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Serotonergic Innervation of the Auditory Brainstem of the Mexican Free-Tailed Bat, *Tadarida brasiliensis*

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ABSTRACT

Anatomical and electrophysiological evidence suggests that serotonin alters the processing of sound in the auditory brainstem of many mammalian species. The Mexican free-tailed bat is a hearing specialist, like other microchiropteran bats. At the same time, many aspects of its auditory brainstem are similar to those in other mammals. This dichotomy raises an interesting question regarding the serotonergic innervation of the bat auditory brainstem: Is the serotonergic input to the auditory brainstem similar in bats and other mammals, or are there specializations in the serotonergic innervation of bats that may be related to their exceptional hearing capabilities? To address this question, we immunocytochemically labeled serotonergic fibers in the brainstem of the Mexican free-tailed bat, *Tadarida brasiliensis*. We found many similarities in the pattern of serotonergic innervation of the auditory brainstem in *Tadarida* compared with other mammals, but we also found two striking differences. Similarities to staining patterns in other mammals included a higher density of serotonergic fibers in the dorsal cochlear nucleus and in granule cell regions than in the ventral cochlear nucleus, a high density of fibers in some periolivary nuclei of the superior olive, and a higher density of fibers in peripheral regions of the inferior colliculus compared with its core. The two novel features of serotonergic innervation in *Tadarida* were a high density of fibers in the fusiform layer of the dorsal cochlear nucleus relative to surrounding layers and a relatively high density of serotonergic fibers in the low-frequency regions of the lateral and medial superior olive. J. Comp. Neurol. 435:78–88, 2001.

Indexing terms: cochlear nucleus; superior olivary complex; lateral lemniscus; inferior colliculus

Neuromodulators can cause the functional reconfiguration of neural circuits in both invertebrates and vertebrates (for examples, see reviews in Harris-Warrick et al., 1992; Sillar et al., 1998). In mammals, the neuromodulator serotonin can induce plasticity in the central representation of many different sensory modalities, including nociception, vision, and somatosensation (see, e.g., Rogawski and Aghajanian, 1980; Iwayama et al., 1989; Bassant et al., 1990; Waterhouse et al., 1990; Huang et al., 1993; Storer and Goadsby, 1997; Edagawa et al., 1999). Several lines of evidence suggest that serotonin modulates the central processing of audition, too. One of these lines of evidence is anatomical number of immunohistochemical studies of serotonin have demonstrated that serotonergic fibers are present in every major auditory nucleus of the brainstem (Steinbusch, 1981; Willard et al., 1984; Klepper and Herbert, 1991; Thompson et al., 1994, 1995; Thompson and Thompson, 1995; Kaiser and Covey, 1997). Furthermore, these serotonergic fibers often show interesting and nonuniform patterns of staining both between and within auditory nuclei, suggesting that the degree of serotonergic modulation is greater in some auditory regions than others.

A second line of evidence that serotonin is a neuromodulator in the auditory system is electrophysiological. Serotonin has been shown to change auditory evoked potentials from the level of the brainstem to the level of the cortex (Ehlers et al., 1991; Revelis et al., 1998). In addi-

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tion, serotonin has been shown to change the firing patterns of single auditory neurons in several brainstem auditory nuclei in different mammalian species (Faingold et al., 1991; Ebert and Ostwald, 1992; Wang and Robertson, 1997; Fitzgerald and Sanes, 1999; Hurley and Pollak, 1999). In the inferior colliculus (IC) of the Mexican free-tailed bat, *Tadarida brasiliensis*, serotonin changes the selectivity of individual neurons for different types of sounds (Hurley and Pollak, 1999).

Mexican free-tailed bats, as microchiropteran bats, are useful models for the study of brainstem auditory processing. These mammals have an exquisitely developed sense of hearing that allows them to locate their insect prey through echolocation (Simmons et al., 1978, 1979). The echolocation signals used by Mexican free-tailed bats are downward frequency-modulated sweeps (Simmons et al., 1978, 1979). In addition, they have a wide repertoire of communication calls (Gelfand and McCracken, 1986; Balcombe and McCracken, 1992). Reflecting this heavy reliance on sound, most brainstem auditory nuclei in bats are hypertrophied compared with those of other mammals. At the same time, however, many aspects of the bat brainstem auditory system are fundamentally mammalian, with most of the same circuitry and response properties that are found in the auditory systems of other mammals (Pollak et al., 1977; Bodenhamer et al., 1979; Grothe et al., 1994, 1997; Park et al., 1996, 1998). Whether the serotonergic innervation of the brain of the free-tailed bat reflects the general mammalian plan or exhibits some specializations that are unique to bat remains an outstanding issue.

To address this issue, we immunohistochemically labeled serotonin fibers in the brainstem of the Mexican free-tailed bat. There were many similarities between the patterns of serotonergic fibers in the brainstem of the free-tailed bat and previously reported staining patterns in other species. As is the case in other species, there were striking differences in the densities of serotonergic fibers both within and between auditory nuclei in the Mexican free-tailed bat. However, there were also two notable features of serotonin immunohistochemistry in the Mexican free-tailed bat that have not been reported previously in other animals.

Abbreviations

AN	auditory nerve
AVCN	anteroventral cochlear nucleus
$AVCN_A$	anterior AVCN
$AVCN_P$	posterior AVCN
C	core (octopus cell region of the posteroventral cochlear nucleus)
DC	dorsal cortex
DCN	dorsal cochlear nucleus
DMPO	dorsomedial periolivary nucleus
DNLL	dorsal nucleus of the lateral lemniscus
DPO	dorsal periolivary nucleus
EC	external cortex
FCL	fusiform cell layer
IC	inferior colliculus
ICc	central nucleus of the IC
INLL	intermediate nucleus of the lateral lemniscus
LNTB	lateral nucleus of the trapezoid body
LSO	lateral superior olive
MNTB	medial nucleus of the trapezoid body
MSO	medial superior olive
PVCN	posteroventral cochlear nucleus
VCN	ventral cochlear nucleus
VMPO	ventromedial periolivary nucleus
VNLL	ventral nucleus of the lateral lemniscus
VNTB	ventral nucleus of the trapezoid body
VPO	ventral periolivary nucleus

MATERIALS AND METHODS

Seven bats were used for serotonin immunohistochemistry. Five of these bats were also used in a parallel tract-tracing study, which is not reported here. These five animals were used within 24–72 hours of tract-tracer injection. The care and use of the animals followed a protocol approved by the University of Texas at Austin Institutional Animal Care and Use Committee.

Animals were deeply anesthetized with methoxyflurane inhalant (Metofane; Schering-Plough, Madison, NJ) and injected intraperitoneally with 0.05–0.1 mL ketamine (Vetamine; Schering-Plough). They were then transcardially perfused with 0.1 M phosphate-buffered saline (PBS), pH 7.2, at 4°C containing 1–2% lidocaine until the blood cleared. This was followed by 4% paraformaldehyde in PBS for 20 minutes. Brains were postfixed overnight in the same fixative, then sequentially equilibrated in 10%, 20%, and 30% sucrose in PBS. Brains were sectioned in the transverse plane at 40-μm intervals on a sliding microtome and collected in 0.1 M PBS. Slices were placed in four parallel treatment groups, so that every fourth slice received the same treatment.

Brain slices were rinsed in 0.1 M PBS with 0.3% Triton X-100 (PBSTx; Sigma, St. Louis, MO) and then blocked in 5% normal goat serum (NGS; Sigma) and 0.1% bovine serum albumin (BSA; Sigma). They were then blocked with avidin alone and biotin alone (avidin/biotin blocking kit; Vector Laboratories, Ingold, CA) and incubated in primary rabbit antiserotonin antibody (Eugene Tech, Ridgefield Park, NJ) at 1:20,000 or 1:10,000 dilution on a shaker at 4°C for 1–2 days. After incubation in the primary antibody, sections were rinsed in PBSTx and incubated for 1 hour at room temperature in biotinylated goat anti-rabbit antibody (Vector Laboratories) diluted 1:400 in 2% NGS for 1 hour at room temperature. Sections were then rinsed in PBS and run through an avidin-biotin reaction (Vectastain peroxidase kit; Vector Laboratories), rinsed in phosphate buffer (PB), pH 7.2, and reacted with diaminobenzidene dihydrochloride at 0.5 μg/mL plus 0.3% hydrogen peroxide in PB. Sections were rinsed and mounted on slides coated with 3% gelatin. Some slides were counterstained with cresyl violet or neutral red to label cell bodies. Controls using preadsorbed primary antibody were performed previously (Thompson et al., 1994).

Slides were viewed and photographed in transmitted light optics on a Nikon light microscope (Tokyo, Japan). Subsequent to this, some of the images were enhanced with Adobe PhotoShop software (Adobe Systems, Mountain View, CA). For some images, the brightness ranges were narrowed to enhance contrast. When these manipulations were performed, they were performed uniformly across an entire image. Serotonin-positive fibers were drawn in representative sections under a ×100 oil-immersion objective with a drawing tube attachment. For the figures, individual pages of the original reconstructions were reduced in size by a photocopier and taped together. Outlines of nuclear subdivisions were added to the figure after the final reduction. Alternatively, pages of

the reconstruction were scanned and assembled using Adobe PhotoShop software.

A subjective measurement of the density of serotonergic fibers in different brainstem regions was made by categorizing the density into three subjective levels represented by crosses, with one cross representing the lowest fiber density and three crosses representing the highest density. The parcellation and nomenclature of the cochlear nucleus was based on Zook and Casseday (1982) and Osen (1969); that of the superior olivary complex was based on Grothe et al. (1994), that of the lateral lemniscus was based on Zook and Casseday (1982) and Covey and Casseday (1986), and that of the IC was based on Faye-Lund and Osen (1985) and Oliver and Huerta (1992).

RESULTS

Cochlear nucleus

The cochlear nucleus is composed of a number of distinct regions that vary in afferent input, efferent output, and cell type (Fekete et al., 1982; Merchán et al., 1985; Zook and Casseday, 1985; Cant, 1992). Reflecting this range, the density of serotonergic-immunoreactive fibers varied strikingly between different nuclear subdivisions.

Overall, serotonergic fibers were much denser in the dorsal cochlear nucleus (DCN) than in the divisions of the ventral cochlear nucleus (VCN; Fig. 1). Even within the DCN, however, there were distinct differences in the degree of serotonergic staining between different cytoarchitecturally distinct layers. The granule cell regions and fusiform cell layer exhibited particularly strong immunoreactivity compared with the molecular layer and deeper layers of the DCN, which had roughly equivalent amounts of serotonin immunoreactivity when judged on a subjective scale with three levels of fiber density (Fig. 2A, Table 1). Serotonergic fibers within all regions of the DCN had a similar appearance, in that fibers exhibited a beaded morphology indicative of en passant varicosities (Fig. 2B). However, there were also small differences in fiber appearance between different regions. The fibers in the granule cell region were particularly enriched in varicosities, and many varicosities could be observed in close apposition to cresyl violet-stained cell bodies (Fig. 2C). In the molecular and fusiform layers, fibers coursed parallel to the surface of the cochlear nucleus (Fig. 2A).

Compared with the DCN, the immunoreactive fibers in the VCN were extremely sparse (Fig. 1, Table 1). This was true for the posteroventral cochlear nucleus (PVCN), which contains the cell bodies of octopus neurons (Cant, 1992), for the posterior portion of the anteroventral cochlear nucleus (AVCN), and for the auditory nerve region. However, slightly higher fiber densities were found in the anterior AVCN and the AVCN small cell cap that decreased somewhat in more anterior sections. As was the case for granule cell regions in the DCN, the granule cell regions adjacent to the VCN exhibited a high density of serotonergic fibers.

Superior olivary complex

The superior olivary complex comprises an intricate group of nuclei and includes the principal olivary nuclei [the lateral superior olive (LSO), medial superior olive (MSO), and medial nucleus of the trapezoid body] as well as periolivary nuclei. The presence or absence as well as the location of these nuclei vary widely between mammalian species. In the Mexican free-tailed bat, these nuclei were classified previously by Grothe et al. (1994) based on their location, afferent inputs, efferent outputs, and on the presence of acetylcholinesterase staining (Warr, 1975, 1980).

Serotonergic fibers were distributed nonuniformly in the superior olivary complex (Fig. 3, Table 1). Overall, several periolivary nuclei showed the densest staining. The ventromedial periolivary nucleus (just ventral to the MSO) and the lateral nucleus of the trapezoid body (immediately lateral to the LSO) had the densest serotonin immunoreactivity of the periolivary nuclei. These were followed by the dorsal periolivary nucleus (just dorsal to the LSO) and the dorsomedial periolivary nucleus (just lateral to the MSO). Within the dorsomedial periolivary nucleus, the fiber density was denser dorsolaterally. The ventral periolivary nucleus had an intermediate density of serotonergic fibers.

Of the principal olivary nuclei, the fiber density in the medial nucleus of the trapezoid body was comparatively low, with a slightly higher density ventrolaterally and dorsomedially. The LSO and MSO themselves each had a nonuniform pattern of serotonin immunoreactivity. The LSO had a higher density of beaded, immunoreactive fibers in its lateral limb (Fig. 2D), and some of the varicosities were in close apposition to cresyl violet-stained cell bodies (Fig. 2E). The MSO showed a higher serotonergic fiber density in its most dorsal quarter. It is interesting to note that these densely stained regions in both the LSO and the MSO correspond to the regions containing neurons that respond to the lowest frequency sounds within each nucleus.

Lateral lemniscus

In bats, there are three well-defined nuclei of the lateral lemniscus, the ventral (VNLL), intermediate (INLL), and dorsal (DNLL) nuclei, each of which has at least one complete tonotopic map (Aitkin et al., 1970; Covey and Casseday, 1991; Markowitz and Pollak, 1993; Yang et al., 1996). Within the INLL and VNLL, there was little variation in the density of serotonergic fibers (Fig. 4, Table 1). As in other auditory nuclei, beaded fibers were present in all nuclei of the lateral lemniscus (e.g. Fig. 2F).

The DNLL had a somewhat columnar arrangement of immunoreactive fibers, a pattern that followed the organization of the cell bodies within this nucleus. The highest density of immunoreactive fibers occurred in the columns of cell somata, whereas the regions with least immunoreactivity corresponded to ascending fiber tracts.

An interesting feature of serotonin immunoreactivity located lateral to the INLL was a strip containing a high density of fibers at the very lateral edge of the brainstem. This high-density strip was discontinued adjacent to the ventral boundary of the INLL and was not present lateral to the VNLL. Dorsally, this strip was continuous with high-density fiber staining lateral to the DNLL, in the region of the sagulum, and farther dorsally with the brachium of the IC.

IC

The IC can be divided into several subdivisions based on innervation patterns and cytoarchitecture (Faye-Lund and Osen, 1985; Oliver and Huerta, 1992). There were regional differences in serotonin-immunoreactive fiber

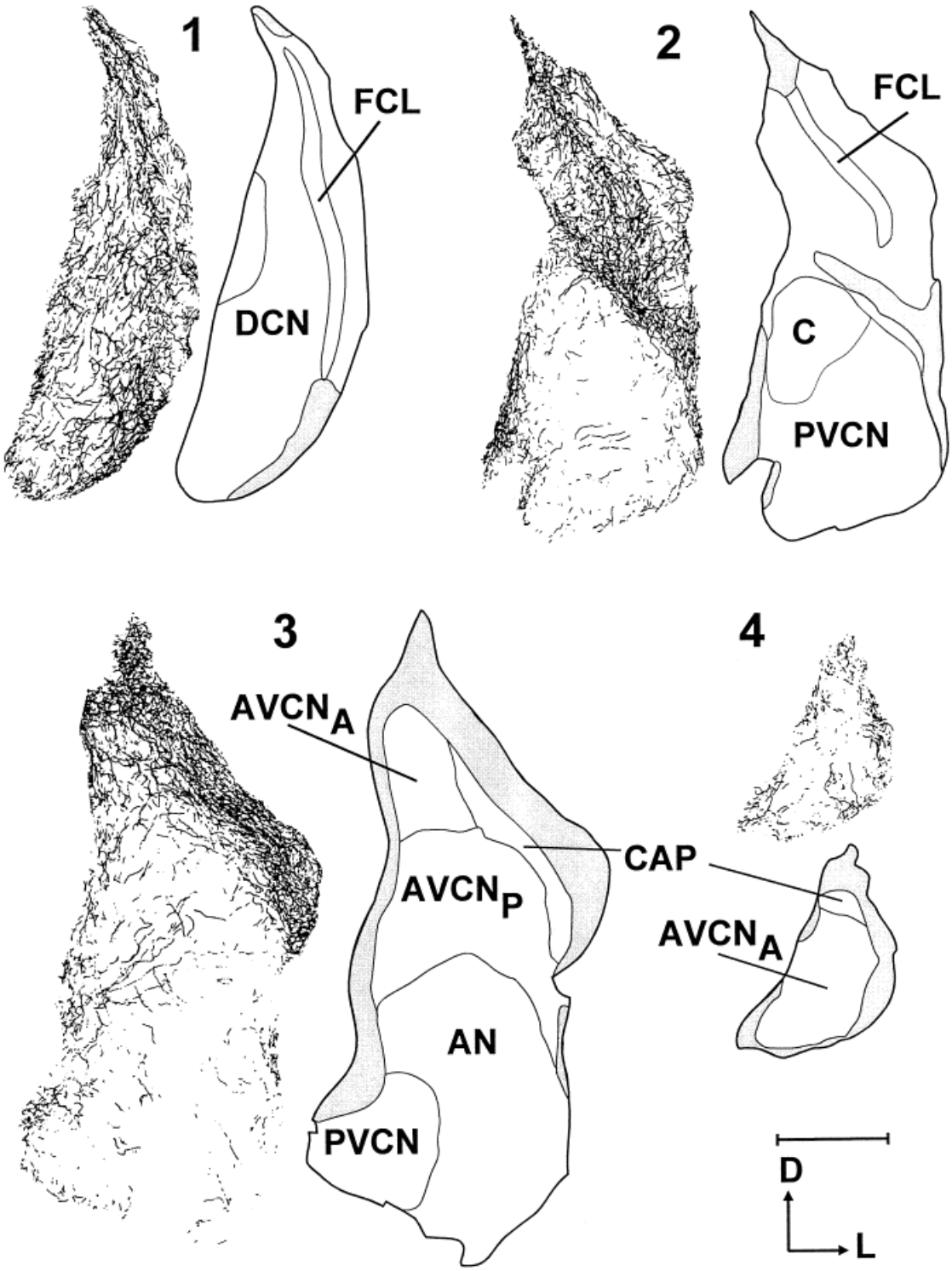

Fig. 1. Camera lucida reconstruction of serotonin-immunopositive fibers in representative sections of the right cochlear nucleus of *Tadarida*. Sections **1–4** represent sequentially more rostral transverse sections that contain the major subdivisions of the cochlear nucleus. There are more fibers in the dorsal cochlear nucleus (DCN) than in the ventral cochlear nucleus (VCN). A dense band of fibers also is present in the fusiform cell layer (FCL) of the DCN. The gray-shaded areas represent granule cell and external areas. Lateral is to the right, and dorsal is to the top. For other abbreviations, see list. Scale bar = 300 μm.

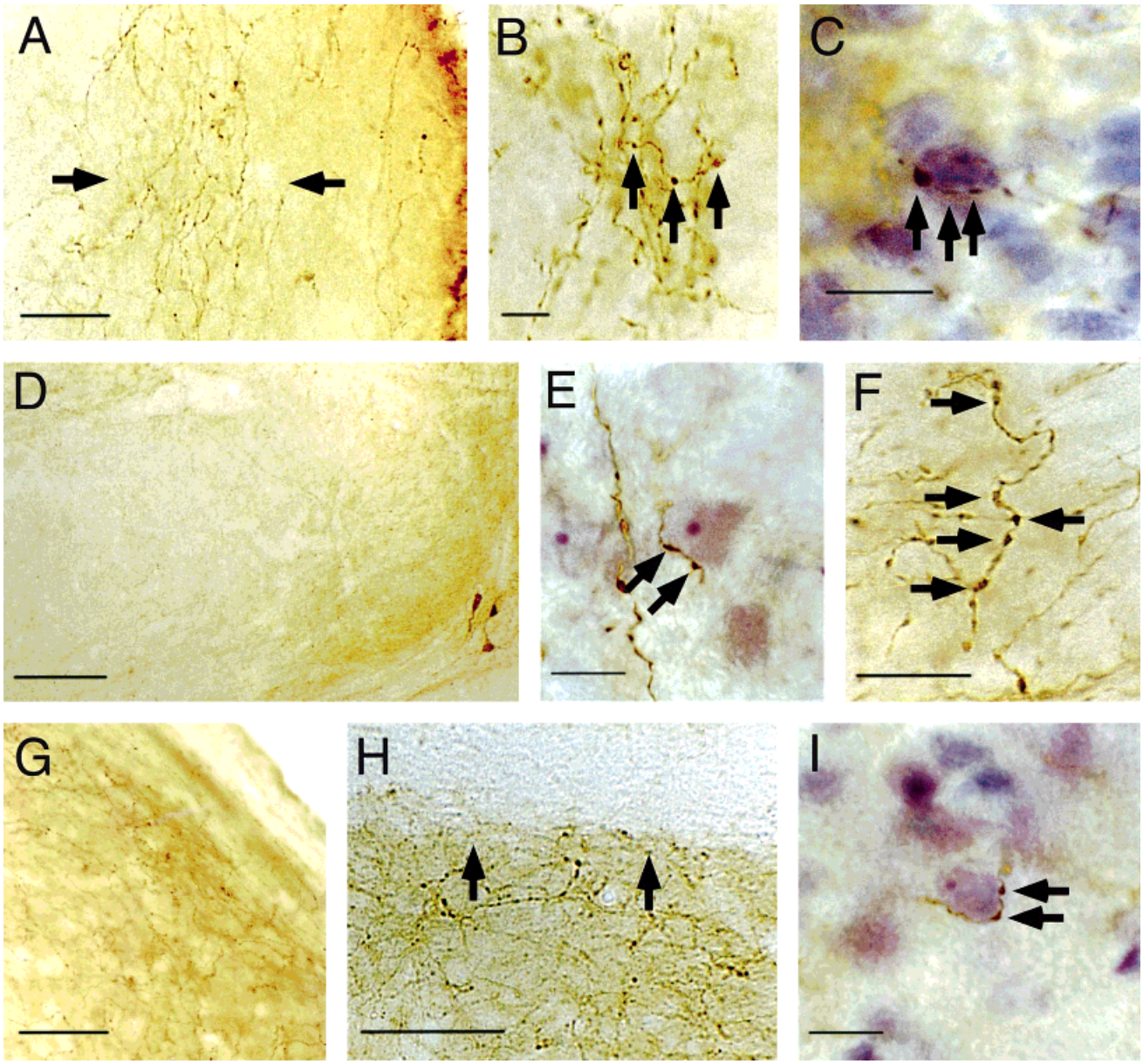

Fig. 2. Photomicrographs of transverse sections of *Tadarida* brainstem showing immunoreactive fibers. **A:** Section through the DCN showing a high density of serotonergic fibers in the fusiform layer between the two arrows. Dorsal is to the top, and lateral is to the right. **B:** A fiber within the fusiform layer of the DCN has a beaded appearance typical of en passant terminals. Arrows indicate some varicosities. **C:** Varicosities (arrows) in close apposition to cresyl violet-stained cell bodies in the granule cell region located between the DCN and the VCN. **D:** A section through the lateral superior olive (LSO) showing the relatively high density of immunoreactive fibers in its lateral limb (to the right). Dark-stained cell bodies at the lower right are lateralized serotonin neurons. Dorsal is to the top. **E:** Varicosities (arrows) in close apposition to a cresyl violet-stained cell body in the lateral limb of the LSO. **F:** Beaded fibers in the ventral nucleus of the lateral lemniscus (VNLL). Arrows indicate some varicosities. **G:** Serotonergic fiber staining within the left inferior colliculus (IC). Dorsal is to the top, and lateral is to the left. **H:** The commissure of the IC has a low density of serotonergic fibers. Dorsal is to the top, and lateral is to either side. **I:** Serotonergic varicosities (indicated by arrows) occur in close apposition to cresyl violet-stained cell bodies in the IC. Scale bars = 50 μm in A,H, 20 μm in B,G; 15 μm in F; 10 μm in C,E,I; 100 μm in D.

density within the IC. The densest areas of immunoreactivity were in peripheral regions of the nucleus (Fig. 5, Table 1). The dorsal cortex and lateral external divisions of the nucleus, including the brachium of the IC, had high fiber densities. In these regions, the most peripheral fibers tended to be oriented parallel to the surface of the brain and also were enriched in varicosities with beaded morphology (Fig. 2G). In contrast, in the ventromedial region of the IC as well as in a region laterally, serotonergic fibers were very sparse. This general pattern also was found in more caudal sections of the IC, with a higher density region of serotonergic fibers surrounding a sparser core.

TABLE 1. Relative Densities of Serotonin-Immunoreactive Fibers in Brainstem Nuclei[1]

Region	Density of fibers						
	Bat 1	Bat 2	Bat 3	Bat 4[2]	Bat 5	Bat 6	Bat 7
CN							
Granule cell regions	+++	+++	++	++	+++	+++	+++
DCN							
Layer 1	++	++	+	+	+	++	++
Layer 2	+++	+++	++	++	++	+++	+++
Deep layers	++	++	+	+	+	++	++
VCN							
AVCN anterior	++	++	++	+	++	++	++
AVCN posterior	+	+	+	+	+	++	+
AN	+	+	+	+	+	+	+
Small cell cap	++	++	++	++	++	++	++
PVCN	+	+	+	+	+	+	+
SOC							
LSO							
Lateral	+++	+++	++	++	++	+++	+++
Medial	++	+	+	+	+	+	+
MSO							
Dorsal	+++	++	+	++	+	++	++
Ventral	++	+	+	+	+	+	+
DPO	+++	++	++	++	+++	+++	++
VPO	++	++	+	+	++	+	++
VMPO	+++	++	+	+	+	+++	++
DMPO	+++	++	+	++	++	+++	++
MNTB	++	+	+	+	+	++	++
LNTB	+++	+++	++	++	+++	+++	+++
VNTB	++	++	+	+	+	+++	++
LL							
DNLL	+	++	+	+	+	++	+
INLL	++	++	+	+	++	++	++
VNLL	++	++	+	+	++	++	++
IC							
Dorsal	+++	+++	++	++	+++	+++	+++
Ventromedial	+	+	+	+	+	+	+

[1]Seven columns represent relative staining in different nuclei for seven individual bats (*Tadarida bresiliensis*). There was a generally consistent pattern of staining from bat to bat. Symbols +, ++, and +++ indicate minimum, intermediate, and maximum fiber density, respectively. CN, cochlear nucleus; DCN, dorsal CN; VCN, ventral CN; AVCN, anteroventral CN; AN, auditory nerve; SOC, superior olivary complex; LSO, lateral superior olive; MSO, medial superior olive; DPO, dorsal periolivary nucleus; VPO, ventral periolivary nucleus; VMPO, ventromedial periolivary nucleus; DMPO, dorsomedial periolivary nucleus; MNTB, medial nucleus of the trapezoid body; LNTB, lateral nucleus of the trapezoid body; VNTB, ventral nucleus of the trapezoid body; LL, lateral lemniscus; DNLL, dorsal nucleus of the LL; INLL, intermediate nucleus of the LL; VNLL, ventral nucleus of the LL; IC, inferior colliculus.
[2]The overall number of labeled fibers in this bat was low, so that only two density levels could be distinguished.

The regions above and below the commissure of the IC had a high fiber density, but the commissural tracts themselves were devoid of serotonergic fibers (Fig. 2H). As in other nuclei, serotonergic varicosities were seen in close apposition to cell bodies in the IC (Fig. 2I).

DISCUSSION

We have observed serotonergic projections in all nuclei of the auditory brainstem of the free-tailed bat. Within many of these nuclei, serotonergic fibers were regionalized, sometimes dramatically. With two exceptions, these patterns of serotonergic staining are similar to what has been reported in four other mammalian species, including one bat. One of these exceptions occurs in the cochlear nucleus, and the other occurs in the superior olivary nuclei. Here, we discuss the species differences and similarities in the serotonergic innervation of these regions, ascending the auditory neuraxis from the cochlear nucleus to the IC, and go on to explore possible functional aspects of these patterns of innervation.

Comparison of serotonergic innervation of auditory nuclei in different species

Cochlear nucleus. In the cochlear nucleus, there are several aspects of serotonergic fiber regionalization that are similar between all mammalian species studied: rat, guinea pig, opossum, and two species of bat (the Mexican free-tailed bat and the big brown bat, *Eptesicus fuscus*; Steinbusch, 1981; Willard et al., 1984; Klepper and Herbert, 1991; Thompson et al., 1994, 1995; Kaiser and Covey, 1997). The cochlear nucleus had a very clear-cut regionalization of serotonergic fibers compared with other brainstem auditory nuclei in all animals studied. More specifically, the DCN had a higher density of serotonergic fibers than the divisions of the VCN in all species studied. The PVCN also had a lower fiber density than the AVCN. Within the AVCN, the density of fibers was greater rostrally and diminished caudally.

The novel feature of serotonin fiber staining in the cochlear nucleus of the Mexican free-tailed bat is in the relative density of fibers in different layers of the DCN. In rat, guinea pig, and opossum, the density of serotonergic fibers is greatest in the most peripheral molecular layer of the DCN and is less in the fusiform cell layer (Willard et al., 1984; Klepper and Herbert, 1991; Thompson et al., 1995). In guinea pig and rat, serotonergic fibers are noticeably sparser in the fusiform cell layer than in the surrounding layers of the DCN (Klepper and Herbert, 1991; Thompson et al., 1995). In marked contrast, the fiber density in the fusiform cell layer in the Mexican free-tailed bat is much higher than that of the molecular layer, although the molecular layer still contains a fairly high density of fibers. This dense region of fibers in the fusiform cell layer is also continuous with the high fiber densities of adjacent granule cell regions. The fusiform cell layer contains the cell bodies of fusiform cells, which are one of the major output cell types of the DCN, as well as those of granule cells and cartwheel cells (Cant, 1992). It also contains the apical dendrites of elongate cells (Kane, 1974). Thus, serotonin potentially may modulate many different types of neurons in this layer (Golding and Oertel, 1997).

Superior olivary complex. The differential innervation of nuclei within the superior olivary complex has been reported in rat, guinea pig, cat, and bush baby (Steinbusch, 1981; Thompson et al., 1994; Thompson and Schofield, 2000). Aspects of serotonin immunoreactivity that are similar in these species include a relatively higher density of fibers in periolivary nuclei, particularly in the lateral nucleus of the trapezoid body, ventral nucleus of the trapezoid body, and dorsomedial periolivary nucleus, compared with the principal nuclei.

However, one striking difference in the Mexican free-tailed bat is the regionalization of serotonin fibers within the LSO and MSO. In cat, rodents, and bush baby, the LSO and MSO are innervated uniformly and sparsely by serotonergic fibers. In the free-tailed bat, the LSO contains a high density of serotonergic fibers laterally, whereas the MSO contains a high density dorsally. These high-density regions in both nuclei coincide with the cell bodies of the neurons responding to the lowest frequencies within both nuclei.

Lateral lemniscus. The serotonergic innervation of the nuclei of the lateral lemniscus has been reported previously in detail in the cat (Thompson et al., 1994) and the

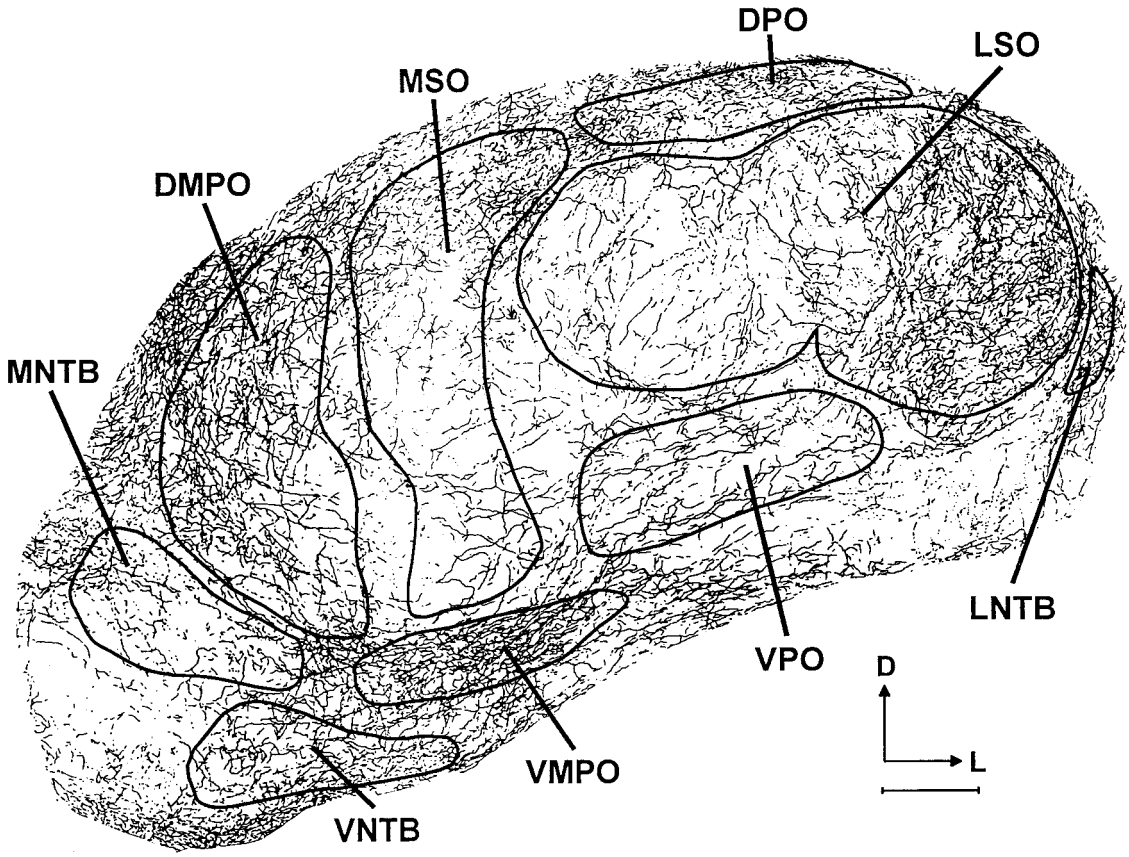

Fig. 3. Camera lucida reconstruction of serotonin-immunopositive fibers from a representative section through the right superior olivary complex. Fibers are relatively dense in periolivary regions, including the dorsal, dorsomedial, and ventromedial regions. In the principal nuclei, fibers are most dense in the lateral limb of the lateral superior olive and the dorsal part of the medial superior olive. Nuclei are classified according to Grothe et al. (1994). Dorsal is to the top, and lateral is to the right. For abbreviations, see list. Scale bar = 100 μm.

big brown bat, *Eptesicus* (Kaiser and Covey, 1997). In the big brown bat, both divisions of the VNLL had a lower density of serotonergic fibers than the intermediate and dorsal nuclei of the lateral lemniscus (Kaiser and Covey, 1997). In the free-tailed bat, all nuclei of the lateral lemniscus had approximately the same density of serotonergic fibers. However, in other regards, the lemniscal staining was similar in the two bats, including the columnar organization of fibers in the DNLL.

IC. Finally, the pattern of serotonergic innervation of the IC was very similar across species. In the three other species in which the serotonergic innervation of the IC was studied, rat, guinea pig, and big brown bat, the most densely innervated regions of the IC were the regions surrounding the central nucleus, just as in the free-tailed bat. (Klepper and Herbert, 1991; Thompson et al., 1994; Kaiser and Covey, 1997). In addition, in all of these species, there was also a region of very low fiber density in the ventral and/or lateral parts of the nucleus.

In summary, although many aspects of serotonergic innervation of the auditory brainstem are consistent across species, several are not. Key features of serotonergic innervation of auditory nuclei in the free-tailed bat that have not been reported previously include the strikingly dense innervation of the fusiform layer of the DCN and the selectively heavy innervation of the low-frequency regions of the superior olives.

Some functional implications of serotonin innervation patterns

The selective innervation of certain auditory nuclei and, furthermore, of subdivisions of these nuclei, suggests that serotonin may selectively modulate some auditory regions and, thus, play particular roles in auditory processing. However, the diverse patterns of serotonin immunoreactivity within the auditory brainstem do not suggest any single hypothesis regarding the function of serotonin in auditory processing. Rather, different patterns of serotonin immunoreactivity are consistent with different hypotheses about serotonin function.

Serotonin modulates integration of inputs

One of these hypotheses that was proposed by Klepper and Herbert (1991) is that serotonin modulates auditory neurons that integrate inputs from many different sources, including higher auditory centers. We found evidence to support this hypothesis in the Mexican free-tailed bat. In several nuclei, serotonergic fibers were densest in regions that integrate inputs from many sources, both higher and lower in the neuraxis. In the cochlear nucleus, the highest fiber density by far was seen in granule and fusiform cell regions. Granule cell regions receive information from many different sources, including the auditory nerve (Shore and Moore, 1998), local circuits within

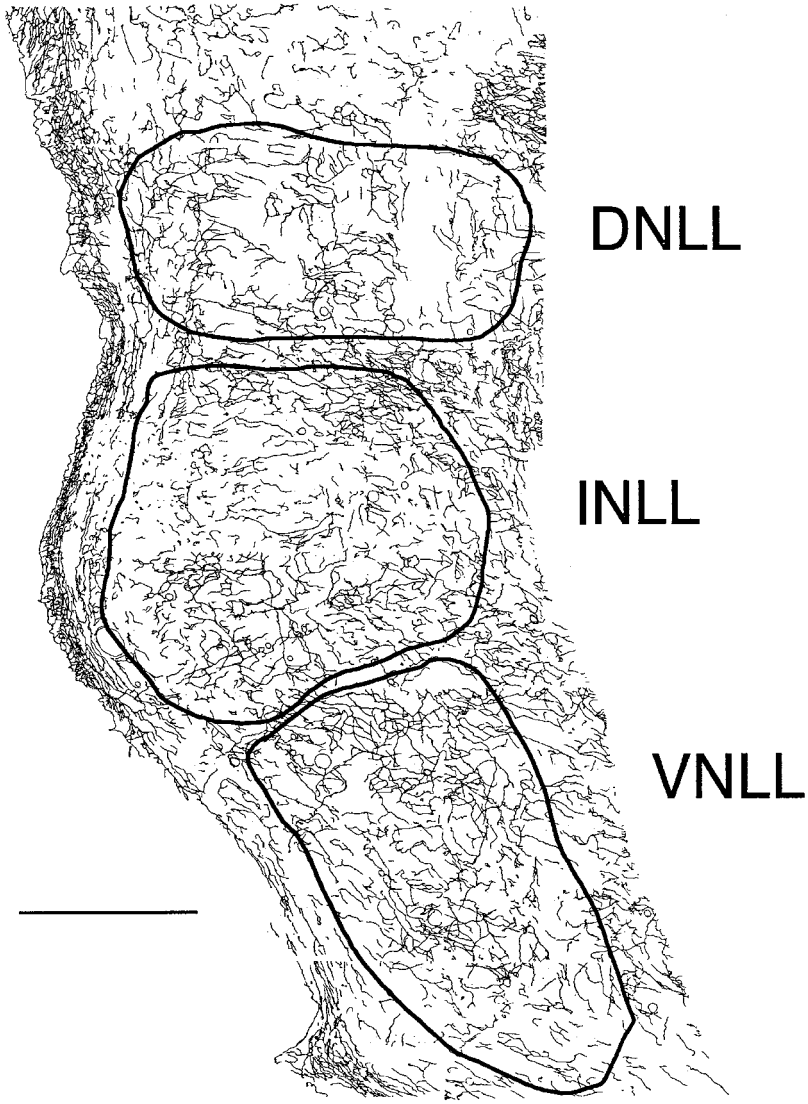

Fig. 4. Camera lucida reconstruction of serotonin-immunopositive fibers from a transverse section through right lateral lemniscus showing the dorsal (DNLL), intermediate (INLL), and ventral (VNLL) nuclei. The density of fibers is similar between the three regions. Note the columnar pattern of staining in the dorsal nucleus of the lateral lemniscus, where serotonin-positive fibers are present in the cellular areas but absent in the areas in which lemniscal fibers travel. Dorsal is to the top, and lateral is to the left. Scale bar = 200 μm.

the cochlear nucleus (Adams, 1983), other brainstem auditory nuclei including the IC (Malmierca et al., 1996), olivocochlear neurons (Benson and Brown, 1990), cortex (Weedman and Ryugo, 1996), and even nonauditory nuclei (Young et al., 1995). Granule cells project to fusiform cells in layer 2 (Cant, 1992; Davis et al., 1996), and granule cell bodies are also present in layer 2 (Cant, 1992). Thus, it is possible that the dense serotonergic innervation of layer 2 in the bat is a consequence of the presence of granule cells in this layer. The fusiform cells of layer 2 themselves integrate inputs from a similar array of auditory and nonauditory sources (Kane, 1977; Conlee and Kane, 1982; Merchán et al., 1985; Young et al., 1995; Schofield and Cant, 1996; Golding and Oertel, 1997). The peripheral regions of the IC, which also were rich in serotonergic fibers in the free-tailed bat, receive inputs from almost all lower auditory nuclei as well as from cortex and from nonauditory regions of the brain (Oliver, 1987; Shneiderman et al., 1988; Sato and Ohtsuka, 1996; Druga et al., 1997; Winer et al., 1998). One region that projects to the dorsal cortex of the IC (Henkel and Shneiderman, 1988), the nucleus sagulum, also had an extremely high density of serotonergic fibers.

Serotonin modulates low-frequency regions

A second hypothesis that we propose is that serotonin modulates low-frequency pathways in some nuclei. In two major nuclei of the superior olivary complex in the Mexican free-tailed bat, the LSO and MSO, serotonergic fibers were notably densest in the regions of the nuclei that contain neurons that respond to relatively low-frequency sounds. For the LSO, this is the lateral portion of the nucleus, whereas, for the MSO, this is the dorsal portion (Schwartz, 1992). The lowest reported best frequencies of

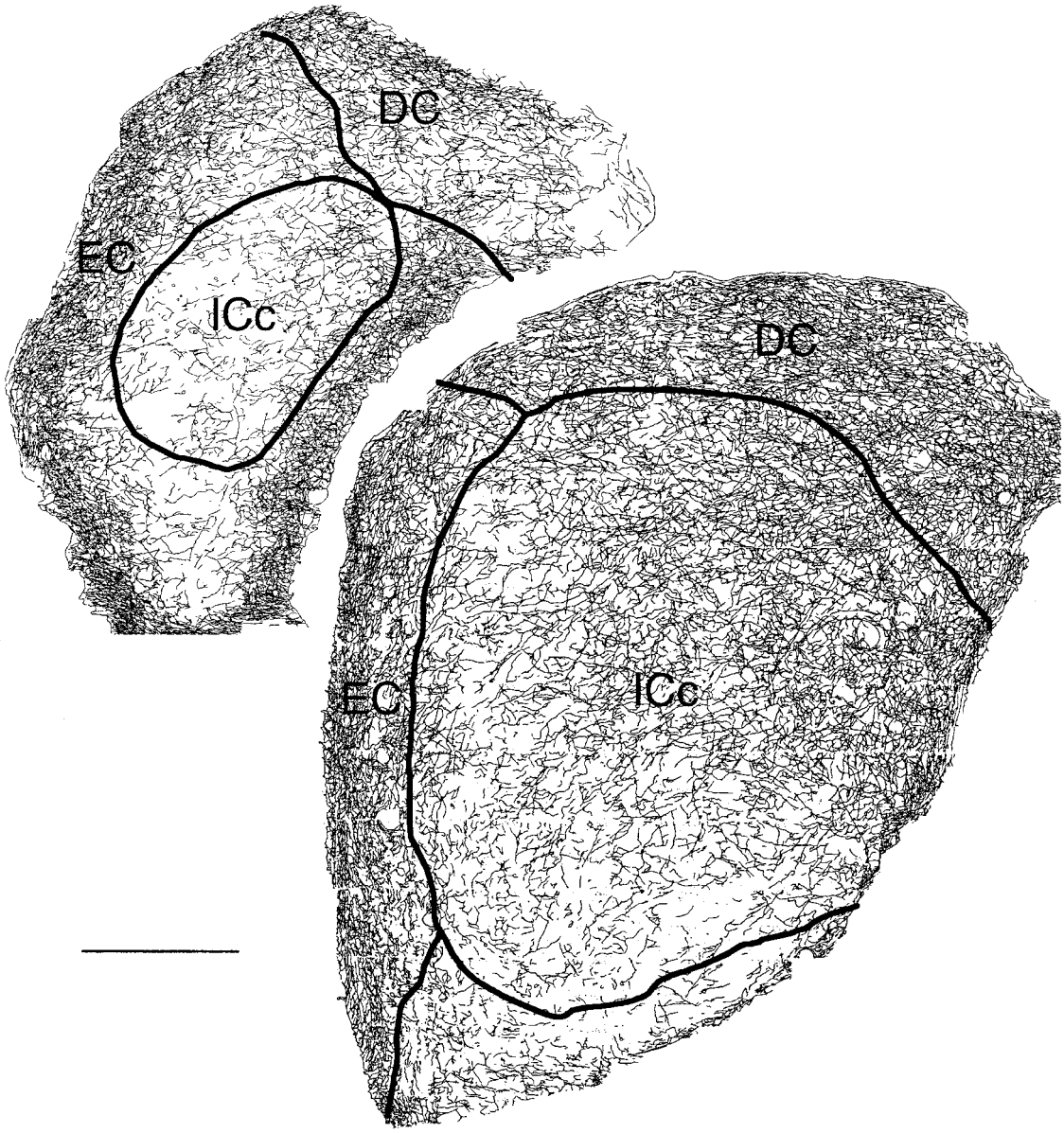

Fig. 5. Camera lucida reconstruction of serotonin-immunopositive fibers from a transverse section through the right inferior colliculus. Fibers are densest in the dorsal (DC) and external (EC) cortices and in the dorsomedial part of the central nucleus. The reconstruction on the top left was taken from a more caudal slice within the same brain. Dorsal is to the top, and lateral is to the left. For other abbreviations, see list. Scale bar = 400 μm.

neurons in these nuclei are 8 kHz for the LSO (Park et al., 1996) and 9 kHz for the MSO (Grothe et al., 1997). In the IC, serotonergic fibers were denser in the dorsal portion of the central nucleus, which contains the neurons that respond to relatively lower frequencies, than in the ventral portion (Bodenhamer and Pollak, 1981).

The LSO and MSO are thought to be involved in the localization of sound in azimuthal space through detecting intensity differences or timing differences between the two ears, respectively (Irvine, 1992; Park et al., 1997; Grothe and Park, 1998). Thus, the presence of serotonergic fibers in these nuclei may indicate that the localization of sound is subject to serotonergic modulation. In brain slices from young gerbils, serotonin does reduce the excitatory inputs coming from the ipsilateral side of the brain more than inhibitory inputs coming from the MNTB, a phenomenon that, in intact animals, would create a difference in interaural intensity coding (Fitzgerald and Sanes, 1999). However, why the potential modulation should be greater at lower frequencies and why this should be true in the free-tailed bat but not in other mammals remain unclear. Could the selective serotonergic innervation of low-frequency regions underlie a differential modulation of different functional classes of sound that are specific to the free-tailed bat? Within the free-tailed bat's vocal repertoire, the first harmonics of many communication calls tend to span a range of relatively low frequencies, whereas the first harmonics of echolocation

calls sweep from high to low frequencies (Simmons et al., 1978, 1979; Gelfand and McCracken, 1986; Balcombe and McCracken, 1992). However, there is a significant amount of frequency overlap in the ranges of these calls. Thus, it is difficult to imagine that this selective innervation pattern by serotonin could underlie a selective serotonergic modulation of echolocation sounds versus nonecholocation sounds. Still, the possibility remains that this selective innervation of low-frequency regions may underlie a selective modulation of the central processing of some other class of low-frequency sounds. An alternative possibility is that, at least in the IC, serotonergic innervation of low-frequency regions reflects the neurotransmitter phenotype. In these two nuclei, the pattern of serotonergic fibers coincides well with the pattern of GABAergic puncta seen in two other bat species (Vater et al., 1992).

Serotonin modulates inputs descending to cochlear/cochlear nucleus

Yet a third hypothesis regarding the function of serotonergic innervation of the auditory brainstem is that serotonin modulates inputs descending from periolivary regions to the cochlea (olivocochlear neurons) and cochlear nucleus. Olivocochlear neurons are found in some nuclei in the superior olivary complex and in periolivary regions. Exactly where they can be found varies widely between mammalian species (Aschoff and Ostwald, 1987), but the serotonin system projects to olivocochlear neurons in bush baby and rat (Thompson and Thompson, 1995; Woods and Azerado, 1999). In the free-tailed bat, some of the regions that have been shown to contain olivocochlear efferents either through tracing from the cochlea or by staining for acetylcholinesterase also show dense serotonergic projections (Aschoff and Ostwald, 1987; Grothe et al., 1994). For example, olivocochlear cell bodies can be found in the free-tailed bat ventral to the dorsomedial periolivary nucleus and within the ventromedial periolivary nucleus and dorsal periolivary nucleus. These regions also have a high density of serotonergic fibers relative to surrounding regions. Inputs descending to the cochlear nucleus also can be found in periolivary regions with a high density of serotonergic fibers. For example, serotonergic axon terminals contact neurons in the ventral and lateral nuclei of the trapezoid body that project to the cochlear nucleus (Thompson and Schofield, 2000). All of this suggests that the serotonergic system may widely innervate the descending auditory pathway comprised of cells in the superior olive that terminate in the cochlea and/or cochlear nucleus. The fact that serotonin can indeed affect the firing of neurons in some periolivary regions (Wang and Robertson, 1997) lends strength to this hypothesis.

Thus, the pattern of serotonergic innervation of the auditory brainstem is consistent with many hypotheses regarding serotonin function in auditory processing. To test these specific hypotheses in the future will require electrophysiological and anatomical experiments done in tandem in the same preparations. It will be especially interesting to discover whether those aspects of serotonergic innervation that are particular to bats are reflected functionally.

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