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Dense serotonergic innervation of principal nuclei of the superior olivary complex in mouse

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Abstract

To evaluate species differences in the serotonergic innervation of the superior olivary complex, serotonergic fibers and varicosities were labeled with immunohistochemistry in mouse. Many immunoreactive fibers and varicosities were observed in two of the three principal nuclei, in addition to some periolivary nuclei. This pattern of staining differs greatly from that observed in other mammals in which periolivary, but not principal nuclei are richly innervated by serotonin (5-HT). These results indicate a functional relationship between the 5-HT system and both the ascending and descending auditory systems in the mouse.

Keywords: Serotonin; Superior olivary complex; Lateral superior olive; Medial superior olive; Auditory brainstem; Ascending auditory pathways

Serotonin (5-HT) is a major neuromodulator that is found throughout the auditory brainstem of all mammalian species that have been examined. Both within and between auditory brainstem nuclei, serotonergic inputs are nonuniformly distributed, creating a pattern of innervation that suggests that 5-HT targets certain neuronal groups. The patterns of serotonergic inputs are generally similar between different mammalian species. For example, there are more 5-HT fibers in the dorsal, as opposed to the ventral cochlear nucleus [4,6,14]. In the superior olivary complex (SOC), the density of 5-HT terminal fibers is much lower in the principal SOC nuclei than in the surrounding periolivary nuclei [4,13,15,17]. This general mammalian 5-HT staining pattern is consistent despite interspecific variations in the SOC nuclei, including the size of the principal nuclei [12].

However, variation in this pattern does exist. A recent detailed investigation in the bat suggested that 5-HT innervation densities within SOC nuclei may vary in some species. In the bat, 5-HT fibers were unevenly distributed within two principal SOC nuclei, the lateral (LSO) and medial (MSO) superior olives [4]. In an effort to further evaluate species differences in the 5-HT innervation of the SOC, we investigated the 5-HT staining pattern in the mouse SOC. In addition to being well-studied models of the auditory brainstem, mice are well-suited to exploration of the genetic and developmental factors influencing the distribution of 5-HT in the SOC. In this study, we found that the 5-HT innervation of the SOC departs markedly from that reported in other mammals, including the bat. The particular pattern of staining observed suggests that, unlike other species, 5-HT has a modulatory role in two major SOC nuclei of the adult mouse.

Serotonergic terminal fibers and varicosities were labeled immunohistochemically in the brainstems of C3H/HeJ mice (Jackson Labs). Mice of this inbred strain maintain normal hearing throughout their lifespan [19]. The mice were cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Five adult mice ranging in ages from 5 to 10 months of age were overdosed with sodium pentobarbital (200 μg/g, i.p.) and transcardially perfused first with saline (7 ml, room temperature) and then 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). Some mice were perfused with the pH switch protocol, which includes the addition of 0.05% glutaraldehyde [16]. The brains were removed and post-fixed for 1–3 days at 4 °C. The brains were then placed in 20% sucrose in

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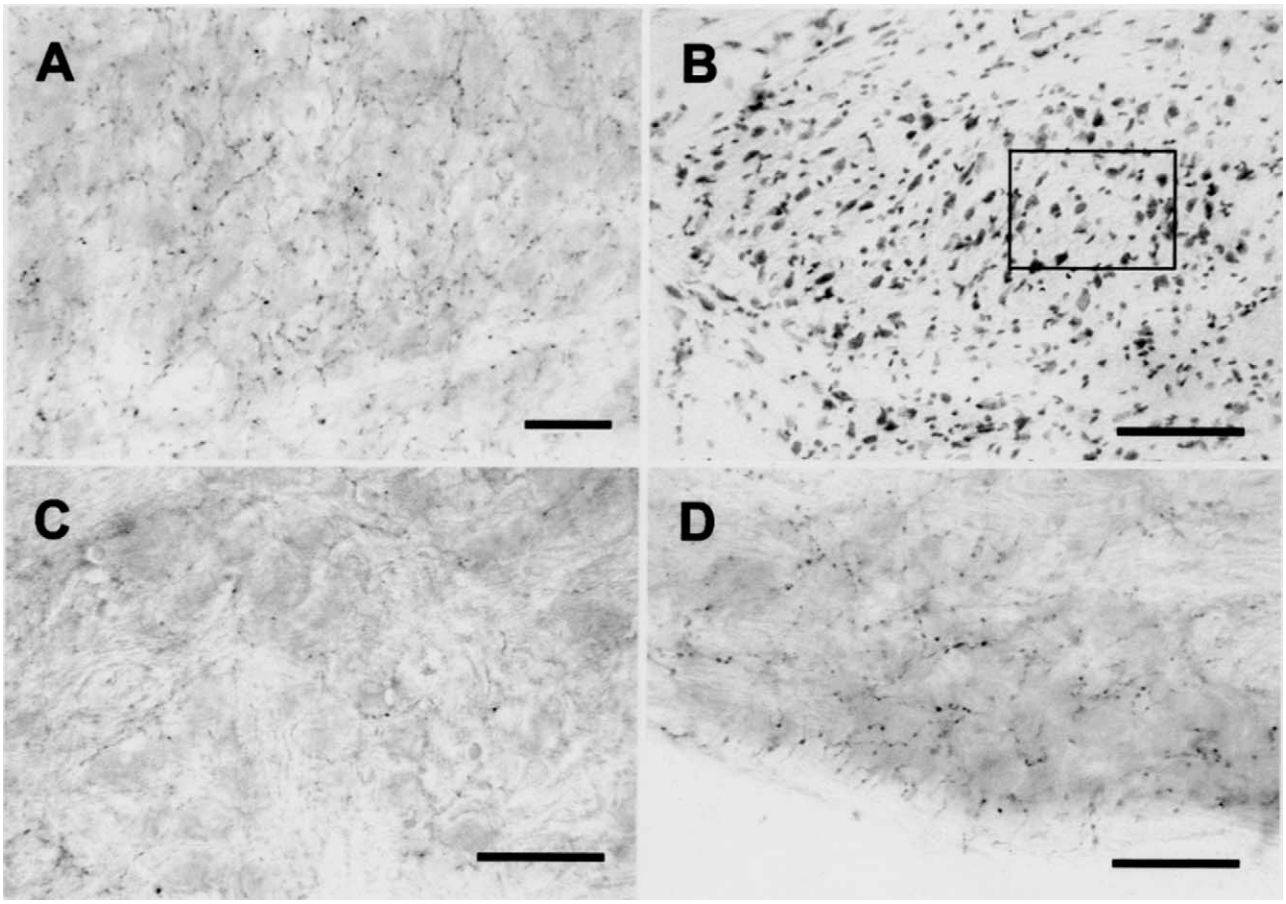

Fig. 1. Photomicrographs of 5-HT-IR terminal fibers and varicosities located within the SOC. (A) Immunoreactive terminal fibers and varicosities in the LSO. The picture was taken from the dorsomedial part of the LSO as indicated by the box drawn on an adjacent Nissl-stained section (B). The density of labeled fibers in the LSO and MSO was similar to that in some periolivary nuclei. For comparison, the photomicrograph in (D) illustrates 5-HT-IR terminal fibers and varicosities in one periolivary region, the ventral nucleus of the trapezoid body. As shown in (C), 5-HT-IR fibers were relatively sparse in the medial nucleus of the trapezoid body. All photomicrographs were from the same animal. Scale bars: (B) 60 μm; (A,C,D) 30 μm.

PB at 4 °C until equilibrated. Frozen sections were cut perpendicular to the brainstem at 30 μm thick on a sliding microtome, collected in cryoprotectant (30% ethylene glycol and 20% glycerol in 0.05 M PB, pH 7.4), and stored at −23 °C until processed.

The free-floating sections were rinsed in PBS, placed in H_2O_2 (0.5% H_2O_2 in PBS) to block endogenous peroxidase, and then rinsed in PBS. Non-specific staining was then blocked by incubating the sections in PBS containing 0.3% Triton-X and 3% powdered milk (PBS-TX-Milk), and then avidin and biotin solutions (Vector Labs, as per kit instructions). The sections were then placed in PBS-TX-Milk containing anti-5-HT (1:100,000, ImmunoStar) or anti-5-HT transporter (1:3500, Chemicon), 0.05% normal goat serum, and 0.1% sodium azide for 3 days at 4 °C. The sections were then rinsed in PBS-TX-Milk and incubated in PBS-TX-Milk containing the secondary antibody (biotinylated goat anti-rabbit, 1:8000) and 2% normal goat serum for 60 min. After rinses, the sections were incubated in the ABComplex (Vector Labs) diluted in PB with 0.3 M NaCl per kit instructions for 45 min, rinsed again, and then incubated in DAB/H_2O_2 (0.05% DAB + 0.03% H_2O_2 in Tris saline buffer, pH 7.6) for 15 min. After final rinses in Tris saline buffer, the sections were blotted onto glass slides, air-dried, and coverslipped. Some sections were counterstained with cresyl violet acetate. The sections were viewed and images digitized under a light microscope (Olympus) with a Spot camera and imaging software. The nomenclature of the mouse SOC as described by Ollo and Schwartz [9] was used. For negative controls, some sections were incubated without primary antibody and others were incubated in the 5-HT transporter antibody pre-adsorbed with peptide (Chemicon).

With this protocol, 5-HT-immunoreactive (5-HT-IR) terminal fibers and varicosities were observed throughout the SOC. In all mice, many 5-HT-IR fibers were observed in the LSO (Fig. 1A). Within the LSO, the 5-HT-IR fibers were not restricted to any location, but seemed to fill the entire nucleus. The IR terminal fibers and varicosities were typical of those described in other regions, e.g. thin fibers with swellings. Many fibers were also observed throughout the MSO and these had a density similar to those in the LSO. Immunoreactive terminal fibers were sparse in the medial nucleus of the trapezoid body (MNTB, Fig. 1C). Immuno-

reactive fibers were also observed in the periolivary regions. Among the periolivary regions, the ventral nucleus of the trapezoid body contained the densest IR terminal fibers and varicosities (Fig. 1D). In the superior paraolivary nucleus, 5-HT-IR fibers were sparse. In the cochlear nuclei, the dorsal cochlear nucleus contained relatively more 5-HT-IR fibers than the ventral cochlear nucleus. Other regions of the brainstem, the facial and trigeminal motor nuclei, as well as the raphe cell groups, were more heavily innervated by 5-HT-IR terminal fibers than auditory regions.

The overall staining intensity of the immunolabeled fibers varied across mice but the relative density of 5-HT fibers was similar in all mice. Therefore, even if fiber labeling was less intense, the same distribution pattern was observed. The pattern of staining was similar for the 5-HT antibody and the 5-HT transporter antibody. Immunoreactivity was absent in both types of negative controls.

The current finding that serotonergic terminal fibers and varicosities are present in the mouse LSO and MSO indicates that 5-HT is likely to modulate the responses of neurons found in these nuclei. In the LSO, potential targets of this modulation include at least two types of neurons: (1) principal neurons that project to the inferior colliculus; and (2) lateral olivocochlear neurons that project to the cochlea [2]. It is possible that other cell types may be modulated as well given the possibility that additional morphologically defined cell types exist in other rodents. For example, in the C57BL/6 mouse, the LSO contains three different types of neurons [9], in the rat LSO seven different cell types have been described [10], and in the gerbil LSO there are at least three other types of neurons besides the principal and lateral olivocochlear neurons [3]. The other LSO cell types are likely to be interneurons that project to areas within the LSO or to other areas of the SOC. The cell types of the MSO have not been described in detail in the mouse but in other species, the MSO contains both principal and marginal neurons [11]; each of these would be a putative target of 5-HT in the mouse. Further studies will be necessary to identify which of the cell types within the LSO and MSO are post-synaptic targets of 5-HT.

The current study indicates differences in the density of 5-HT terminal fibers among the nuclei of the SOC in mouse. In other mammalian species, the density of 5-HT fibers and/or varicosities also varies within the SOC, but the distribution patterns of the fibers are different from those in mouse. In cat, guinea pig, and bush baby, there are more 5-HT terminal fibers in periolivary regions than in principal nuclei [15,17]. In the rat, there are conflicting data. Steinbusch [13] reported a similar pattern as that found in cat, e.g. relatively more fibers in the periolivary regions than in the principal nuclei. In contrast, Woods and Azeredo [18] reported equal numbers of 5-HT varicosities in the principal and periolivary nuclei. The discrepancy between these two studies could be due to different methods of detection and analysis.

In the Mexican free-tailed bat, the results were somewhat intermediate. Although more fibers were located in periolivary regions, some parts of the LSO (lateral limb) and MSO (dorsal pole) contained more 5-HT fibers compared to other parts of the same nucleus [4]. This suggests that any modulatory effects of 5-HT in the bat may be frequency-specific, because the lateral LSO and dorsal MSO contain the cell bodies of neurons that have relatively low best frequencies [1]. Frequency-specific modulation is unlikely to occur in mouse because of the uniform distribution of fibers in the mouse LSO found in this study. Interestingly, the interspecific variability in reported patterns of 5-HT-IR fibers in the SOC is greater than for some auditory nuclei like the inferior colliculus, in which the patterns of serotonergic fiber staining are quite similar in different species of mammal [4].

The current results strongly indicate that in the adult mouse, both ascending and descending auditory pathways originating in the SOC receive input from the 5-HT system. While the specific source(s) of these inputs is not known, it is likely that the activity of the 5-HT inputs is highly correlated with arousal and attention level [5,7]. It is possible that via these inputs, the 5-HT system modulates the outputs of the SOC to help attend to certain stimuli so that the appropriate behavioral response can be made. The current study also suggests that the particular SOC efferent pathways receiving 5-HT inputs differ among species and hence, any process of sensory filtering performed by the 5-HT system may also vary among species.

Besides implicating 5-HT as a neuromodulator in principal and periolivary nuclei of the SOC in the adult mouse, the current results provide further rationale for studying the role of 5-HT as a developmental signal in the auditory brainstem. As with other brain targets of the 5-HT system [8], it is likely that the SOC nuclei innervated by 5-HT terminal fibers and endings rely on 5-HT for their normal development. Thus, the current results provide a strong basis for examining the developmental role of 5-HT not only in periolivary regions, but also in two principal nuclei of the mouse SOC.

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