei, and none in the ventral division (V). This finding suggests that the injection was restricted to SF. **D–G**: Anterograde labeling was lighter than that from the AI and AII injections; this was most marked in caudal sections. The transport was somewhat heavier in the central and medial sagulum, and sparser dorsally and ventrally in most sections. Note the focal, patchy quality of the input. A few axons (G) were followed toward the pontine nuclei from the ventral margin of the sagulum. Three-day survival, 0.6 µl. For abbreviations, see list.

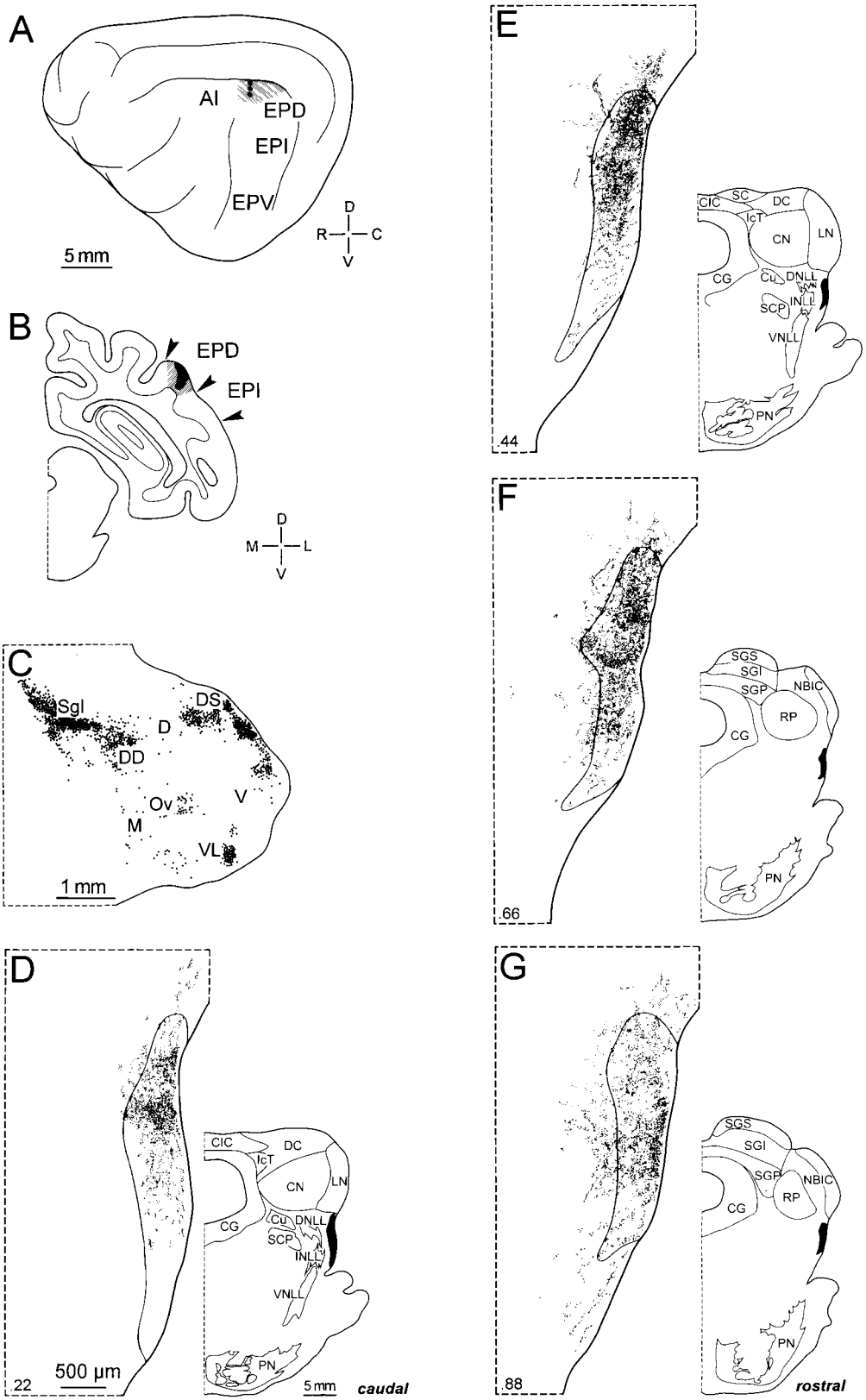

Fig. 9. Projections from the dorsal part of the posterior ectosylvian area (EPD) to the sagulum shown with wheat germ agglutinin conjugated to horseradish peroxidase. **A**: The injections extended to the crest of the posterior ectosylvian gyrus, but did not enter the lateral suprasylvian visual association areas in the medial bank of the posterior suprasylvian sulcus. **B**: The tracer avoided the intermediate part of the posterior ectosylvian gyrus and did not invade the ventral bank of the suprasylvian sulcus or the white matter. **C**: All medial geniculate subdivisions had some labeled neurons; these were concentrated in the suprageniculate (Sgl), dorsal superficial (DS) and ventrolateral nuclei (VL), each of which has strong affiliations with the lateral tegmental part of the tectothalamic pathway (Morest, 1965). **D–G**: The labeling was the most intense in the present study. It was located preferentially in the dorsal two-thirds of the nucleus and was lighter in the dorsomedial sagulum. Dense foci of input were separated by regions of lighter transport. Most of the ventral one-third of the sagulum was virtually devoid of terminals; this experiment had labeling in the most rostral part of the sagulum of any in this series. Four-day survival, 0.6 µl. For abbreviations, see list.

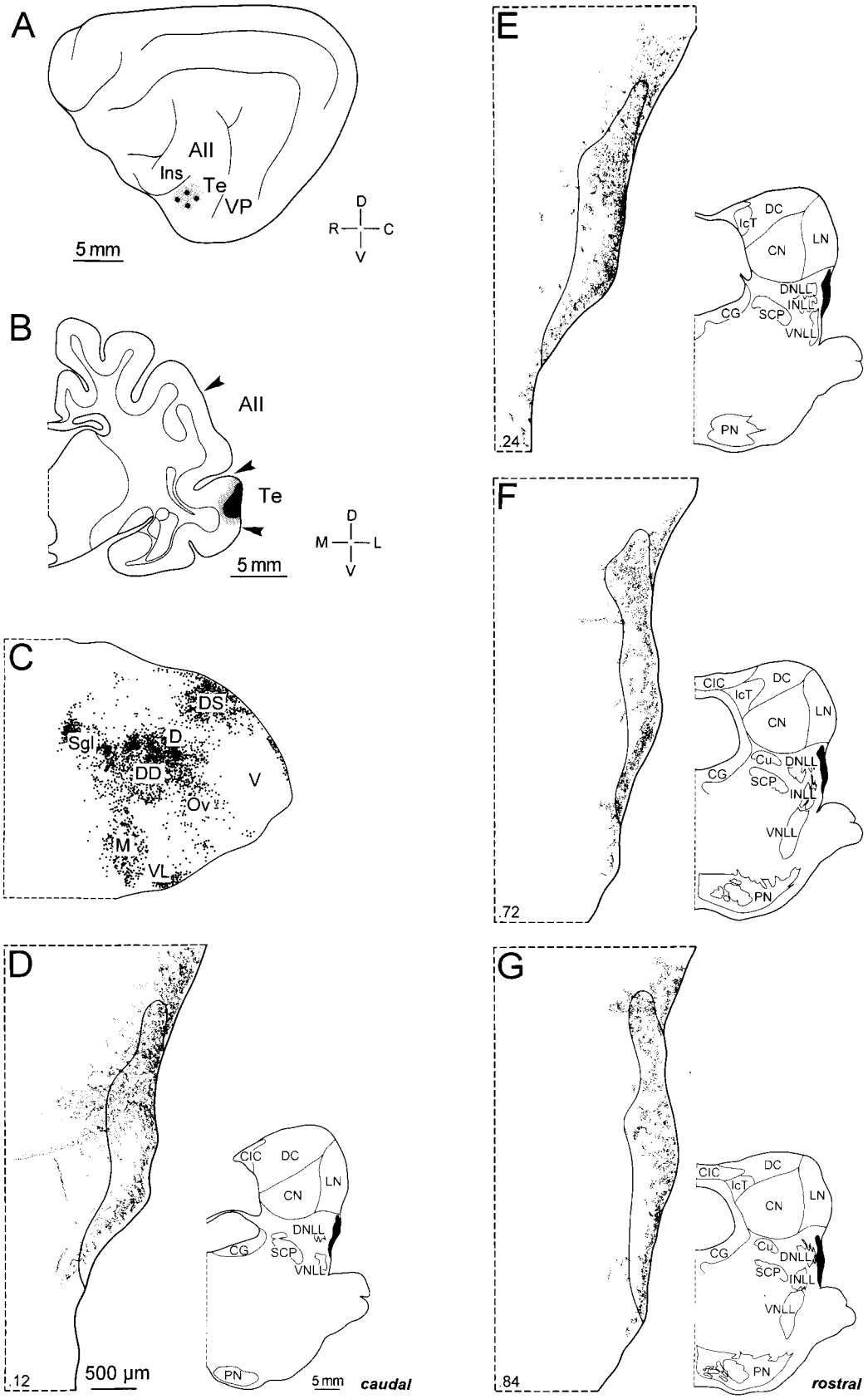

Fig. 10. Projections to the sagulum from the temporal cortex (Te). **A**: Four deposits of wheat germ agglutinin conjugated to horseradish peroxidase filled much of the temporal cortex caudal to the pseudosylvian sulcus. **B**: The tracer was limited to the ventral bank of the sylvian sulcus, and it did not enter the white matter. **C**: Retrogradely labeled cells were found in the suprageniculate nucleus (Sgl), the superficial (DS) and deep dorsal (DD) nuclei, and the medial (M) division. Their location was consistent with that from prior tract tracing studies (Winer et al., 1977). **D–G**: Sagulum input was dense and, unlike the pattern in the prior experiments, formed a dorsoventral band concentrated in the lateral sagulum (Figs. 6–9). This case had the greatest amount of labeling in the ventral and lateral sagulum in our series, suggesting that the entire sagulum is a recipient of cortical input. Four-day survival, 0.6 µl. For abbreviations, see list.

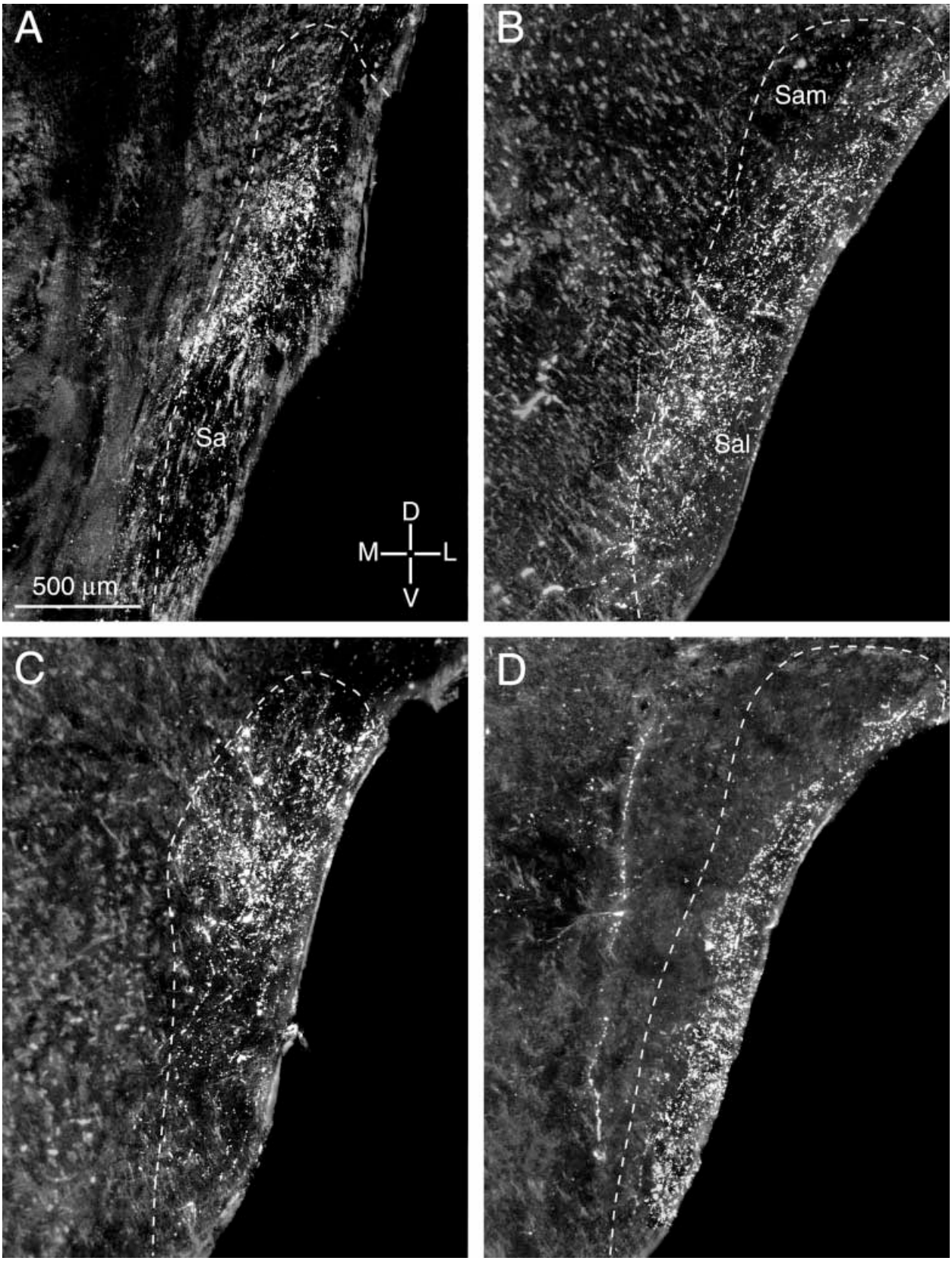

Fig. 11. Representative anterograde labeling in the sagulum after auditory cortical injections of wheat germ agglutinin conjugated to horseradish peroxidase. **A**: The terminal field formed by corticosagular axons arising in AI was located in the central sagulum, and situated medially. Three-day survival, 0.6 µl. **B**: The labeling from the AII injection covered more of the nucleus, and it extended farther laterally and ventrally than in the previous experiment. The density of the projection, however, was comparable to that of the AI injection (Fig. 6). There was little labeling in the dorsomedial sagulum (A,B: Sam). Four-day survival, 0.6 µl. **C**: Although restricted to the dorsal half of the sagulum in this caudal section, the labeling from EPD formed conspicuous focal patches. Three-day survival, 0.6 µl. **D**: The corticosagular transport from Te differed from all other experiments and was concentrated in the lateral portion of the nucleus, abutting the brainstem surface. Four-day survival, 0.6 µl. Polarized light microscopy. Planachromat, N.A. 0.12, ×80. For abbreviations, see list.

from AI, and it was focused more laterally and ventrally (Figs. 7E, 11B). The labeling spanned the dorsoventral extent of the sagulum in caudal sections (Fig. 7D,E), but it was heaviest ventrally in rostral sections (Figs. 7G, 11B). As in the previous experiment (Fig. 6), labeling was entirely ipsilateral, density was variable, and within a given region, patches of dense input adjoined projection-free areas (Fig. 7F,G). The density of preterminal labeling dorsal to the sagulum suggests that the corticofugal axons probably entered (Fig. 7D,F) by crossing the long axis of lateral lemniscal fibers in the neuropil (see Figs. 1, LL, and 7G, ventral part of sagulum).

Deposits in the suprasylvian fringe area (SF) dorsal and medial to AI (Fig. 8A,B) labeled neurons in many parts of the auditory thalamus, including the medial division and the dorsal, deep dorsal, and suprageniculate nuclei (Fig. 8C). The injection was restricted because the ventral division of the medial geniculate body contained no labeled cells, as would have been expected had AI been involved, although there may be slight involvement of sulcal visual areas. The labeling in the sagulum was lighter than that in the previous cases (Figs. 6, 7), especially in the caudalmost one-third (Fig. 8D). As in the AI experiment (Fig. 6), the transport was concentrated centrally in the sagulum along the dorsoventral axis and was heaviest medially (Fig. 8F,G). In this experiment, the patches were less prominent and fewer in number than in the other cases, and preterminal fibers formed linear fascicles within the sagulum (Fig. 8E).

Injections in the dorsal part of the posterior ectosylvian gyrus (EPD) labeled neurons in every medial geniculate subdivision, with the largest number in the suprageniculate and superficial dorsal nuclei (Fig. 9C, Sgl, DS) and only a few in the ventral division (Fig. 9C, V). This field had the strongest projection to the sagulum (Fig. 9D–G) in the series, although the deposits were no larger than those in several of the other cases and the thalamic labeling was less than that in the AI (Fig. 6C) and AII (Fig. 7C) experiments. The input was concentrated in the dorsolateral part of the nucleus throughout its caudorostral extent (Figs. 9D–F, 11C), and it was uniformly dense along the mediolateral axis. The most ventral third was almost entirely without input along the length of the sagulum. This finding suggests an areal, and possibly a regional, segregation of input. Besides the characteristic transport above the sagulum, some labeling was present in the neuropil at the junction with the lateral lemniscal nuclei (Fig. 9G); this finding suggests again that corticosagular afferents entered the nucleus from its anterodorsal pole.

Several injections in temporal cortex (Fig. 10A,B, Te) labeled neurons in auditory thalamic subdivisions whose nuclear distribution was consistent with previous data on the thalamocortical cells of origin projecting to Te (Winer et al., 1977). Such neurons were abundant in the suprageniculate nucleus, the superficial and deep dorsal nuclei, and the medial division (Fig. 10C). The anterograde transport in the sagulum departed from the pattern for AI (Fig. 6), AII (Fig. 7), SF (Fig. 8), and EPD (Fig. 9). The bulk of the labeling was concentrated on the perimeter of the most lateral part of the nucleus (Figs. 10D–G, 11D), whereas the medial region was virtually free of anterograde transport other than preterminal fragments (Fig. 10D,G). The input was also robust caudally, extending farther posterior than in any other experiment (Fig. 10D), and somewhat less rostrally.

The anterograde transport of WGA-HRP from other cortical fields was considerably smaller and these experiments are not illustrated here. To summarize briefly (see Fig. 13), the projection from the anterior auditory field (AAF) was concentrated medially, and formed a few isolated patches of labeling near the central sagulum along its dorsoventral extent. Axons arising in the ventral posterior area (VP) terminated chiefly in rostral regions, where the sparse labeling formed a lateral focus oriented dorsoventrally; the strength of the projection was slightly greater than that from AAF. The axons originating from insular cortex (Ins) terminated diffusely in the dorsolateral sagulum; the strength of the projection was moderate, like that from AI. Injections in the intermediate part of the posterior ectosylvian gyrus (EPI) produced weak anterograde labeling in the dorsal sagulum, which was heavier in rostral sections. The connection from field P to the sagulum was not evident with WGA-HRP injections, perhaps due to the comparatively small size of this area or variability in its locus; however, corticosagular axons arising in P were labeled when a more sensitive tracer, BDA, was used (see below).

Corticosagular axons

BDA labeled the corticofugal axons in a Golgi-like manner, filling them by diffusion and revealing their terminal configurations in considerable detail. As in the WGA-HRP material, preterminal axons entered the sagulum dorsomedially, then formed a terminal plexus within it (Fig. 12A). Axonal endings consisted of three categories: en passant boutons, terminal profiles, and large, complex en passant endings. The vast majority of preterminal axons were thin, <1 μm in diameter, followed a dorsoventral orientation, and had swellings at irregular intervals (Fig. 12B, arrowheads). These en passant boutons were similar in size, ranging from 1 to 1.5 μm. Along their course, some fibers gave off single, short lateral branches approximately 5- to 10-μm long, which ended in a terminal bouton (Fig. 12B, hollow arrow). Other axons terminated abruptly, forming finger-like clusters of three to five branches, each decorated with a terminal bouton at its free end (Fig. 12C). Far rarer were the globular en passant endings with complex terminals. These terminals were elaborate and consisted of a central stem from which several boutons of various sizes and shapes arose. A large fusiform swelling was embedded in five to eight sessile or slightly pedunculated boutons and formed a tight cluster, like a rosette (Fig. 12D, upper part), the whole terminal measuring 8- to 10-μm long. These axonal endings resembled cerebellar mossy fibers and did not differ with regard to their cortical areal origin.

DISCUSSION

Corticosagular projection

Projections to the sagulum arose from the nine auditory cortical fields studied (Fig. 13). In five areas, the projection was substantial; in four others, it was appreciably lighter. This finding suggests that, although the corticosagular projection is a feature common to almost all areas recognized as auditory cortex, areal differences in the strength of input could reflect differing degrees of corticosagular influence. As a rule, the nontonotopic fields made a slightly larger contribution. That such widespread input has not been described before may reflect its broad origins, which

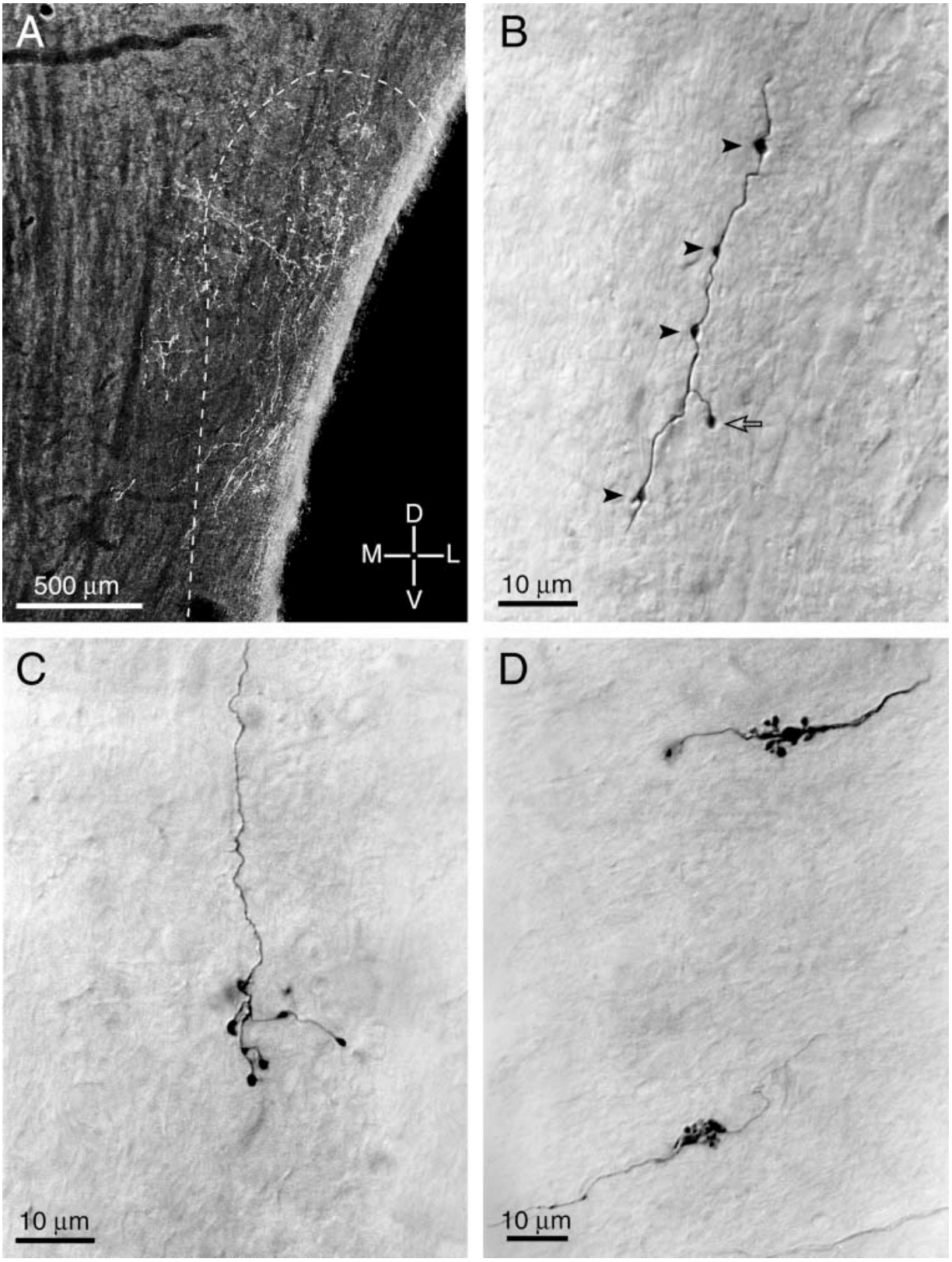

Fig. 12. Terminal morphology of auditory corticofugal axons in the sagulum after injections of biotinylated dextran amines (BDA). **A**: Global view of a corticofugal terminal plexus after insular cortex deposits; the axons entered the nucleus from its dorsomedial aspect and branched, often at right angles, within it (compare with Fig. 11). Seven-day survival, 1.32 µl of 20% BDA/saline, pressure injection. **B**: Many corticosagular axons had classic en passant endings (arrowheads) like these from a fiber projecting from field P; the single, short lateral branches of such fibers ended in a bouton (hollow arrow). Eight-day survival, 0.96 µl of 20% BDA/saline, pressure injection. **C**: The next most common type also had en passant endings; many axons formed tufts of three to five branches, each ending in a terminal bouton within the sagulum, like these from AII. Three-day survival, 0.6 µl of 10% BDA/saline, pressure injection. **D**: Two axons labeled by an AI injection. The largest (upper) has a thick preterminal trunk and large, irregular en passant excrescences like those of cerebellar mossy fibers; although relatively rare, such endings arose from many areas. Six-day survival, 1.32 µl of 20% BDA/saline, pressure injection. Protocol for A: Darkfield microscopy. Planachromat, N.A. 0.12, ×80. Protocol for panels B–D: Differential interference contrast (Nomarski) optics. Planapochromat, N.A. 1.4, ×1,250. D, dorsal; L, lateral; M, medial; V, ventral.

are revealed only by large injections of sensitive tracers. The collective projections from all areas and their convergence onto the sagulum must be massive.

A second issue is whether this labeling represents axon terminals or fibers projecting elsewhere. We reject the interpretation that they are preterminal axons for three reasons. The labeling that we classified as terminal usually ends abruptly at the ventral pole of the sagulum, making it unlikely that these are fibers of passage coursing to remote targets such as the pontine nuclei. The labeling forms irregular clusters of boutons, which is consistent with an interpretation of a terminal plexus. In experiments which used BDA, fine axons have abundant terminal boutons; these boutons are linked by the thin, parallel preterminal processes passing through the transitional neuropil between the lateral nucleus of the inferior colliculus and the sagulum. Such terminal boutons were present even after comparatively small cortical injections (Fig. 12).

The virtual absence of labeling in the most rostral 15–20% of the sagulum suggests that it is a separate region largely devoid of input or that areas other than those injected here have projections to it. The heterogeneity of sagulum cytoarchitecture (in which at least two divisions and several types of neuron are distinguished) suggests that other parts may be identified as the varieties of sagulum neurons can be classified more definitively and potential borders identified with more confidence. It has been suggested that the larger neurons in the dorsomedial sagulum are a lateral extension of the dorsal nucleus of the lateral lemniscus, which encroaches into the sagulum's neuropil (Adams and Mugnaini, 1984; Shneiderman et al., 1988; Hutson et al., 1991). Dorsomedial sagulum neurons are appreciably larger than those elsewhere in the nucleus (Morest and Oliver, 1984; Hutson et al., 1991; present results). We found that this region receives appreciable cortical input, much like the ventrolateral sagulum, whereas such terminal labeling is never present in the dorsal nucleus of the lateral lemniscus. Either the dorsomedial sagulum is not part of the dorsal nucleus of the lateral lemniscus, or it is a part that receives cortical input, or it is an independent entity. We favor the first conclusion.

The cortical areas with the strongest projections are the secondary and association fields, whose tonotopic arrangement is less orderly than those in areas AI, AAF, P, and other related territories (Reale and Imig, 1980). This finding is consistent with the idea that the sagulum has connectional affiliations with the extralemniscal auditory pathway, and it suggests a role in polymodal processing. In view of the preponderance of input from nontonotopical cortical areas, it would be somewhat surprising if future physiologic studies were to reveal a tonotopic organization within the sagulum. Insofar as a tonotopic organization exists, it would suggest that the sagulum has closer functionally affiliations with the central nucleus of the inferior colliculus and the dorsal nucleus of the lateral lemniscus, both of which show a high degree of tonotopy. If a tonotopic organization is absent, it suggests that the corticosagular nontonotopic projections prevail at the expense of tonotopy. Considering only the spatial overlap in terminal labeling at similar anteroposterior levels from tonotopic (Fig. 6F) and nontonotopic (Figs. 7F, 9F) cortical areas, it would seem unlikely that there is a tonotopic arrangement. This hypothesis remains to be examined directly.

Cortical modulation of the auditory brainstem and thalamus

Corticofugal auditory projections in the cat reach the medial geniculate body (Andersen et al., 1980a; Rouiller and de Ribaupierre, 1990; Bajo et al., 1995), the inferior colliculus (Diamond et al., 1969; Rockel and Jones, 1973; Andersen et al., 1980b), the superior olivary complex (Granstrem, 1988), and the sagulum (present results). Auditory cortex also projects to the superior colliculus (Meredith and Clemo, 1989; Harting et al., 1992) and the pontine nuclei (Brodal, 1972). This finding suggests that almost all levels of the ascending auditory system (and many structures with only remotely auditory affiliations) at or above the level of the midbrain have some role in polymodal processing and are under the influence of corticofugal input.

In the absence of physiologic studies on the sagulum, its connections and intrinsic circuitry may shed some light on its roles. Besides the lemniscal pathway to the cortex, an extralemniscal pathway (Morest, 1965; Winer, 1992) exists that partially bypasses the inferior colliculus and may subserve the auditory-evoked cortical responses preserved after interruption of the brachium of the inferior colliculus (Galambos et al., 1961; Adrian et al., 1966). The brachium is not essential for orientation to, or localization of, sound (Kryter and Ades, 1943; Jane et al., 1965; Thompson and Masterton, 1978), although large lesions of the brachium can result in auditory deficits of >30 dB in carnivores (Heffner and Heffner, 1984). The sagulum may contribute to extralemniscal activation by means of monosynaptic and widespread projections both to the inferior colliculus and the medial geniculate body (Hutson et al., 1991; and present account, Fig. 13). Cortical input to the sagulum thus represents the first significant opportunity for the auditory cortex to directly influence the ascending auditory system.

Because the sagulum projects to the medial and dorsal divisions of the medial geniculate body (Morest, 1965; Calford and Aitkin, 1983; Whitley and Henkel, 1984), the sagulothalamic outflow may have access by means of these nuclei to much of the auditory cortex. Perhaps the only pathways ipsilateral to the medial geniculate body that remain intact after inferior colliculus ablation and section of its commissure arise from the intermediate nucleus of the lateral lemniscus and the sagulum (Hutson et al., 1991).

A combined electrophysiologic and connectional study found that neurons in the dorsal division of the medial geniculate body discharge at long latencies and prefer a broad range of frequencies or respond selectively to complex sounds (Aitkin et al., 1981). This finding may reflect the sagulum's influence on neurons in the caudodorsal nucleus of the dorsal division, cells that are broadly tuned and could act as long-latency novelty detectors (Calford and Aitkin, 1983) and that are dependent on input from the pericentral nucleus of the inferior colliculus and the sagulum.

Cortical influence on premotor systems

Besides its role in the auditory system, the sagulum projects to both the superior colliculus (Hutson et al., 1991) and a lateral midbrain tegmental region, the paralemniscal zone (Henkel, 1981). The latter area receives input from the deep layers of the superior colliculus and a broad

constellation of other structures implicated in gaze control such as the nucleus prepositus hypoglossi, the paramedian pontine reticular formation, some interabducens neurons, the medial vestibular nucleus, and subthalamic regions (Henkel, 1981). The sole auditory projection to the paralemniscal zone is the sagulum. Paralemniscal neurons project upon the facial motor nucleus (Henkel and Edwards, 1978), thus providing sensory information for pinna orientation to sound source. Cortical input to both the deep layers of the superior colliculus (Meredith and Clemo, 1989; Harting et al., 1992) and the sagulum (present results) could provide this system with an auditory frame of reference critical for calibrating visual spatial orientation to sound. The sagulum also has a direct projection from the globus pallidus (Shinonaga et al., 1992) that could underlie long-term motor strategies with regard to auditory space. That the sagulum is active during vocalization in primates suggests that it may have a role in communicative aspects of auditory behavior (Jürgens et al., 1996).

Other evidence implicates the sagulum in motor responses such as the acoustic startle reflex. The sagulum projects to the pretectum and the midbrain reticular formation (Kudo et al., 1983). The pretectum has a role on sensorimotor integration (Carpenter and Pierson, 1973; Sprague et al., 1973; Berkley and Mash, 1978), whereas the midbrain reticular formation is involved in polysensory processing (Scheibel et al., 1955). In an echolocating bat (*Pteronotus parnellii*), the projection from the central nucleus of the inferior colliculus to the pretectum was as large as that to the auditory thalamus, attesting to the close relation between the lemniscal auditory pathway and the premotor system (Wenstrup et al., 1994). Both the pretectum and midbrain reticular formation respond to auditory stimulation (Amassian and DeVito, 1954; Scheibel et al., 1955), and lesions to them profoundly affect the acoustic startle reflex (Jane et al., 1965; Groves et al., 1974; Jordan and Leaton, 1982). Thus, some aspects of the startle reflex may be under corticosagular influence.

Neurochemical observations

A moderate number of glutamic acid decarboxylase–positive cells occur in the sagulum (Adams and Mugnaini, 1984). A postembedding immunocytochemical study found a few GABA-immunoreactive cells and many more glycine-positive cells (Stanforth et al., 1995). Some neurons colocalize both GABA and glycine though the interpretation of such colocalization is uncertain (Winer et al., 1995). It has been proposed that the sagulum projection to the inferior colliculus might be glycinergic (Stanforth et al., 1995). In our material glycine-positive cells are rare, at most one per section, and in most sections there were none; these cells almost always colocalize GABA. The primary target of sagulocollicular projections are the dorsal cortex and lateral nucleus, which receive a minute glycinergic input compared with the central nucleus (Henkel and Shneiderman, 1988; J.A. Winer and D.T. Larue, unpublished observations). It seems equally unlikely that there is any glycinergic component in the sagulothalamic system. Transmitter-specific labeling studies indicate that the majority of sagulothalamic neurons are labeled by [^{3}H]aspartate (Saint Marie, 1996 [chinchilla]). Moreover, neither thick, free-floating nor semithin, plastic embedded material from several hundred sections processed for glycine reveal glycinergic axon terminals in any auditory thalamic subdivision (Winer et al., 1995 [bat]; D.T. Larue and J.A. Winer, unpublished observations [rat]). Abundant glycinergic fibers and a few immunopositive neurons were seen in the inferior colliculus with two antibodies, one of which we used (Dr. Wenthold's Glycine II) and another that we did not. No or few glycinergic fibers, and no neurons, are immunostained in the rat auditory thalamus (Rampon et al., 1996). We conclude that intrinsic glycinergic neurons play little, if any, significant role in sagulum function, although glycinergic puncta from extrinsic sources may influence sagulum neurons. In contrast, GABAergic neurons and puncta are relatively abundant and suggest a degree of intrinsic organization not unlike that in the medial geniculate body (Winer, 1992). The predominance of GABA is quintessentially a forebrain pattern to which the thalamus in general and the medial geniculate body in particular are no exception.

Comparison with other auditory corticofugal systems

The present results complement a parallel series of studies in which auditory cortical projections onto the inferior colliculus were examined by using an experimental approach similar to that in the present study, including the same tracers (Winer et al., 1998). These studies found that each of the three principal parts of the inferior colliculus received a different pattern of corticofugal input. Thus, the lateral nucleus and one or more parts of the dorsal cortex were labeled to varying degrees by every injection, whereas the central nucleus received a far lighter projection (except at its dorsomedial or caudal and rostral borders, where heavier projections to adjoining areas seemed to extend into the central nucleus). A further parallel with the corticosagular projection was that the labeling from deposits in nontonotopic cortical areas had focal and topographic relations with their targets. Another similarity was that the collicular targets with the largest cortical input received the sparsest input from the auditory brainstem (Aitkin, 1986). This finding suggests that either ascending or descending projections are prominent in some nuclei, unlike the medial geniculate body, for example, in which ascending (Calford and Aitkin, 1983) and descending (Andersen et al., 1980b) systems each terminate.

A further set of auditory cortical projection patterns to the medial geniculate body offered the closest concordance with the present results in the sagulum (Diehl and Winer, 1996). The parallel findings in the two studies were that each cortical field had corticothalamic and corticosagular targets. A second parallel was that this projection was focal and topographic in each experiment, even if the axis of topical representation is uncertain. A third result was that the projection from nonprimary areas was, on the whole, larger (or at least more divergent) than that from primary areas. A fourth outcome was that the corticofugal axons ending in an area often had diverse morphologies, suggesting that more than one type of cortical projection neuron was involved or that a single type had heterogeneous terminal patterns. The many parallels between the corticothalamic and corticosagular projection systems suggest that the sagulum might be regarded appropriately from a functional perspective as the most remote midbrain nucleus in which auditory cortical input predominates.

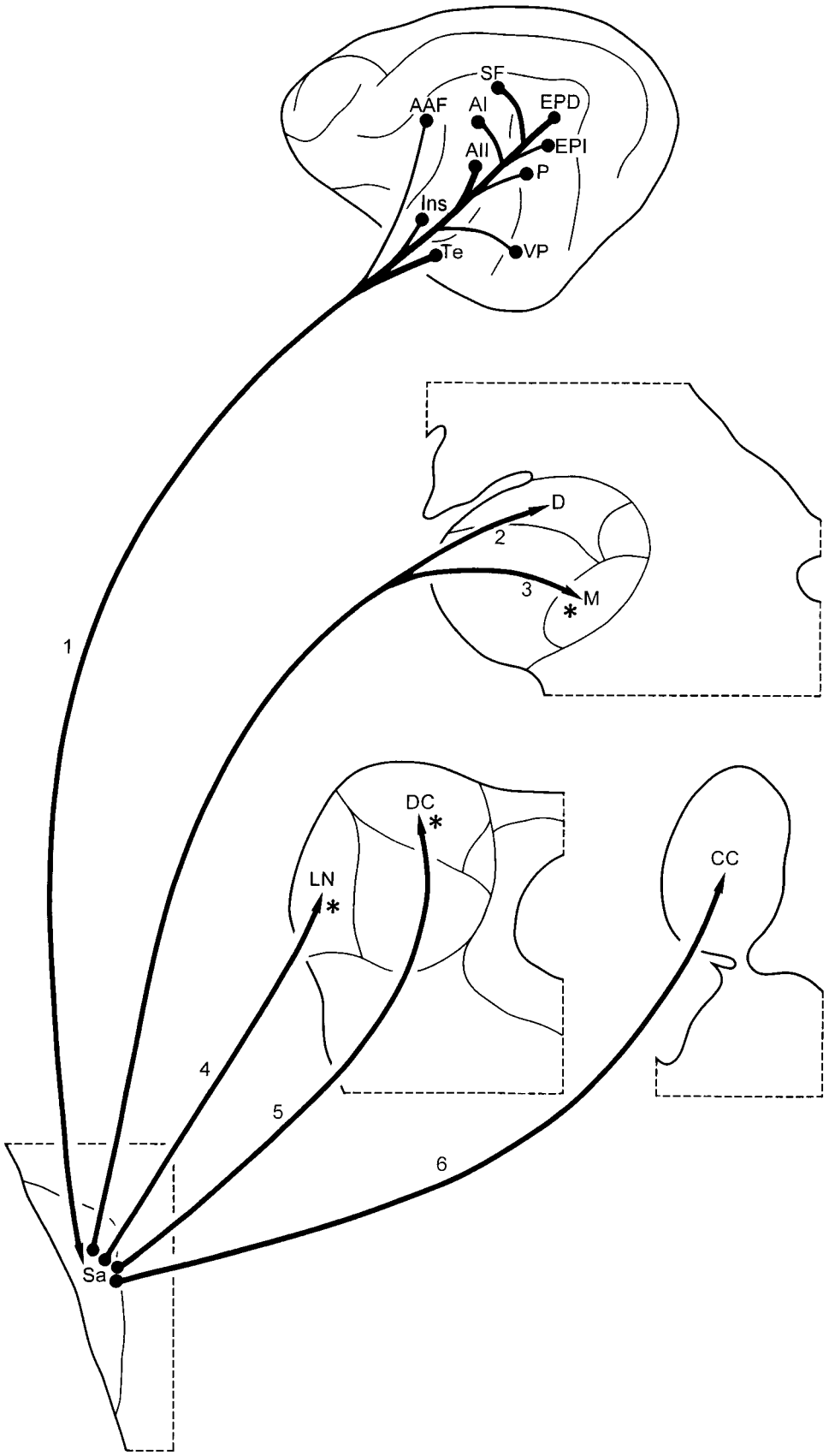

Fig. 13. Schematic representation of the primary connections of the sagulum with the auditory midbrain, the thalamus, and the cortical areas projecting to the sagulum. The tectosagular projection is omitted. The sagulum receives descending inputs from a wide array of auditory cortical areas (1). The sagulum sends ascending projections ipsilaterally to the dorsal (2) and medial (3) subdivisions of the medial geniculate body, and to much of the inferior colliculus, including the lateral nucleus (4) and the dorsal (5) and caudal (6) cortices. Contralaterally (asterisks), the sagulum projects to the medial division of the medial geniculate body and the dorsal cortex and lateral nucleus of the inferior colliculus. The principal references documenting these projections are: 1) present results; 2) Aitkin et al. (1981), Calford and Aitkin (1983), Henkel (1983), Whitley and Henkel (1984), and present results; 3) Henkel (1983), Whitley and Henkel (1984), Hutson et al. (1991), and present results; 4) Henkel and Shneiderman (1988); 5) Brunso-Bechtold et al. (1981), Henkel and Shneiderman (1988), Hutson et al. (1991), and present results; and 6) Henkel and Shneiderman (1988). For abbreviations, see list.

Speculations on functional organization

Although there are no physiological data available on the functional properties of sagulum neurons, circumstantial evidence suggests that they may be part of the monaural system (Kelley et al., 1992 [chinchilla]). Several brainstem nuclei that encode monaural information (such as the medial and ventral nuclei of the trapezoid body, anterolateral periolivary nucleus, and ventral nucleus of the lateral lemniscus) have many calbindin-immunoreactive neurons, which has been used as a marker to denote monaural affiliations. In contrast, neurons in the nuclei preferentially responsive to binaural input (medial and lateral superior olivary nuclei, and the dorsal nucleus of the lateral lemniscus) are largely calbindin-negative. All of the principal cells of the sagulum are calbindin-positive (Kelley et al., 1992). If the sagulum is dedicated to monaural processing, then perhaps the projection from the auditory cortex arises primarily from bands of monaural neurons, which are segregated spatially in columns interspersed between binaural interaction bands (Imig and Adrián, 1977; Imig and Brugge, 1978; Imig and Reale, 1981). Because these monaural representations account for only 6–7% of the neurons in AI (Hall and Goldstein, 1968; Phillips and Irvine, 1983) and approximately 10% in area P (Orman and Phillips, 1984), and 6.9–41% in area AII (Schreiner and Cynader, 1984), their density and preferential distribution is consistent with the finding that the projection to the sagulum, at least from AII, exceeds that from AI, and may contribute to a monaural descending system. The clustered and widely distributed zones of monaural cortical neurons reported in these physiological studies could help to account for the irregular, patchy, and periodic distribution of corticosagular terminal labeling seen in the present experiments. Whether neurons in nonprimary cortical areas form the aural bands characteristic of those found in AI is unknown. If they do have such periodic distributions, or if they are dispersed even more diffusely, this arrangement could help account for the unusual configuration of the terminal fields. In any case, the projections from a tonotopically organized field (AI; Fig. 6) were not as focal as those from temporal cortex (Te; Fig. 10), an area whose tonotopic organization is modest (Sindberg and Thompson, 1962).

Our data suggest that the sagulum provides a parallel excitatory pathway influencing both midbrain and thalamic nuclei while receiving a wide range of descending cortical input from many areas. The sagulum thus occupies a unique position in the sensorimotor midbrain, linking the auditory brainstem and forebrain to premotor circuits. A more precise understanding of its role will require physiological investigations abetted by immunoconnectional studies.

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LITERATURE CITED

Adams, J.C. (1981) Heavy metal intensification of DAB-based HRP reaction product. J. Histochem. Cytochem. *29:*775.

Adams, J.C. and E. Mugnaini (1984) Dorsal nucleus of the lateral lemniscus: A nucleus of GABAergic projection neurons. Brain Res. Bull. *13:*585–590.

Adrian, H.O., J.M. Goldberg, and J.F. Brugge (1966) Auditory evoked cortical potentials after lesion of brachium of the inferior colliculus. J. Neurophysiol. *29:*456–466.

Aitkin, L.M. (1986) The Auditory Midbrain. Structure and Function in the Central Auditory Pathway. Clifton, NJ: Humana Press.

Aitkin, L.M., M.B. Calford, C.E. Kenyon, and W.R. Webster (1981) Some facets of the organization of the principal division of the cat medial geniculate body. In J. Syka and L.M. Aitkin (eds): Neuronal Mechanisms of Hearing. New York: Plenum Press, pp. 163–181.

Amassian, V.E. and R.V. DeVito (1954) Unit activity in reticular formation and nearby structures. J. Neurophysiol. *17:*575–603.

Andersen, R.A., P.L. Knight, and M.M. Merzenich (1980a) The thalamocortical and corticothalamic connections of AI, AII, and the anterior auditory field (AAF) in the cat: Evidence for two largely segregated systems of connections. J. Comp. Neurol. *194:*663–701.

Andersen, R.A., R.L. Snyder, and M.M. Merzenich (1980b) The topographic organization of corticocollicular projections from physiologically identified loci in the AI, AII, and anterior auditory cortical fields of the cat. J. Comp. Neurol. *191:*479–494.

Bajo, V.M., E.M. Rouiller, E. Welker, S. Clarke, A.E.P. Villa, Y. de Ribaupierre, and F. de Ribaupierre (1995) Morphology and spatial distribution of corticothalamic terminals originating from the cat auditory cortex. Hear. Res. *83:*161–174.

Berkley, K.J. and D.C. Mash (1978) Somatic sensory projections to the pretectum in the cat. Brain Res. *158:*445–449.

Berman, A.L. and E.G. Jones (1982) The Thalamus and Basal Telencephalon of the Cat: A Cytoarchitectonic Atlas with Stereotaxic Coordinates. Madison and London: The University of Wisconsin Press.

Bowman, E.M. and C.R. Olson (1988a) Visual and auditory association areas of the cat's posterior ectosylvian gyrus: Thalamic afferents. J. Comp. Neurol. *272:*15–29.

Bowman, E.M. and C.R. Olson (1988b) Visual and auditory association areas of the cat's posterior ectosylvian gyrus: Cortical afferents. J. Comp. Neurol. *272:*30–42.

Brodal, P. (1972) The corticopontine projection in the cat: The projection from the auditory cortex. Arch. Ital. Biol. *110:*119–144.

Brunso-Bechtold, J.K., G.C. Thompson, and R.B. Masterton (1981) HRP study of the organization of auditory afferents ascending to central nucleus of inferior colliculus in cat. J. Comp. Neurol. *197:*705–722.

Calford, M.B. and L.M. Aitkin (1983) Ascending projections to the medial geniculate body of the cat: Evidence for multiple, parallel auditory pathways through the thalamus. J. Neurosci. *3:*2365–2380.

Carpenter, M.B. and R.J. Pierson (1973) Pretectal region and the pupillary light reflex: An anatomical analysis in the monkey. J. Comp. Neurol. *149:*271–300.

Diamond, I.T., E.G. Jones, and T.P.S. Powell (1969) The projection of the auditory cortex upon the diencephalon and brainstem in the cat. Brain Res. *15:*305–340.

Diehl, J.J. and J.A. Winer (1996) Contrasting auditory corticofugal projections to the inferior colliculus and the medial geniculate body. Proc. Soc. Neurosci. Abstr. *22:*1069.

Feliciano, M., E. Saldaña, and E. Mugnaini (1995) Direct projections from the rat primary auditory neocortex to nucleus sagulum, paralemniscal regions, superior olivary complex and cochlear nuclei. Audit. Neurosci. *1:*287–308.

Galambos, R., R.E. Myers, and G.C. Sheatz (1961) Extralemniscal activation of auditory cortex in cats. Am. J. Physiol. *200:*23–28.

Granstrem, É.É. (1988) Ultrastructural organization of efferent fiber endings in zones A_1 and A_4 of the cat auditory cortex in the superior olivary complex. Neurosci. Behav. Physiol. *18:*109–114.

Groves, P.M., C.J. Wilson, and R.D. Boyle (1974) Brainstem pathways, cortical modulation, and habituation of the acoustic startle response. Behav. Biol. *10:*391–418.

Hall, J.L. and M.H. Goldstein, Jr. (1968) Representation of binaural stimuli by single units in primary auditory cortex of unanesthetized cats. J. Acoust. Soc. Am. *43:*456–461.

Harting, J.K., B.V. Updyke, and D.P. Van Lieshout (1992) Corticotectal projections in the cat: Anterograde transport studies of twenty-five cortical areas. J. Comp. Neurol. *324:*379–414.

Heffner, R.S. and H.E. Heffner (1984) Hearing loss in dogs after lesions of the brachium of the inferior colliculus and medial geniculate. J. Comp. Neurol. *230:*207–217.

Henkel, C.K. (1981) Afferent sources of a lateral midbrain tegmental zone associated with the pinnae in the cat as mapped by retrograde transport of horseradish peroxidase. J. Comp. Neurol. *203:*213–226.

Henkel, C.K. (1983) Evidence of sub-collicular auditory projections to the medial geniculate nucleus in the cat: An autoradiographic and horseradish peroxidase study. Brain Res. *259:*21–30.

Henkel, C.K. and S.B. Edwards (1978) The superior colliculus control of pinna movements in the cat: Possible anatomical connections. J. Comp. Neurol. *182:*763–776.

Henkel, C.K. and A. Shneiderman (1988) Nucleus sagulum: Projections of a lateral tegmental area to the inferior colliculus in the cat. J. Comp. Neurol. *271:*577–588.

Hutson, K.A., K.K. Glendenning, and R.B. Masterton (1991) Acoustic chiasm IV: Eight midbrain decussations of the auditory system in the cat. J. Comp. Neurol. *312:*105–131.

Imig, T.J. and H.O. Adrián (1977) Binaural columns in the primary field (A1) of cat auditory cortex. Brain Res. *138:*241–257.

Imig, T.J. and J.F. Brugge (1978) Sources and terminations of callosal axons related to binaural and frequency maps in primary auditory cortex of the cat. J. Comp. Neurol. *182:*637–660.

Imig, T.J. and A. Morel (1984) Topographic and cytoarchitectonic organization of thalamic neurons related to their targets in low-, middle-, and high-frequency representations in cat auditory cortex. J. Comp. Neurol. *227:*511–539.

Imig, T.J. and R.A. Reale (1980) Patterns of cortico-cortical connections related to tonotopic maps in cat auditory cortex. J. Comp. Neurol. *192:*293–332.

Imig, T.J. and R.A. Reale (1981) Ipsilateral corticocortical projections related to binaural columns in cat primary auditory cortex. J. Comp. Neurol. *203:*1–14.

Jane, J.A., R.B. Masterton, and I.T. Diamond (1965) The function of the tectum for attention to auditory stimuli in the cat. J. Comp. Neurol. *125:*165–192.

Jordan, W.P. and R.N. Leaton (1982) Effects of mesencephalic reticular formation lesions on habituation of startle and lick suppression responses in the rat. J. Comp. Physiol. Psychol. *96:*170–183.

Kelley, P.E., R.D. Frisina, M.L. Zettel, and J.P. Walton (1992) Differential calbindin-like immunoreactivity in the brainstem auditory system of the chinchilla. J. Comp. Neurol. *319:*196–212.

Kryter, K.D. and H.W. Ades (1943) Studies on the function of the higher acoustic nervous centers in the cat. Am. J. Psychol. *56:*501–536.

Kudo, M., K. Itoh, S. Kawamura, and N. Mizuno (1983) Direct projections to the pretectum and the midbrain reticular formation from auditory relay nuclei in the lower brainstem of the cat. Brain Res. *288:*13–19.

Larue, D.T. and J.A. Winer (1996) Postembedding immunocytochemistry of large sections of brain tissue: An improved flat-embedding technique. J. Neurosci. Methods *68:*125–132.

Meredith, M.A. and H.R. Clemo (1989) Auditory cortical projection from the anterior ectosylvian sulcus (field AES) to the superior colliculus in the cat: An anatomical and electrophysiological study. J. Comp. Neurol. *289:*687–707.

Merzenich, M.M. and M.D. Reid (1974) Representation of the cochlea within the inferior colliculus of the cat. Brain Res. *77:*397–415.

Mesulam, M.-M. (1978) Tetramethylbenzidine for horseradish peroxidase neurohistochemistry: A non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. J. Histochem. Cytochem. *26:*106–117.

Morel, A. and T.J. Imig (1987) Thalamic projections to fields A, AI, P, and VP in the cat auditory cortex. J. Comp. Neurol. *265:*119–144.

Morest, D.K. (1965) The lateral tegmental system of the midbrain and the medial geniculate body: Study with Golgi and Nauta methods in the cat. J. Anat. *99:*611–634.

Morest, D.K. and D.L. Oliver (1984) The neuronal architecture of the inferior colliculus in the cat: Defining the functional anatomy of the auditory midbrain. J. Comp. Neurol. *222:*209–236.

Oertel, W.H., D.E. Schmechel, M.L. Tappaz, and I.J. Kopin (1981) Production of a specific antiserum to rat brain glutamic acid decarboxylase by injection of an antigen-antibody complex. Neuroscience *6:*2689–2700.

Oliver, D.L., J.A. Winer, G.E. Beckius, and R.L. Saint Marie (1994) Morphology of GABAergic neurons in the inferior colliculus of the cat. J. Comp. Neurol. *340:*27–42.

Orman, S.S. and D.P. Phillips (1984) Binaural interactions of single neurons in posterior field of cat auditory cortex. J. Neurophysiol. *51:*1028–1039.

Phillips, D.P. and D.R.F. Irvine (1983) Some features of binaural input to single neurons in physiologically defined area AI of cat cerebral cortex. J. Neurophysiol. *49:*383–395.

Prieto, J.J., M. Beneyto, M.L. Picó, J.A. Merchán, and J.A. Winer (1996) Nucleus sagulum: A possible substrate for cortical control of the auditory midbrain. Proc. Soc. Neurosci. Abstr. *22:*1071.

Rampon, C., P.H. Luppi, P. Fort, C. Peyron, and M. Jouvet (1996) Distribution of glycine-immunoreactive cell bodies and fibers in the rat brain. Neuroscience *75:*737–755.

Reale, R.A. and T.J. Imig (1980) Tonotopic organization in auditory cortex of the cat. J. Comp. Neurol. *182:*265–291.

Rockel, A.J. and E.G. Jones (1973) The neuronal organization of the inferior colliculus of the adult cat: II. The pericentral nucleus. J. Comp. Neurol. *149:*301–334.

Rouiller, E.M. and F. de Ribaupierre (1990) Arborization of corticothalamic axons in the auditory thalamus of the cat: A PHA-L tracing study. Neurosci. Lett. *108:*29–35.

Saint Marie, R.L. (1996) Glutamatergic connections of the auditory midbrain: Selective uptake and axonal transport of D-[^{3}H]aspartate. J. Comp. Neurol. *373:*255–270.

Scheibel, M.F., A. Scheibel, A. Mollica, and G. Moruzzi (1955) Convergence and interaction of afferent impulses on single units of reticular formation. J. Neurophysiol. *18:*309–331.

Schreiner, C.E. and M.S. Cynader (1984) Basic functional organization of second auditory cortical field (AII) of the cat. J. Neurophysiol. *51:*1284–1305.

Shinonaga, Y., M. Takada, R. Ogawa-Meguro, Y. Ikai, and N. Mizuno (1992) Direct projections from the globus pallidus to the midbrain and pons in the cat. Neurosci. Lett. *135:*179–183.

Shneiderman, A., D.L. Oliver, and C.K. Henkel (1988) Connections of the dorsal nucleus of the lateral lemniscus: An inhibitory parallel pathway in the ascending auditory system? J. Comp. Neurol. *276:*188–208.

Sindberg, R.M. and R.F. Thompson (1962) Auditory response fields in ventral temporal and insular cortex of cat. J. Neurophysiol. *25:*21–28.

Sprague, J.M., G. Berlucchi, and G. Rizzolatti (1973) The role of superior colliculus and pretectum in vision and visually guided behavior. In R. Jung (ed): Handbook of Sensory Physiology, Vol. VII/3, Part B, Central Visual Processing. Berlin: Springer-Verlag, pp. 27–102.

Stanforth, D.A., A. Shneiderman, and R.L. Saint Marie (1995) GABA and glycine immunoreactivities of the nucleus sagulum. Proc. Soc. Neurosci. Abstr. *21:*403.

Thompson, G.C. and R.B. Masterton (1978) Brainstem auditory pathways involved in reflexive head orientation to sound. J. Neurophysiol. *41:*1183–1202.

Wenstrup, J.J., D.T. Larue, and J.A. Winer (1994) Projections of physiologically defined subdivisions of the inferior colliculus in the mustached bat: Targets in the medial geniculate body and extrathalamic nuclei. J. Comp. Neurol. *346:*207–236.

Whitley, J.M. and C.K. Henkel (1984) Topographical organization of the inferior collicular projection and other connections of the ventral nucleus of the lateral lemniscus in the cat. J. Comp. Neurol. *229:*257–270.

Winer, J.A. (1992) The functional architecture of the medial geniculate body and the primary auditory cortex. In D.B. Webster, A.N. Popper, and R.R. Fay (eds): Springer Handbook of Auditory Research, Vol. 1, The Mammalian Auditory Pathways: Neuroanatomy. New York: Springer-Verlag, pp. 222–409.

Winer, J.A. and D.T. Larue (1988) Anatomy of glutamic acid decarboxylase (GAD) immunoreactive neurons and axon terminals in the rat medial geniculate body. J. Comp. Neurol. *278:*47–68.

Winer, J.A., I.T. Diamond, and D. Raczkowski (1977) Subdivisions of the auditory cortex of the cat: The retrograde transport of horseradish peroxidase to the medial geniculate body and posterior thalamic nuclei. J. Comp. Neurol. *176:*387–418.

Winer, J.A., G.D. Pollak, and D.T. Larue (1995) GABA and glycine in the central auditory pathway of the mustache bat: Structural substrates for inhibitory neuronal organization. J. Comp. Neurol. *355:*317–353.

Winer, J.A., R.L. Saint Marie, D.T. Larue, and D.L. Oliver (1996) GABAergic feedforward projections from the inferior colliculus to the medial geniculate body. Proc. Natl. Acad. Sci. USA *93:*8005–8010.

Winer, J.A., D.T. Larue, J.J. Diehl, and B.J. Hefti (1998) Auditory cortical projections to the cat inferior colliculus. J. Comp. Neurol. (in press).

Woolsey, C.N. (1960) Organization of cortical auditory system: A review and a synthesis. In G.L. Rasmussen and W.F. Windle (eds): Neural Mechanisms of the Auditory and Vestibular Systems. Springfield: C.C. Thomas, pp. 165–180.