

Research report

The cellular origin of corticofugal projections to the superior olivary complex in the rat

John R. Doucet*, Liana Rose, David K. Ryugo

Center for Hearing Sciences, Departments of Otolaryngology-Head and Neck Surgery and Neuroscience,
Johns Hopkins University School of Medicine, 720 Rutland Avenue, Baltimore, MD 21205, USA

Accepted 5 October 2001

Abstract

Corticofugal pathways originating in auditory cortex innervate most subcortical auditory nuclei in the ascending pathway [Auditory Neurosci. 1 (1995) 287–308; J. Comp. Neurol. 371 (1996) 15–40]. Our goal is to determine if these projections arise from the same neurons or if different neurons project to each of the separate structures. We also seek to identify the layers and fields of auditory cortex from which these neurons originate. In the present study, we answer these questions with respect to the projections to the superior olivary complex (SOC). Fluorescent retrograde tracers, Fast Blue (FB) or Diamidino Yellow (DiY), were injected into the SOC and the pattern of labeled cells was determined in temporal neocortex. We also injected FB into the granule cell domain (GCD) of the cochlear nucleus. Cortical projections to the GCD derive exclusively from layer V pyramidal cells in primary auditory cortex [Brain Res. 706 (1996) 97–102]. Thus the pattern of labeling produced by injections in the GCD provided a reference for interpreting the labeling after SOC injections. Layer V pyramidal cells project to the SOC, and these neurons were distributed bilaterally in primary and secondary areas of auditory cortex. The projections to the SOC from primary auditory cortex are predominantly uncrossed, whereas those from secondary auditory cortex are nearly equal for the two hemispheres. In animals that received injections of FB in the GCD and DiY in the SOC, cells labeled by each injection had a different laminar distribution and very few cells were double labeled. These data suggest that the cortical pathways ending in the cochlear nucleus and SOC are largely independent. We discuss the implications of these findings with respect to the multifunctional nature of the SOC in brainstem auditory processing. © 2002 Elsevier Science B.V. All rights reserved.

Theme: Sensory systems

Topic: Auditory systems: central anatomy

Keywords: Auditory; Hearing; Corticobulbar projection; Granule cell domain; Cochlear nucleus

1. Introduction

The auditory cortex resides at the summit of the auditory pathway. It interprets ascending information and gives rise to pathways that descend to subcortical auditory nuclei [9,10,28,40]. These corticofugal pathways suggest that auditory cortex not only processes ascending neural information but also actively influences it. One target of these descending pathways is the superior olivary complex (SOC [9,20]). The SOC is a collection of distinct nuclei located rostral to the facial nucleus along the floor of the pons. The neurons in the SOC form circuits that compute

timing and intensity differences between the two ears, stimulus attributes that are crucial in localizing the position of a sound in space [13,14]. They also provide efferent feedback to the cochlea [11,34,35,38,42] and the cochlear nucleus [4,8,26,30,31,39,46], and have been implicated in the pathway mediating the acoustic startle reflex [17,37]. As a first step towards understanding the role of corticofugal inputs within this diverse array of functions, our goal in this study was to define the cortical areas and cells that project to the SOC in the rat.

We addressed these questions by injecting the retrograde tracer Fast Blue (FB) or Diamidino Yellow (DiY) into the SOC and analyzing the distribution of retrogradely labeled cells over broad regions of temporal cortex. FB and DiY are fluorescent tracers that are commonly employed in tract-tracing studies because of their sensitivity and their

*Corresponding author. Tel.: +1-410-955-0026; fax: +1-410-614-4748.

E-mail address: jdoucet@bme.jhu.edu (J.R. Doucet).

ability to be transported effectively over long distances [2,16]. They also are used together in double labeling experiments, because FB and DiY have similar retrograde transport rates and they accumulate in different compartments of the cell – DiY mainly labels the cell nucleus, whereas FB labels the cytoplasm and proximal dendrites of a neuron [16]. In a few experiments, we took advantage of these latter properties by pairing a DiY injection in the SOC with an injection of FB in the granule cell domain (GCD) of the cochlear nucleus (CN). In the rat, corticofugal projections to the GCD arise from layer V pyramidal cells sprinkled within primary auditory cortex [41]. Therefore, the cortical labeling produced by the CN injection provided context for describing the cellular origin of projections to the SOC. Additionally, we looked for double-labeled cells in these experiments in order to determine if the corticofugal pathways to the SOC and CN derive from a common source.

2. Materials and methods

2.1. Surgical preparation and dye injections

The present report is based on data obtained from eight male Sprague–Dawley rats weighing between 250 and 400 g. All animals and procedures were used in accordance with the NIH guidelines and the approval of the Johns Hopkins Medical School Animal Care and Use Committee. At the beginning of each experiment, a rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg) and then given an intramuscular injection of atropine sulfate (0.05 mg) to reduce mucous secretions. When the animal was areflexic to a paw pinch, the soft tissues overlying the dorsal aspect of the skull were removed and the occipital bone to the left of the midline was drilled away in order to expose the posterior aspect of the cerebellar cortex. A portion of the cerebellum was aspirated in order to view the CN and the floor of the IV ventricle. After injecting dye into the SOC or the CN, gel foam was used to fill the space created by the aspirations, the skin sutured, and the animal was allowed to recover.

We injected the retrograde tracer Fast Blue (FB; Sigma, St. Louis, MO, USA; 3% aqueous solution) into the SOC for two rats and Diamidino Yellow (DiY; Sigma, 3% aqueous solution) for three rats. All injections in the SOC (and the CN) were made on the same side of the brainstem (animal's left side). The tip of a 10- μ l Hamilton syringe filled with one of the dyes was placed just posterior to the medial boundary of the dorsal cochlear nucleus (DCN) and advanced through the brainstem. The angle of approach and stereotaxic coordinates were computed using a brain atlas [22]. Two tracks were made through the brainstem that were spaced 0.5 mm apart along the medial/lateral axis. Once the tip of the syringe reached the target, two

deposits of 0.1–0.2 μ l of dye were placed 0.3–0.5 mm apart along the path of the syringe. Finally, in one rat, we used the same protocol and injected FB into the reticular nucleus dorsal to the SOC. This experiment revealed the cortical labeling attributable to dye left along the syringe path.

We describe results from four animals that received an injection of FB in the GCD of the CN. Two of the animals also received a DiY injection in the SOC (these animals were referred to in the preceding paragraph), and the other two received an injection only in the GCD. The tip of a glass electrode (inside tip diameter, 30–50 μ m) was inserted into the granule cell lamina, the region of the GCD that separates the dorsal and ventral regions of the CN. The electrode tip was advanced 400 μ m below the CN surface, and 13 nl of FB was deposited at two depths using an oocyte injector (Drummond Scientific, Broomall, PA, USA). The electrode was then left in place for 5–10 min before withdrawing. We did not inject DiY into the GCD because this dye is quite viscous; therefore, the size of the syringe or electrode required to inject DiY produced an injection site that spilled outside of the cochlear nucleus.

2.2. Tissue processing

Four to 7 days after the injection(s), animals were administered a lethal dose of sodium pentobarbital (100 mg/kg). When the animal was areflexic to a paw pinch, it was transcardially perfused with 25 ml of 0.1 M phosphate-buffered saline (pH 7.4) with 1% sodium nitrite (a vasodilator) followed immediately by 250 ml of 10% formalin in phosphate buffer (PB, pH 7.4). The brain was dissected from the skull and a 28 gauge needle was inserted into the cerebral hemisphere contralateral to the injection site. The brain was postfixed in the fixative solution for 90 min (21°C) and then cryoprotected overnight in a solution of 30% sucrose in PB (4°C). The next day, the brain was sectioned in the coronal plane using a freezing microtome. The section thickness for most of the tissue was 50 μ m, with one 75 μ m section taken every 325 μ m. Upon completion of the sectioning, all of the 75 μ m sections were mounted on subbed slides and coverslipped with Krystalon (Harleco, EM Science, Gibbstown, NJ, USA). The 50 μ m sections that were immediately adjacent to the 75 μ m sections were mounted on subbed slides, air-dried overnight, and stained with cresyl violet.

2.3. Data analysis

2.3.1. Injection sites

In coronal sections through a FB or DiY injection site, the tracer had a brightly fluorescent central region comprised of the dye, necrotic tissue, and intensely labeled cells (Fig. 1: dotted black line in photomicrograph). Surrounding this central area was a halo of labeled neurons and glial cells (Fig. 1: dotted white line in photomicro-

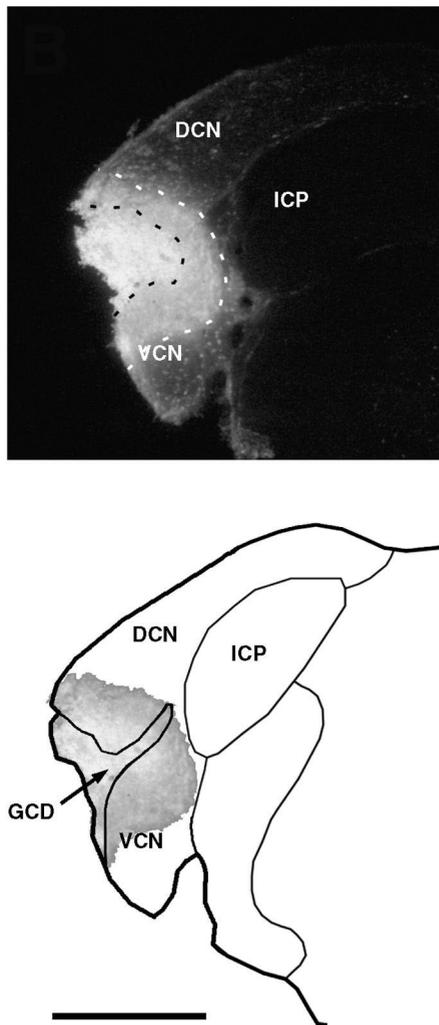


Fig. 1. Representative injection site in the cochlear nucleus (CN). A photomicrograph of the FB injection is shown in the top panel, and its position in the CN is illustrated in the bottom panel. Scale bar equals 1 mm. Abbreviations: DCN, dorsal cochlear nucleus; GCD, granule cell domain; ICP, inferior cerebellar peduncle; VCN, ventral cochlear nucleus.

graph). Axon terminals (or fibers of passage) that are thought to take up the dye lie within or near the central region [6]. Therefore, we summarized the injection sites in the SOC by displaying the center and halo for a series of sections through the brainstem (Fig. 5). First, cresyl violet sections from each case were drawn at low magnification ($2.5\times$ objective) with a drawing tube and used to form an atlas of the SOC. The dye deposit in the corresponding sections from each case was photographed using a cooled, three-chip RGB digital CCD camera (C5810 Hamamatsu) attached to a fluorescent microscope. The brainstem outline along with major internal structures (e.g., facial nucleus), and the injection sites were outlined using computer software (Adobe Photoshop v5.0). These drawings were then scaled and aligned with the appropriate atlas section. The nuclear boundaries and nomenclature for the SOC were adopted from Ref. [9].

2.3.2. Cell distribution and size

We photographed the series of $75\ \mu\text{m}$ sections spanning the rostral/caudal extent of temporal cortex on the same day the sections were mounted and coverslipped. Several pictures ($10\times$ objective) were taken of each hemisphere that collectively circumscribed the cortical regions that contained labeled neurons. Image processing software (Adobe Photoshop v5.0) then was used to build montages, to draw the boundaries of gray and white matter, and to plot the position of each labeled cell.

The distribution of labeled cells within a series of coronal sections was replotted so that it could be displayed as a two-dimensional projection onto the lateral surface of the cortex. A digitized atlas [32] that contained drawings of coronal sections was used as a template. For each case, we found the photographed coronal section that best matched the drawing in the Swanson atlas at $-5.65\ \text{mm}$ with respect to Bregma. The physical distance of the remaining coronal sections from this matched section was then computed. As a result, each photographed coronal section was assigned a distance from Bregma along the rostral/caudal axis. Drawings of the coronal sections that contained the boundaries of cortex and the position of labeled cells were then scaled to fit the appropriate section in the atlas. In Fig. 2, we show a drawing of one cortical hemisphere scaled to fit the atlas at $-5.65\ \text{mm}$ with respect to Bregma. We used the atlas coordinate system to partition the dorsal/ventral axis into $0.2\ \text{mm}$ bins and then counted the number of labeled cells within each bin. These counts were collected from several coronal sections that spanned 5 or 6 mm along the rostral/caudal axis to form lateral views of each hemisphere (see Fig. 2). Note that in Figs. 2, 4, and 8 distance along the rostral/caudal or x -axis is given as the actual physical distance between the sections. The distance along the dorsal/ventral or y -axis refers to Swanson's coordinate system [32].

The size of the cortical neurons labeled with FB injections in the CN or the SOC (one case each) was measured by photographing a total of 50 cells collected from both cortical hemispheres. This number represents a small quantity of the total number of labeled cells, but we limited our choice to those that were bright and well filled (e.g., see cells in Fig. 6C). Cells were photographed at a total magnification of $400\times$ ($40\times$ objective, $\text{NA}=0.75$) and chosen from within Te1 (primary auditory cortex of the rat). The outlines of the somata were traced (Adobe Photoshop, v5.0) and the areas were measured (NIH image, v1.62).

2.3.3. Identifying auditory cortex in the rat

In order to describe the distribution of cortical cells projecting to the SOC, we needed to identify primary and nonprimary auditory areas in the rat. Except for the rhinal fissure, rat cortex lacks any obvious anatomical features (e.g., gyri or sulci) that could be used to localize these areas. Using a variety of criteria such as cytoarchitecture

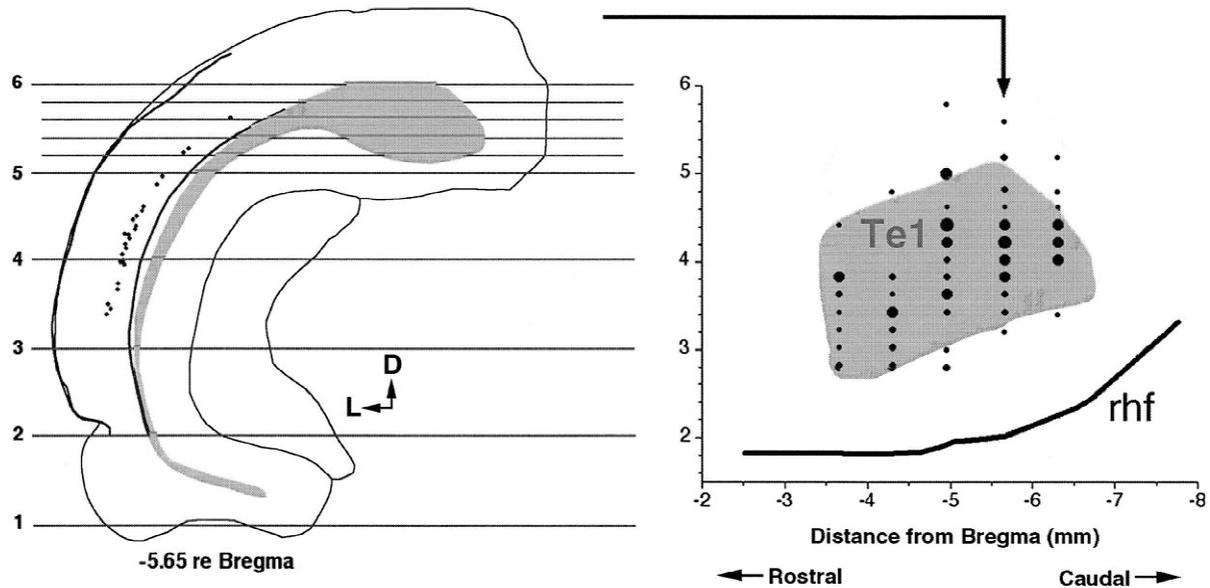


Fig. 2. Illustration of how cells plotted in coronal sections were collected to form a lateral view of their distribution in cortex. For each coronal section, a photographic montage was produced that captured the regions of cortex containing filled neurons. The montage was used to create a line drawing that contained an outline of the section and the position of the labeled cells (left panel; thick line and solid circles). The section (line drawing) was assigned a position along the rostral/caudal axis with respect to Bregma (see text), and the line drawing was then scaled and matched to the appropriate level in a brain atlas [32]. The horizontal lines through the coronal section represent the coordinate system supplied with the Swanson atlas. The coordinate system was used to partition the dorsal/ventral axis into 0.2 mm bins (e.g., horizontal lines between 5 and 6 mm), and the number of cells was counted in each bin. The result for each section was then plotted as a function of distance from Bregma to produce the lateral view of cortex shown on the right. To summarize, the figure on the left shows a match between the data and the atlas at -5.65 mm with respect to Bregma and the arrow denotes the resulting position of the data in the lateral view on the right. In the lateral view, the size of a circle corresponds to the number of neurons observed in a particular 0.2 mm bin along the dorsal/ventral axis. The position of the rhinal fissure and the extent of a prior definition of Te1 (gray area [48]) are also illustrated. This example shows data from the hemisphere ipsilateral to an injection of FB in the CN. Abbreviations: D, dorsal; L, lateral; rhf, rhinal fissure; Te1, temporal cortical area 1 (primary auditory cortex).

and myeloarchitecture, temporal cortex in the rat has been partitioned into at least three distinct regions referred to as Te1, Te2, and Te3 (Fig. 3 [48]). Te1 is primary auditory cortex [7,23,25,27] whereas Te2 and Te3 appear to be secondary auditory areas [8,24,27,48]. The ventral border of Te1 is defined in Nissl-stained sections as a decrease in the density of cells in layer IV and a marked decrease in

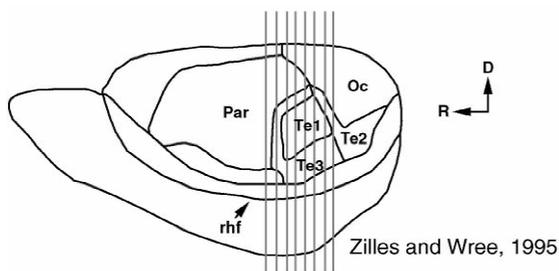


Fig. 3. Lateral view of auditory cortex in the rat. The distribution of retrogradely labeled cells was analyzed for a series of equally spaced coronal sections that spanned temporal cortex. The vertical lines indicate the approximate position of these coronal sections with respect to the subdivisions of rat cortex. The areal borders and nomenclature are taken from the literature (Zilles and Wree [48]). Abbreviations: D, dorsal; Oc, occipital cortex; Par, parietal cortex; R, rostral; rhf, rhinal fissure; Te1, temporal cortical area 1 (primary auditory cortex); Te2, Te3, temporal cortical areas 2 and 3 (secondary auditory cortex).

cortical layer differentiation [47]. We used counterstained sections adjacent to those containing FB or DiY labeled cells to mark the ventral border of Te1.

In addition to cytoarchitecture, the distribution of cortical cells projecting to the CN was used to define Te1. Corticofugal axons originating in Te1 terminate throughout the GCD [9,41]. The GCD is comprised of a shell of small cells surrounding the magnocellular regions of the ventral cochlear nucleus (VCN) and concentrated within layer II of the DCN [19]. Many granule cells are located within a wedge-shaped region separating the VCN and DCN that is referred to as the granule cell lamina. In four experiments, we injected FB into this region of the GCD (Fig. 1). The distribution of labeled cortical cells was plotted on coronal sections, and then projected onto a two-dimensional lateral view of each hemisphere (see Fig. 2).

3. Results

3.1. Injections in the CN and identifying rat auditory cortex

In all four animals that received injections in the CN, the FB injection sites were centered in the granule cell lamina

(Fig. 1) and filled this area from its rostral to caudal borders. The injection sites were confined to the CN and did not leak into structures medial to the nucleus such as the inferior cerebellar peduncle.

In Fig. 2, we show results from the hemisphere ipsilateral to a FB injection site in the CN. We also superimposed Zilles and Wree's [48] boundaries of Te1 (Fig. 3) by aligning their caudal and ventral borders with our data. Note that when the projections to the granule cell lamina are mapped, they coincide quite well with prior definitions of Te1. In Fig. 4, the distributions of labeled cells from all four cases are shown. Since the number of cortical cells projecting to the CN is modest, we pooled data from the two hemispheres (open and closed circles) in order to outline Te1. Data from one animal (2/4/00,B; upper left

panel of Fig. 4) that had the most labeled cells were combined with the pattern of Nissl staining in this case to draw the boundaries of Te1. The region of cerebral cortex outlined in animal 2/4/00,B was then superimposed on the distributions obtained in the other three experiments. Notice that this encircled area of cortex captures the spread of filled cells in the other three experiments. Given this consistency across animals, we used this border to define Te1 for all our experiments.

3.2. Injections in the SOC

Our injections of retrograde tracers into the SOC are summarized in Fig. 5. Corticobulbar projections primarily target three regions in the SOC: the lateral superior olive

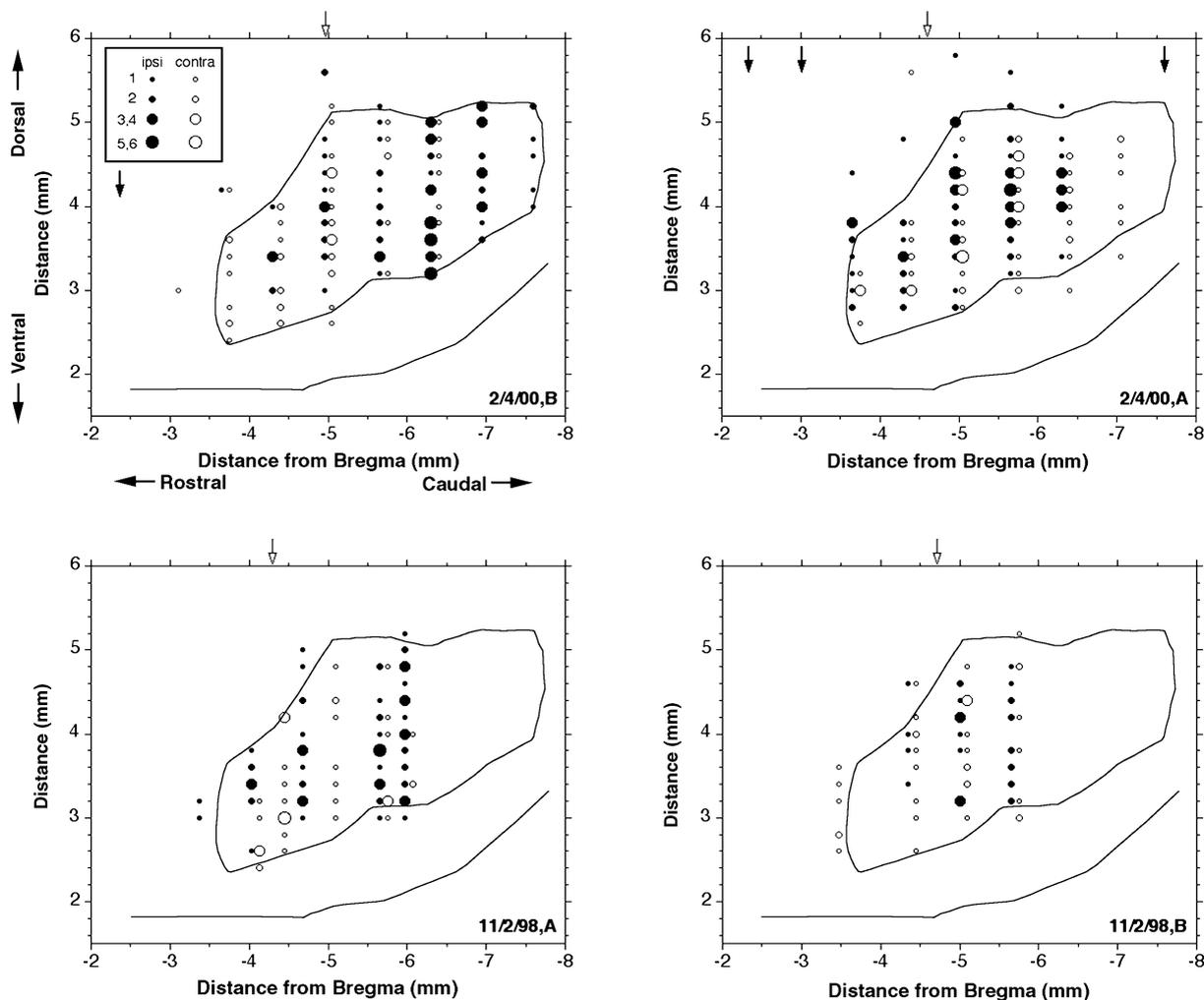


Fig. 4. Defining area Te1 in the rat. This figure displays lateral views of the retrograde labeling in temporal cortex after injections of FB into the CN. Methods for producing these plots are explained in the text and Fig. 2. Each panel shows data from one animal. The distribution of cells labeled in the hemisphere ipsilateral to the injection site is shown with closed circles. Open circles display the distribution in the contralateral hemisphere and they have been shifted slightly along the x-axis for illustration purposes. The number of labeled cells is denoted by the size of the circles (see symbol key). Closed arrows denote the positions of coronal sections that were analyzed but did not contain labeled cells. Open arrows at the top of each plot denote the caudal boundary of the splenium of the corpus callosum. We used the case containing the most labeled cells to trace the boundary of Te1 (2/4/00,B, upper left). The boundary was then copied without modification onto the other three plots. Notice that the definition of Te1 obtained from one case surrounds the distribution of cells in the other three animals. We used this definition of Te1 for analyzing our data.

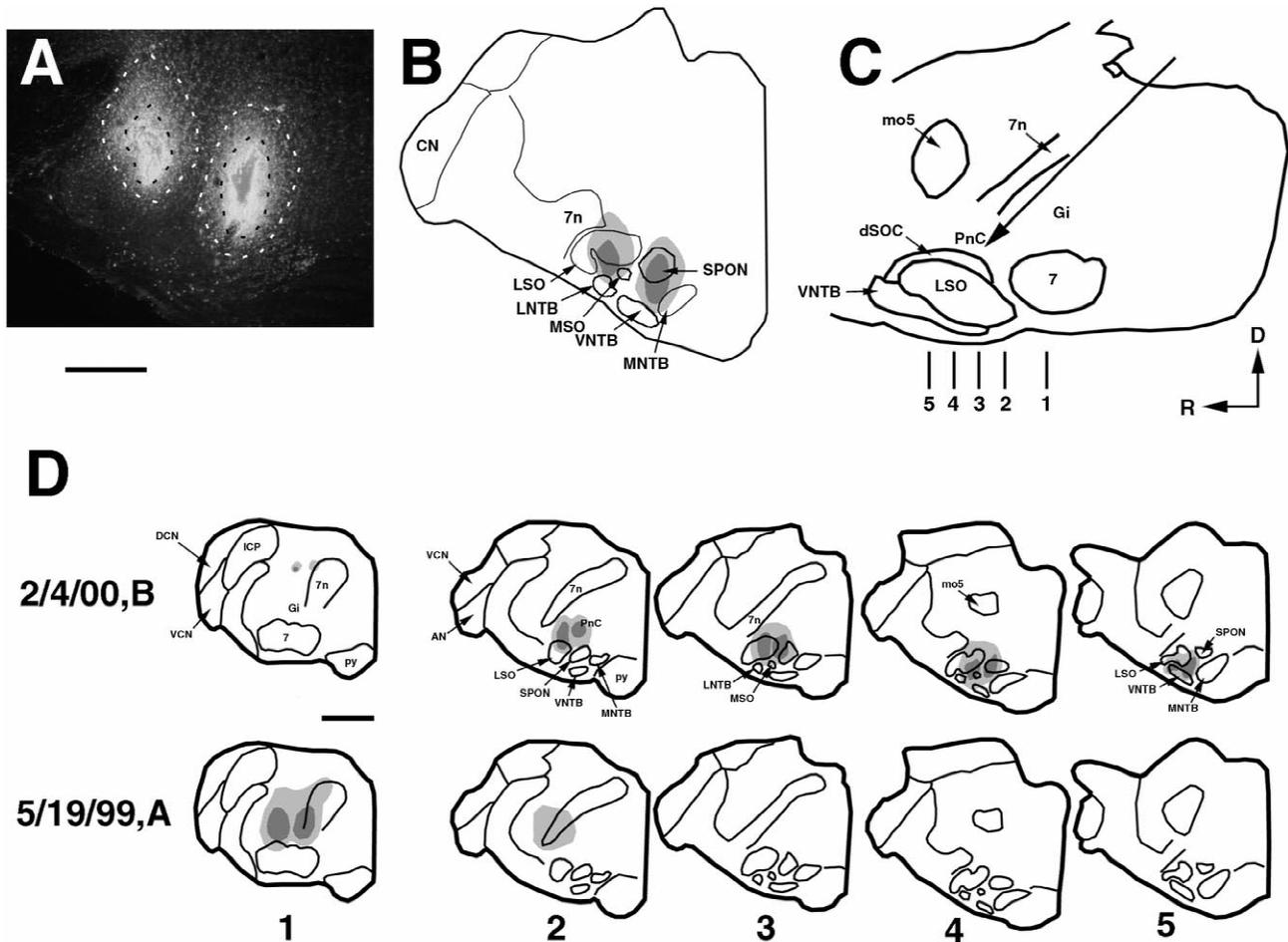


Fig. 5. Representative injection sites in the superior olivary complex (SOC). (A) Fluorescent micrograph of a DiY injection site. The center (black broken line) and halo (white broken line) of the injection site are highlighted. Scale bar equals 250 μm for the photograph and 500 μm for the drawing in (B). (B) Location of injection site shown in (A) with respect to the nuclei of the SOC. Dark gray represents center and light gray denotes halo of the injection site. (C) Sagittal view of the brainstem illustrating the trajectory of the injecting needle (arrow) and the position of the sections shown in (D). The distance between section one and two is 600 μm . The distance between the remaining sections is 250 μm . (D) Series of coronal sections illustrating an injection site that intersected several SOC nuclei (case 2/4/00,B) and a control injection site localized to the reticular nucleus (case 5/19/99,A). The number beneath the drawings refers to the rostral/caudal position of the section as shown in (C). Scale bar equals 1 mm. Abbreviations: AN, auditory nerve; CN, cochlear nucleus; DCN, dorsal cochlear nucleus; dSOC, dorsal ribbon of the superior olivary complex; Gi, gigantocellular reticular nucleus; LSO, lateral superior olive; LNTB, lateral nucleus of the trapezoid body; MNTB, medial nucleus of the trapezoid body; mo5, motor nucleus of the trigeminal nerve; MSO, medial superior olive; PnC, caudal pontine reticular nucleus; py, pyramidal tract; SPON, superior paraolivary nucleus; VCN, ventral cochlear nucleus; VNTB, ventral nucleus of the trapezoid body; 7, facial nucleus; 7n, facial nerve.

(LSO), the ventral nucleus of the trapezoid body (VNTB), and an ill-defined region dorsal to the SOC referred to as 'the dorsal ribbon of the SOC (dSOC)' [9]. In Fig. 5C, these three regions are drawn in a sagittal section through the brainstem along with the path of the syringe used to inject the SOC (arrow). In one animal, the center of the injection site (dark gray) intersected all three of these areas (case 2/4/00,B in Fig. 5D). In the other four experiments, the center and halo of the injection site were confined to the dorsal half of the SOC (e.g., Fig. 5B). In two of these latter four animals, the halo of the injection impinged on the dorsal and lateral borders of the pyramidal tract. Te1 is known to give rise to corticospinal neurons that run in the pyramidal tract [9,20] and these could be a source of corticofugal fibers that are independent of those termi-

nating in the SOC. However, the quantity and distribution of labeled cortical cells were similar in the animals that received an injection in the dorsal half of the SOC. Therefore, all four of these animals were included in the database. All SOC nuclei were injected with dye in at least one experiment. Only the regions rostral to the LSO were not included in our injection sites.

In SOC experiments, some FB or DiY was deposited inadvertently into the reticular nucleus (RN). The spillage of dye into the RN was unavoidable for two reasons: (1) the syringe passed through this area before reaching the SOC (Fig. 5C) and (2) we were attempting to inject dye into the dSOC (e.g., Fig. 5D; section 2 in case 2/4/00,B). Injections of DiY ($n=3$) resulted in a very small amount of dye left along the path of the syringe (e.g., Fig. 5D, section

1 in case 2/4/00,B), whereas more dye was deposited into the RN when using FB. The involved regions included the gigantocellular reticular nucleus (Gi) lying dorsal to the facial nucleus and the caudal pontine reticular nucleus (PnC) lying dorsal to the SOC. In order to distinguish between the retrograde labeling of corticofugal pathways ending in the SOC and inadvertent labeling of axons passing through the Gi and PnC, we injected FB directly into these areas in one experiment (Fig. 5D, case 5/19/99,A). The FB injection site was centered in the Gi but some dye also was deposited in the PnC. We chose FB for this control experiment to try and maximize the retrograde labeling in cortex attributable to dye deposited in the RN.

3.3. Retrograde labeling in temporal cortex after injections in the SOC

An injection of FB or DiY into the SOC produced labeled cells bilaterally in temporal cortex. These injections also labeled cells bilaterally in the inferior colliculus (IC), but with many more cells labeled ipsilateral to the injection site as compared to the contralateral IC. The IC-SOC pathway in rats has been described previously [5,8,36] and thus will not be discussed further. We did not observe retrogradely labeled cells in the medial geniculate body after these injections.

The type of cortical cells that project to the SOC and their laminar distribution are illustrated in Fig. 6. Fig. 6A and B show results from an experiment where FB was injected into the CN and DiY into the SOC. Neurons labeled with injections in the SOC will be referred to as ‘SOC cortical cells’ and those labeled by CN injections as ‘CN cortical cells’. SOC cortical cells, like CN cortical cells, are confined to the deep regions of layer V (Fig. 6B). In both hemispheres, CN cortical cells are found primarily in the deepest part of layer V, whereas SOC cortical cells are distributed more broadly in the middle and deep part of layer V.

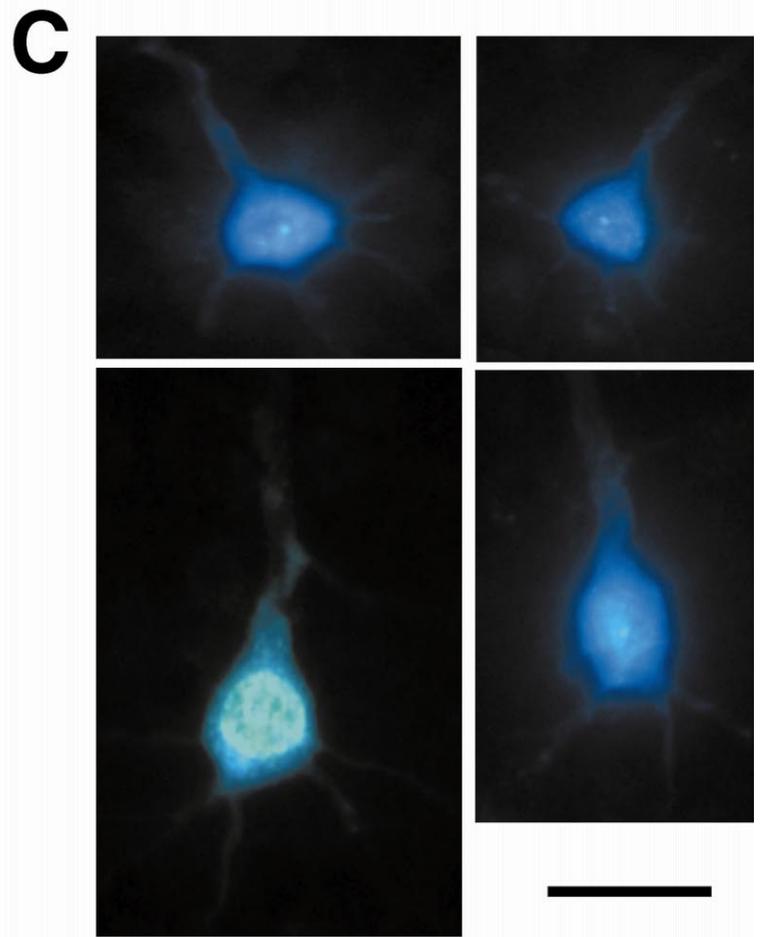
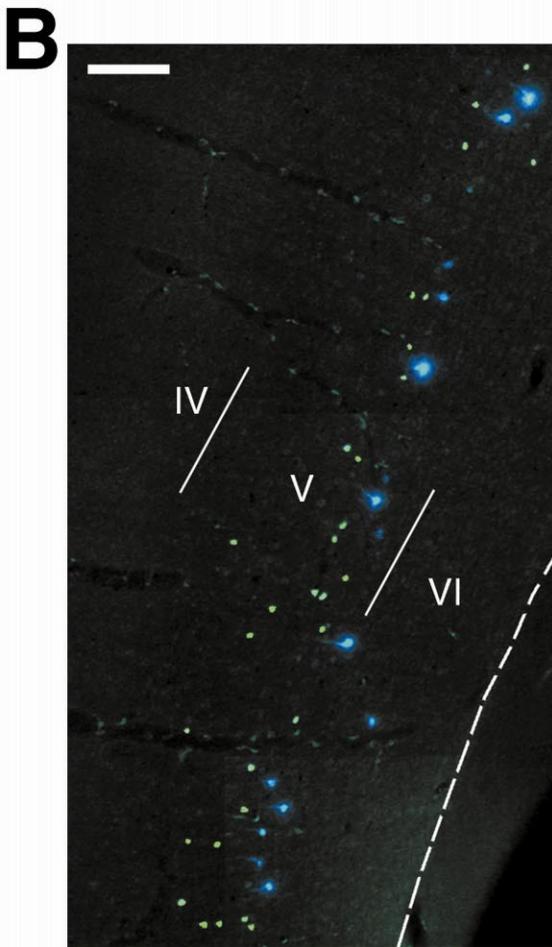
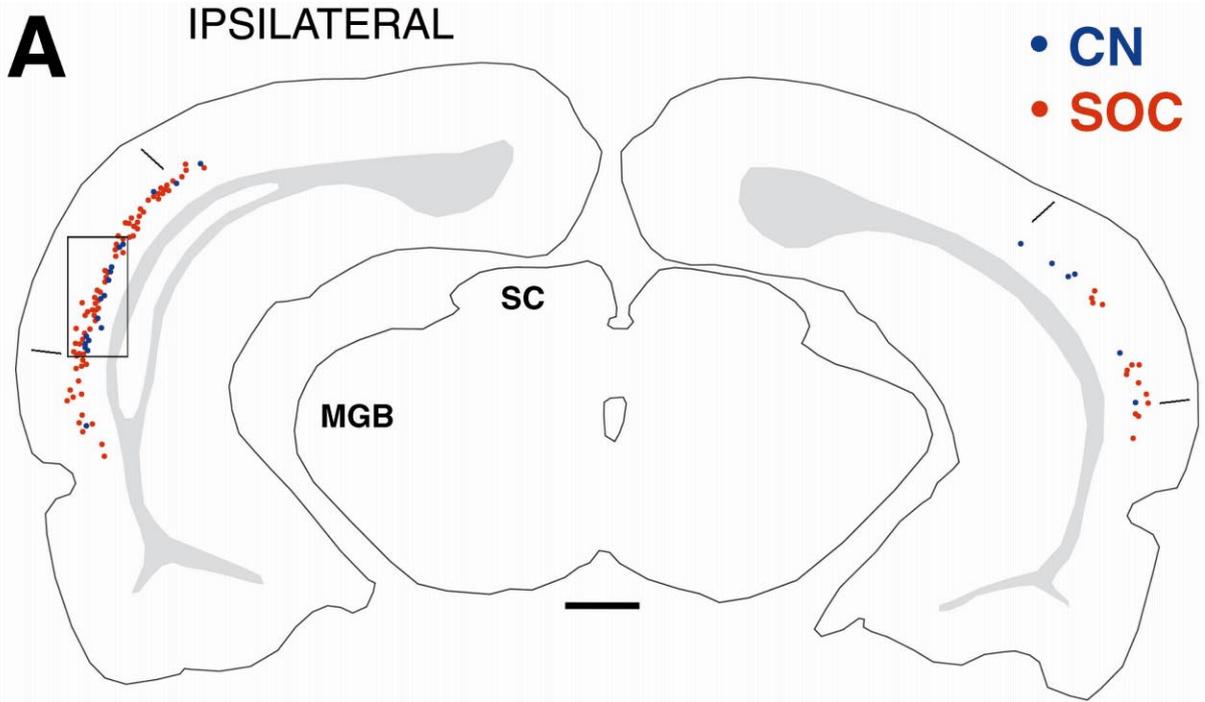
Within Te1, most SOC cortical cells have the classic appearance of layer V pyramidal neurons: a triangular-shaped soma, a large apical dendrite extending towards more superficial layers, and several basal dendrites (Fig. 6C). We measured the somatic silhouette areas for a collection of FB-labeled SOC and CN cortical cells. The mean somatic areas for the two sets of neurons are not statistically different (SOC, $159 \pm 47 \mu\text{m}^2$; CN, 168 ± 55

μm^2 ; $P=0.33$). Given the similarity in size and distribution of SOC and CN cortical cells, one might predict that some of these cells would project to both nuclei, indicating that both corticofugal pathways share a common source. In the two experiments where FB was injected into the CN and DiY into the SOC, we occasionally observed pyramidal cells that were double labeled (Fig. 6C) but such cells were rare. Less than 10% of the total number of labeled cells in Te1 contained both FB and DiY. We measured the somatic areas for a collection of double labeled cells. These neurons were taken from different experiments than those used to form the CN and SOC (single labeled) area distributions. The mean size for double labeled neurons ($191 \pm 56 \mu\text{m}^2$) was not statistically different from single labeled CN cells ($P=0.13$) but they were larger than SOC cortical cells ($P=0.03$).

A typical distribution of SOC cortical cells in temporal cortex is shown in Fig. 7. In this experiment, DiY was injected into the SOC and filled most of the dorsal half of the complex. Several labeled neurons are observed bilaterally within the borders of Te1 (denoted by angled dashes in each hemisphere). Ipsilateral to the injection site, the number of filled cells is roughly constant throughout the rostral/caudal extent of Te1. In the contralateral hemisphere, the number of labeled cells in rostral Te1 (e.g., $-4.3 \text{ mm re: Bregma}$) is noticeably greater than that in caudal Te1 (e.g., $-6.3 \text{ mm re: Bregma}$). Filled cells are also found dorsal, ventral, and rostral to Te1 in sections less than 5 mm from Bregma. We never observed SOC cortical cells caudal to Te1.

It is possible that some of the retrograde labeling in cortex is due to the leakage of FB or DiY into the RN. Prior studies have noted that Te1 does not innervate the Gi or PnC divisions of the RN [21,49]. However, these regions do receive input from parietal cortex that borders Te1 dorsally and rostrally (Par in Fig. 3). This issue is addressed in Fig. 8, where lateral views of cortex illustrate the distribution of SOC cortical cells from three experiments. The top row shows data from a DiY injection in the SOC (case 2/4/00,B, injection site in Fig. 5), the data in the middle are from a FB injection in the SOC (case 4/20/99,B), and the labeling in the bottom row is from a FB injection into the RN (case 5/19/99,A, injection site in Fig. 5). We always observed many more filled cells bilaterally within the core of Te1 when dye was injected into the SOC (top two rows) versus when it was confined

Fig. 6. Retrograde labeling in cortex of one animal that received an injection of FB into the CN and DiY into the SOC. (A) A drawing of a coronal section through temporal cortex showing the distribution of labeled cells. Cells labeled with FB are shown in blue and those filled with DiY are shown in red. The borders of Te1 are illustrated with solid lines. Ipsilateral refers to the side of the brain that received the injections. Notice that the cortical areas projecting to the CN and SOC overlap extensively, indicating that area Te1 (primary auditory cortex) targets both of these nuclei. The scale bar equals 1 mm. (B) A photomontage of the highlighted area in panel A, illustrating the laminar separation of cells projecting to the CN (blue, deep layer V) and to the SOC (yellow–green, middle and deep layer V). Scale bar equals 0.3 mm. (C) Photomicrographs of representative cells within Te1 labeled after injections of FB in the SOC. The double labeled neuron is from a different experiment where FB was injected into the CN and DiY into the SOC (lower left). Scale bar equals 25 μm .



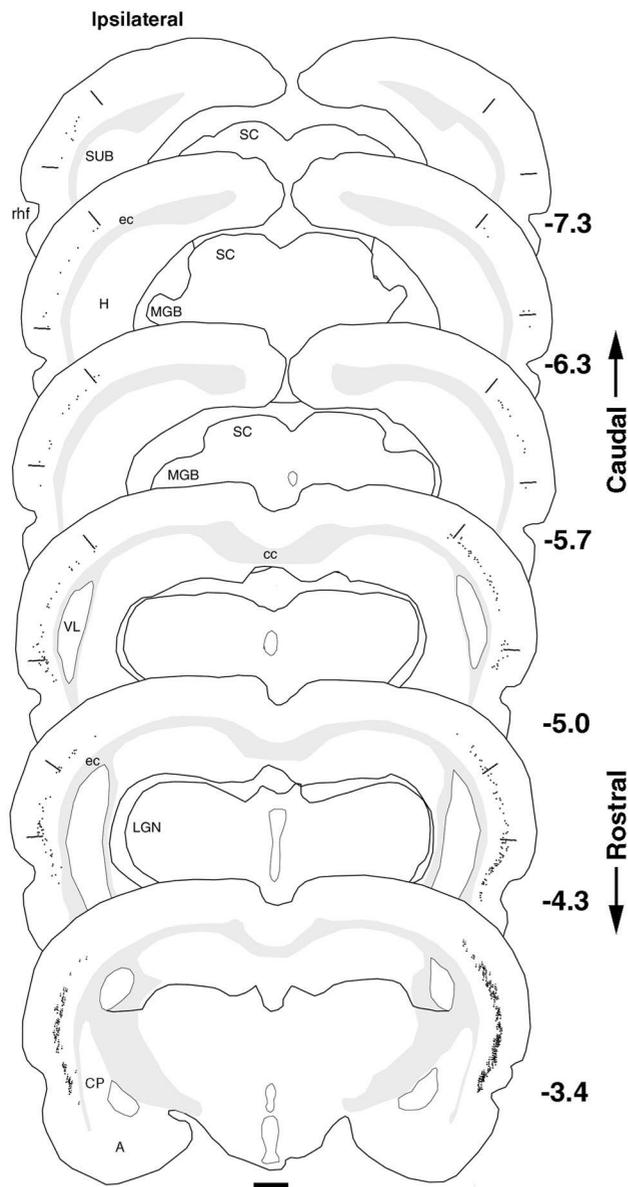


Fig. 7. An atlas showing the distribution of SOC cortical cells in one animal (case 5/19/99,B). Ipsilateral refers to the injected side of the brain. The DiY injection site was centered in the dorsal half of the SOC. The distance of each section from Bregma is given. The solid lines in each cortical hemisphere are the boundaries of Te1. Notice that some SOC cortical cells are observed dorsal, ventral, and rostral to area Te1. Also, the labeling within area Te1 in the contralateral hemisphere appears to be biased towards rostral Te1. Scale bar equals 1 mm. Abbreviations: A, amygdala; cc, corpus callosum; CP, caudate putamen; ec, external capsule; H, hippocampus; LGN, lateral geniculate nucleus; MGB, medial geniculate body; rhf, rhinal fissure; SC, superior colliculus; SUB, subiculum; VL, lateral ventricle.

to the RN (bottom row). Consistent with prior work [21,49], the majority of the filled neurons produced by the RN injection were observed rostral and dorsal to Te1, in parietal cortex. Additionally, the amount of labeling in parietal cortex correlated with the amount of dye that

spilled into the RN after SOC injections. For example, injections of FB in the SOC invariably left more dye in the RN compared to DiY injections. In Fig. 8, notice that there are more labeled neurons rostral and dorsal to Te1 in case 2/4/00,B (DiY) versus that in case 4/20/99,B (FB). These results suggest that the labeling within Te1 is caused by the injection in the SOC, whereas the filled cells dorsal and rostral to Te1 are due to FB or DiY deposited into the RN.

In Fig. 9, the number of labeled neurons in Te1 is plotted for both hemispheres as a function of distance from Bregma. Data from the animal that received an injection in the dSOC, LSO, and VNTB are shown in the top panel (2/4/00,B), and averaged data from all animals are shown in the bottom panel. At each rostral/caudal position, the number of labeled cells observed in the RN experiment was subtracted before computing the average. The data for each animal were used to estimate the total number of neurons that project from Te1 to the SOC. For case 2/4/00,B, approximately 1500 neurons were labeled on the ipsilateral side, whereas 500 were labeled on the contralateral side. The results from this experiment are consistent with the finding that the uncrossed projection from Te1 to the SOC is larger than the crossed projection [9,20]. In the remaining four cases where the injection site was centered in the dorsal half of the SOC (intersecting the LSO and dSOC, see Fig. 5B), the number of filled cells in the ipsilateral hemisphere ranged from 200 to 700 and the amount of ipsilateral and contralateral labeling was approximately equal.

The distribution of filled cells along the rostral/caudal axis of Te1 differed between the two hemispheres. Ipsilateral to the injection site, filled neurons are observed throughout Te1 (Fig. 9). This distribution along the rostral/caudal axis is similar to the pattern observed for CN cortical cells (data not shown). The number of CN and SOC cortical cells appears to correlate with the size of Te1 at each rostral/caudal position. In contrast, contralateral to the injection site, the distribution of filled cells clearly is biased towards rostral Te1. This pattern can be seen in the averaged data shown in Fig. 9 and for the three individual cases illustrated in Figs. 7 and 8. The low number of filled neurons in caudal Te1 did not appear to be attributable to an incomplete filling of the SOC with dye, since this pattern also is observed in case 2/4/00,B where all three of the main targets of the corticofugal pathway were injected.

In addition to the labeling in Te1, SOC cortical cells also were observed ventral to Te1 (gray area in Fig. 8), confirming the results of a prior study [20]. This region coincides with secondary auditory areas [1,24,27,45]. Labeled cells were observed bilaterally and their distribution was biased towards the rostral portion of the cortex ventral to Te1. The gray region in Fig. 8 appears to correspond with a cortical area that has been referred to as Te3 (Fig. 3 [12,47]).

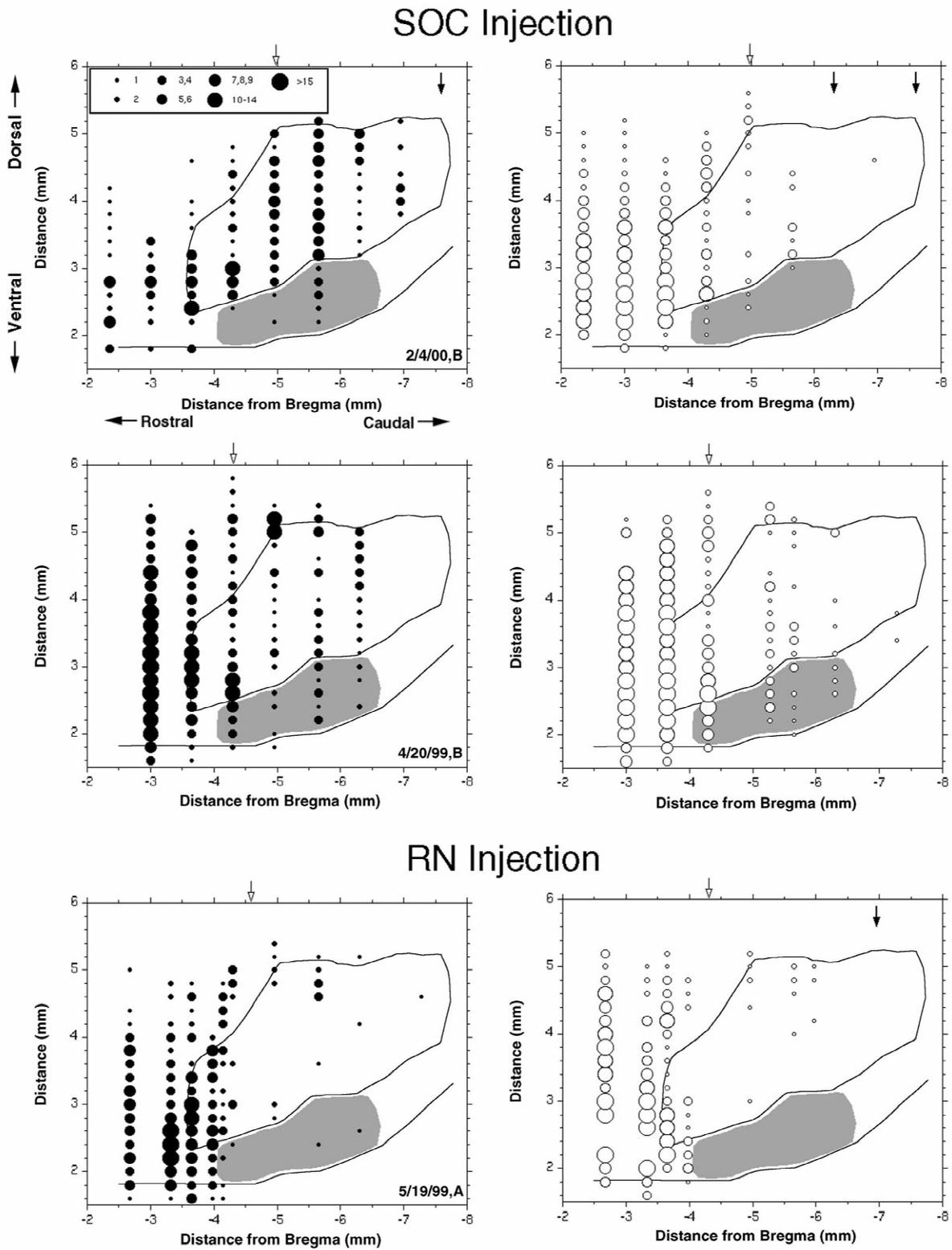


Fig. 8. Lateral views of the retrograde labeling in cortex for three animals. Two had injections centered in the SOC (top two rows) and one received a FB injection directly into the reticular nucleus (bottom row). For each case, the distribution in the hemisphere ipsilateral to the injection site is shown on the left (closed circles) and the size of each circle corresponds to the number of labeled cells observed at a particular location (see symbol key in upper left panel). Other symbols are defined in Fig. 4.

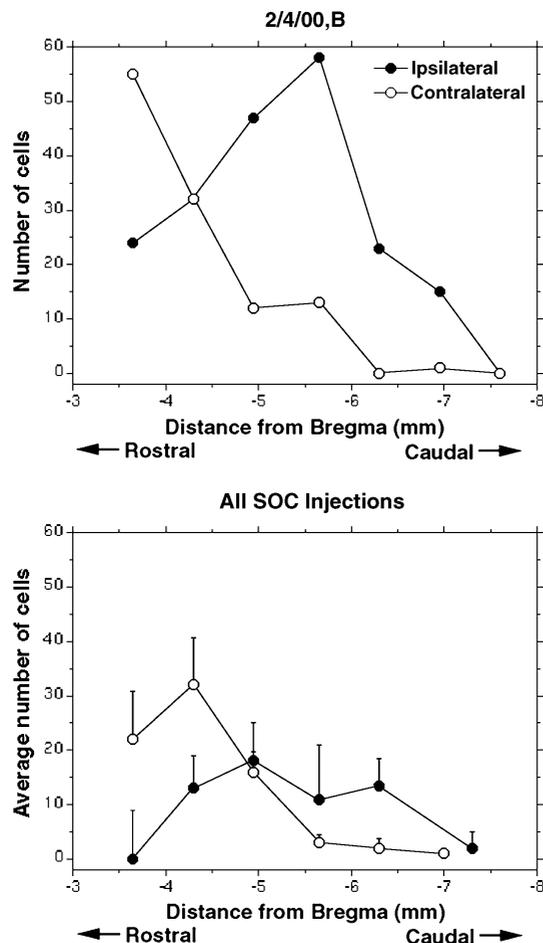


Fig. 9. These plots show the number of SOC cortical cells in Te1 as a function of distance from Bregma. The top panel shows data from one animal while the bottom panel displays averages taken from all our experiments. In the bottom panel, the standard error for each average is denoted. The number of cells labeled after injecting the reticular nucleus was subtracted from all the averages. In case 2/4/00,B; the DiY injection intersected each SOC nucleus that receives projections from Te1 cells (see Fig. 5). Notice that the projection from Te1 to the SOC is mostly ipsilateral in case 2/4/00,B. All regions of ipsilateral Te1 project to the SOC in this case, whereas the distribution on the contralateral side appears to be biased towards rostral Te1. In other animals, less cells were labeled in Te1 overall, but the same pattern of labeling is observed when their data are averaged. Since Te1 is tonotopically organized, these data suggest that the contralateral projection is predominantly from the high frequency regions of primary auditory cortex.

4. Discussion

We have demonstrated that injecting a retrograde tracer into the SOC of the rat fills cortical cells distributed bilaterally in Te1 and the surrounding ventral area. Te1 is primary auditory cortex and regions just ventral to Te1 are thought to be secondary auditory areas [1,12,23–25,45,48]. The results of this study confirm the findings of previous studies that used anterograde and retrograde techniques to demonstrate that primary and secondary auditory cortex project to the SOC [9,20,21,33,49]. We extended these

observations by describing the number and distribution of SOC cortical cells in the two hemispheres as well as by identifying the source of the projections as layer V pyramidal neurons. SOC cortical cells are found bilaterally in ventral temporal association areas and appear to be confined to area Te3. Finally, we have shown that the source of the corticofugal pathways ending in the CN and the SOC are largely independent.

The labeling in Te1 and Te3 (we will hereafter refer to the gray region in Fig. 8 as Te3) does not appear to be due to contamination caused by FB or DiY deposited into the RN. First, after examining all of our cases, the number of SOC cortical cells in Te1 and Te3 did not correlate with the amount of tracer deposited into the RN. Second, injection of FB directly into the Gi and PnC divisions of the RN produced labeled cells that were distributed primarily dorsal and rostral to Te1. Only a few scattered cells were found within Te3 and the core of Te1 (Fig. 8). Third, previous studies of the corticobulbar projections from Te1 failed to find a projection to the Gi and PnC regions of the RN [21,49]. For these reasons, we conclude that our description of the pattern of labeling in Te1 and Te3 is indicative of their projections to the SOC.

4.1. Topography of corticofugal projections to the SOC

The neurons within the three main targets of the cortical pathway ending in the SOC – the dSOC, LSO, and VNTB – probably subserve different functions in acoustic processing. Thus it is important to know if there is topography in the cortical projections to these SOC nuclei. We have too few cases and our injections are too large to be able to address this question definitively. However, we did observe that an injection involving the VNTB (case 2/4/00,B in Fig. 5) produced over twice as many labeled neurons in ipsilateral Te1 compared to the other four animals where the dSOC and the LSO were injected. This result suggests that the VNTB receives a particularly large input from auditory cortex. A prior study in rats appears to show more terminal labeling in the VNTB than in the dSOC or LSO after anterograde tracers were injected into Te1 (see Figs. 2 and 6 in Ref. [9]). In guinea pigs, the VNTB is reported to receive the majority of cortical input to the SOC [33]. The VNTB is a heterogeneous collection of neurons that project their axons to the cochlea, cochlear nucleus, inferior colliculus, and within the SOC (e.g., Ref. [39]). Thus cortical input to the VNTB could play an important role in signal processing in the brainstem and midbrain. One cell type targeted by cortical cells in the VNTB appears to be the medial olivocochlear neuron [20]. The axons of these cells form synapses at the base of outer hair cells, suggesting that the influence of corticofugal pathways extends to the cochlea.

Te1 projections to the SOC are primarily uncrossed [9,20,21] and all frequency regions of Te1 project bilaterally to the SOC [9,20]. We confirmed both of these results.

For example, in the experiment where DiY was injected into the dSOC, LSO, and VNTB (case 2/4/00,B in Fig. 9), three times as many labeled cells were found in Te1 ipsilateral to the injection compared to contralateral Te1. Furthermore, collecting the labeled cells from all five animals, SOC cortical cells are distributed throughout the rostral/caudal extent of Te1 in both hemispheres. Te1 is tonotopically organized with rostral areas being more sensitive to high frequencies and caudal areas responding preferentially to low frequencies [29]. Thus our results demonstrate that all frequency regions of Te1 project bilaterally to the SOC.

Our data further suggest a clear difference in the frequency organization of the crossed and uncrossed projection. The uncrossed projection to the SOC appears to derive equally from all frequency regions of Te1 (e.g., Fig. 9). In contrast, the source of the crossed projection to the SOC is biased towards cells in the rostral (high frequency) regions of Te1. Unfortunately, two studies that injected anterograde tracers into different rostral/caudal positions of Te1 did not show the labeling in the contralateral SOC as a function of the position of the injection site [9,20]. However, we should note that neither study reported any prominent difference in the quantity of axonal labeling in the contralateral SOC as the injection site was moved along the rostral/caudal axis. One reason may be that the size of the crossed projection to the SOC suggested by our data is fairly modest (<1000 cells). Also, it is difficult to quantify the topography of projections from one brain region to another using anterograde tracing techniques (due to differences in injection size, efficacy of tracer pickup across experiments, etc.). Physiological experiments that assess the effect on SOC neurons of stimulating different areas of Te1 along the rostral/caudal axis (either with electrical stimulation or changing sound frequency) may be better suited to revealing the topography of the cortical input that we have described here.

Primary auditory cortex in the rat is thought to be completely surrounded by secondary auditory areas (e.g., Fig. 3 [1,12,24,27,48]). The number of areas and their borders are not well defined, but most of the cited studies agree that regions caudal to Te1 (area Te2) are distinct from those ventral and rostral to Te1. We have shown that an area that appears to overlap with Te3 projects bilaterally to the SOC. Corticofugal projections to the CN arise only from primary auditory cortex [41], whereas those to the IC arise from Te1, Te2, and Te3 [12]. The areal extent of cortical input to the SOC appears to reflect its intermediate position between the CN and IC in the hierarchical organization of the auditory pathway. The region of the SOC targeted by cells in Te3 is unknown because studies that injected anterograde tracers into Te3 described only corticothalamic and corticocollicular projections [1,12,24]. It is interesting to note; however, that a major target of Te3 in the inferior colliculus is the intercollicular zone (IZ) in the rostral IC [12]. In the ferret, IZ neurons provide a large

portion of IC input to the superior colliculus (SC) and they have been suggested to convey acoustic information that is used to create spatially-tuned neurons in the SC [15]. Perhaps Te3 contains cells that are specialized to encode space. The projections to the SOC and the IZ would then reflect a modulation of downstream neurons that participate in creating spatially tuned neurons. More data are necessary on the targets of cortical neurons in the SOC to test such hypotheses, but the study of these corticofugal pathways should provide insight into the functional organization of both the SOC and auditory cortex.

4.2. Corticobulbar pathways ending in the CN and the SOC

Pyramidal cells in layer V of Te1 project to a number of cortical and subcortical nuclei such as the contralateral Te1 [10], inferior colliculus [3,10], the cochlear nucleus [41], the pontine nuclei [43], and the striatum [18]. We have demonstrated that they also project to the SOC. An important question for elucidating the function of these descending pathways is whether layer V cells project to one target or multiple targets. After making a FB injection in the CN and a DiY injection in the SOC, we found only a few cortical cells that contained both dyes. Furthermore, the laminar distributions of CN and SOC cortical cells were different. CN cortical cells were observed in the deepest regions of layer V, whereas SOC cortical cells were more broadly distributed within layer V (Fig. 6). Together, these data suggest that the corticofugal pathways ending in the CN and the SOC predominantly derive from two different populations of layer V pyramidal cells.

One reservation of this conclusion is that our injections almost certainly did not label all CN and SOC cortical cells. We are probably underestimating the number of pyramidal cells that project to both nuclei. Two animals received a FB injection in the GCD and a DiY injection in the SOC. Combining the labeled cells in the two hemispheres, the number of CN cortical cells was approximately equal in the two rats (1192 vs. 1070), whereas the number of SOC cortical cells in one rat (case 2/4/00,B) was more than twice the number in the other animal (2057 vs. 881). The percentage of filled cells that were double labeled was less than 10% for both animals. These data indicate that filling more CN and SOC cortical cells does not necessarily result in more double labeled neurons.

Our proposal of two independent corticofugal pathways is based on experiments in which the injections in the CN and SOC were made on the same side of the brainstem. It is conceivable that placing the injections on opposite sides of the brainstem might have revealed more double labeled cells. This suggestion implicitly assumes that CN cortical cells (or SOC cortical cells) that project to the ipsilateral and contralateral CN (SOC) derive from different populations of pyramidal neurons. This assumption might be true for the pathway ending in the SOC, given the difference in

the rostral/caudal distribution of SOC cortical cells in the two hemispheres (see Fig. 9). However, the size and laminar distribution of CN cortical cells are similar in the two hemispheres. Furthermore, the rostral/caudal distribution of CN cortical cells ipsilateral and contralateral to the injection site is nearly identical. These observations suggest that CN cortical cells in the two hemispheres derive from the same population(s) of pyramidal cells, and that our experiments have revealed the degree of overlap between the cortical pathways ending in the SOC and the CN. In other words, we argue that injecting the left CN and the right SOC in the same animal would not produce significantly more double labeled cells.

Our data are consistent with the hypothesis that pyramidal cells tend to project to a single target. This hypothesis also is supported from observations on cortico-collicular and cortico-striatal pathways [18] as well as cortico-collicular and cortico-commissural projections [10]. In the cat, layer V contains many distinct anatomical classes of pyramidal and nonpyramidal cells [44]. Perhaps each class primarily projects to a single target. It should be noted, however, that the cortico-collicular cells are broadly distributed within layer V and far outnumber the cortical neurons projecting to the CN or SOC. Thus an important test of this ‘one class-one target’ hypothesis is to determine whether cortical cells projecting to the IC send collaterals to the SOC and CN.

Acknowledgements

We thank Hugh Cahill for technical assistance. Portions of these data were presented in preliminary fashion at the 27th Annual Meeting of the Society for Neuroscience, New Orleans, LA, October 1997. This work was supported by NIH/NIDCD grants DC00232 and DC04395 awarded to D.K.R. and DC04505 awarded to J.R.D.

References

- [1] P. Arnault, M. Roger, Ventral temporal cortex in the rat: connections of secondary auditory areas Te2 and Te3, *J. Comp. Neurol.* 302 (1990) 110–123.
- [2] M. Bentivoglio, H.G. Kuypers, C.E. Catsman-Berreoets, H. Loewe, O. Dann, Two new fluorescent retrograde neuronal tracers which are transported over long distances, *Neurosci. Lett.* 18 (1980) 25–30.
- [3] B.D. Beyerl, Afferent projections to the central nucleus of the inferior colliculus in the rat, *Brain Res.* 145 (1978) 209–223.
- [4] M.C. Brown, M.C. Liberman, T.E. Benson, D.K. Ryugo, Brainstem branches from olivocochlear axons in cats and rodents, *J. Comp. Neurol.* 278 (1988) 591–603.
- [5] A. Caicedo, H. Herbert, Topography of descending projections from the inferior colliculus to auditory brainstem nuclei in the rat, *J. Comp. Neurol.* 328 (1993) 377–392.
- [6] F. Conde, Further studies on the use of the fluorescent tracers fast blue and diamidino yellow: effective uptake area and cellular storage sites, *J. Neurosci. Methods* 21 (1987) 31–43.
- [7] H. Faye-Lund, The neocortical projection to the inferior colliculus in the albino rat, *Anat. Embryol.* 173 (1985) 53–70.
- [8] H. Faye-Lund, Projection from the inferior colliculus to the superior olivary complex in the albino rat, *Anat. Embryol.* 175 (1986) 35–52.
- [9] M. Feliciano, E. Saldaña, E. Mugnaini, Direct projections from the rat primary auditory neocortex to nucleus sagulum, paralemniscal regions, superior olivary complex and cochlear nuclei, *Auditory Neurosci.* 1 (1995) 287–308.
- [10] K.D. Games, J.A. Winer, Layer V in rat auditory cortex: projections to the inferior colliculus and contralateral cortex, *Hear. Res.* 34 (1988) 1–25.
- [11] J.J. Guinan, B.E. Norris, S.S. Guinan, Single auditory units in the superior olivary complex. II. Locations of unit categories and tonotopic organization, *Int. J. Neurosci.* 4 (1972) 147–166.
- [12] H. Herbert, A. Aschoff, J. Ostwald, Topography of projections from the auditory cortex to the inferior colliculus in the rat, *J. Comp. Neurol.* 304 (1991) 103–122.
- [13] D.R.F. Irvine, The auditory brainstem: A review of the structure and function of auditory brainstem processing mechanisms, in: D. Ottonson (Ed.), *Progress in Sensory Physiology*, Vol. 7, Springer-Verlag, Berlin, 1986, pp. 1–279.
- [14] D.R.F. Irvine, Physiology of the auditory brainstem, in: A.N. Popper, R.R. Fay (Eds.), *The Mammalian Auditory Pathway: Neurophysiology*, Springer-Verlag, New York, 1992, pp. 153–231.
- [15] Z.D. Jiang, D.R. Moore, A.J. King, Sources of subcortical projections to the superior colliculus in the ferret, *Brain Res.* 755 (1997) 279–292.
- [16] K. Keizer, H.G. Kuypers, A.M. Huisman, O. Dann, Diamidino yellow dihydrochloride (DY-2HCl); a new fluorescent retrograde neuronal tracer, which migrates only very slowly out of the cell, *Exp. Brain Res.* 51 (1983) 179–191.
- [17] K. Lingenhohl, E. Friauf, Giant neurons in the rat reticular formation: a sensorimotor interface in the elementary acoustic startle circuit?, *J. Neurosci.* 14 (1994) 1176–1194.
- [18] T. Moriizumi, T. Hattori, Pyramidal cells in rat temporoauditory cortex project to both striatum and inferior colliculus, *Brain Res. Bull.* 27 (1991) 141–144.
- [19] E. Mugnaini, W.B. Warr, K.K. Osen, Distribution and light microscopic features of granule cells in the cochlear nuclei of cat, rat, and mouse, *J. Comp. Neurol.* 191 (1980) 581–606.
- [20] W.H. Mulders, D. Robertson, Evidence for direct cortical innervation of medial olivocochlear neurones in rats, *Hear. Res.* 144 (2000) 65–72.
- [21] D.B. Newman, S.K. Hilleary, C.Y. Ginsberg, Nuclear terminations of corticoreticular fiber systems in rats, *Brain Behav. Evol.* 34 (1989) 223–264.
- [22] G. Paxinos, C. Watson, *The Rat Brain in Stereotaxic Coordinates*, 4th Edition, Academic Press, San Diego, CA, 1998.
- [23] M. Roger, P. Arnault, Anatomical study of the connections of the primary auditory area in the rat, *J. Comp. Neurol.* 287 (1989) 339–356.
- [24] L.M. Romanski, J.E. LeDoux, Information cascade from primary auditory cortex to the amygdala: corticocortical and corticoamygdaloid projections of temporal cortex in the rat, *Cereb. Cortex* 3 (1993) 515–532.
- [25] L.M. Romanski, J.E. LeDoux, Organization of rodent auditory cortex: anterograde transport of PHA-L from MGv to temporal neocortex, *Cereb. Cortex* 3 (1993) 499–514.
- [26] A.F. Ryan, E.M. Keithley, Z.X. Wang, I.R. Schwartz, Collaterals from lateral and medial olivocochlear efferent neurons innervate different regions of the cochlear nucleus and adjacent brainstem, *J. Comp. Neurol.* 300 (1990) 572–582.
- [27] D.K. Ryugo, An attempt towards an integration of structure and function in the auditory system, Doctoral Thesis, University of California, Irvine, CA, 1976.
- [28] E. Saldaña, M. Feliciano, E. Mugnaini, Distribution of descending

- projections from primary auditory neocortex to inferior colliculus mimics the topography of intracollicular projections, *J. Comp. Neurol.* 371 (1996) 15–40.
- [29] S.L. Sally, J.B. Kelly, Organization of auditory cortex in the albino rat: sound frequency, *J. Neurophysiol.* 59 (1988) 1627–1638.
- [30] B.R. Schofield, Superior paraolivary nucleus in the pigmented guinea pig: separate classes of neurons project to the inferior colliculus and the cochlear nucleus, *J. Comp. Neurol.* 312 (1991) 68–76.
- [31] B.R. Schofield, Projections to the cochlear nuclei from principal cells in the medial nucleus of the trapezoid body in guinea pigs, *J. Comp. Neurol.* 344 (1994) 83–100.
- [32] L.W. Swanson, *Brain Maps: Structure of the Rat Brain*, 2nd Edition, Elsevier, Amsterdam, 1998.
- [33] A.M. Thompson, B.R. Schofield, Afferent projections of the superior olivary complex, *Microsc. Res. Tech.* 51 (2000) 330–354.
- [34] D.E. Vetter, J.C. Adams, E. Mugnaini, Chemically distinct rat olivocochlear neurons, *Synapse* 7 (1991) 21–43.
- [35] D.E. Vetter, E. Mugnaini, Distribution and dendritic features of three groups of rat olivocochlear neurons. A study with two retrograde cholera toxin tracers, *Anat. Embryol.* 185 (1992) 1–16.
- [36] D.E. Vetter, E. Saldaña, E. Mugnaini, Input from the inferior colliculus to medial olivocochlear neurons in the rat: a double label study with PHA-L and cholera toxin, *Hear. Res.* 70 (1993) 173–186.
- [37] T. Wagner, P.K. Pilz, M. Fendt, The superior olivary complex is necessary for the full expression of the acoustic but not tactile startle response in rats, *Behav. Brain Res.* 108 (2000) 181–188.
- [38] W.B. Warr, Olivocochlear and vestibular efferent neurons of the feline brain stem: their location, morphology and number determined by retrograde axonal transport and acetylcholinesterase histochemistry, *J. Comp. Neurol.* 161 (1975) 159–181.
- [39] W.B. Warr, J.E. Beck, Multiple projections from the ventral nucleus of the trapezoid body in the rat, *Hear. Res.* 93 (1996) 83–101.
- [40] D.L. Weedman, D.K. Ryugo, Projections from auditory cortex to the cochlear nucleus in rats: synapses on granule cell dendrites, *J. Comp. Neurol.* 371 (1996) 311–324.
- [41] D.L. Weedman, D.K. Ryugo, Pyramidal cells in primary auditory cortex project to cochlear nucleus in rat, *Brain Res.* 706 (1996) 97–102.
- [42] J.S. White, W.B. Warr, The dual origins of the olivocochlear bundle in the albino rat, *J. Comp. Neurol.* 219 (1983) 203–214.
- [43] R. Wiesendanger, M. Wiesendanger, The corticopontine system in the rat. I. Mapping of corticopontine neurons, *J. Comp. Neurol.* 208 (1982) 215–226.
- [44] J.A. Winer, J.J. Prieto, Layer V in cat primary auditory cortex (AI): cellular architecture and identification of projection neurons, *J. Comp. Neurol.* 434 (2001) 379–412.
- [45] J.A. Winer, S.L. Sally, D.T. Larue, J.B. Kelly, Origins of medial geniculate body projections to physiologically defined zones of rat primary auditory cortex, *Hear. Res.* 130 (1999) 42–61.
- [46] I.M. Winter, D. Robertson, K.S. Cole, Descending projections from auditory brainstem nuclei to the cochlea and cochlear nucleus of the guinea pig, *J. Comp. Neurol.* 280 (1989) 143–157.
- [47] K. Zilles, Anatomy of the neocortex: cytoarchitecture and myeloarchitecture, in: B. Kolb, R.C. Tees (Eds.), *The Cerebral Cortex of the Rat*, MIT Press, Boston, MA, 1990, pp. 77–112.
- [48] K. Zilles, A. Wree, Cortex: areal and laminar structure, in: G. Paxinos (Ed.), *The Rat Nervous System*, 2nd Edition, Academic Press, San Diego, CA, 1995, pp. 649–685.
- [49] E.A. Zimmerman, W.W. Chambers, C.N. Liu, An experimental study of the anatomical organization of the corticobulbar system in the albino rat, *J. Comp. Neurol.* 123 (1964) 301–324.