Neuroscience 136 (2005) 883–894

LAMINAR INPUTS FROM DORSAL COCHLEAR NUCLEUS AND VENTRAL COCHLEAR NUCLEUS TO THE CENTRAL NUCLEUS OF THE INFERIOR COLLICULUS: TWO PATTERNS OF CONVERGENCE

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Abstract—The central nucleus of the inferior colliculus is a laminated structure composed of oriented dendrites and similarly oriented afferent fibers that provide a substrate for tonotopic organization. Although inputs from many sources converge in the inferior colliculus, how axons from these sources contribute to the laminar pattern has remained unclear. Here, we investigated the axons from the cochlear nuclei that terminate in the central nucleus of the cat and rat. After characterization of the best frequency of the neurons at the injection sites in the cochlear nucleus, the neurons were labeled with dextran in order to visualize their axons and synaptic boutons in the central nucleus. Quantitative methods were used to determine the size and distribution of the boutons within the laminar organization. Two components in the laminae were identified: (1) a narrow axonal lamina that included the largest fibers and largest boutons; (2) a wide axonal lamina, surrounding the narrow lamina, composed of thin fibers and only small boutons. The wide lamina was approximately 30–40% wider than the narrow lamina, and it often extended more than 100 μm beyond the larger boutons on each side. The presence of both thick and thin fibers within the acoustic striae following these injections suggests that large and small fibers/boutons within these bands may originate from different neuronal types in the dorsal and ventral cochlear nucleus. We conclude that the narrow laminae that contain large fibers and boutons originate from larger cell types in the cochlear nucleus. In contrast, the wide lamina composed exclusively of small boutons may represent an input from other, perhaps smaller neurons in the cochlear nucleus. Thus, two types of inferior colliculus laminar structures may originate from the cochlear nucleus, and the small boutons in the wide laminae may contribute a functionally distinct input to the neurons of the inferior colliculus. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: fibrodendritic lamina, auditory pathway, tonotopic organization, terminal boutons.

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Abbreviations: AVCN, anteroventral cochlear nucleus; BDA, biotinylated-dextran; DAS, dorsal acoustic stria; DCN, dorsal cochlear nucleus; FD, FITC-dextran; IC, inferior colliculus; PVCN, posteroventral cochlear nucleus; TRD, TRITC-dextran; VCN, ventral cochlear nucleus.

0306-4522/05$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.04.040

A distinct feature of the central nucleus of the inferior colliculus (IC) is its laminar organization (Oliver and Morest, 1984; Morest and Oliver, 1984; Malmierca et al., 1993a,b, 1995b). These fibrodendritic laminae are made of flat, disk-shaped neurons and axonal afferents originating in lower auditory centers (Osen, 1972; Brunso-Bechtold et al., 1981; Whitley and Henkel, 1984; Coleman and Clerici, 1987; Oliver and Shneiderman, 1989; Oliver and Beckius, 1993; Merchan et al., 1994, 1996; Oliver et al., 1995, 1997, 1999; Henkel, 1997; Malmierca et al., 1998; Shneiderman et al., 1999; Henkel et al., 2003). The laminae are thought to be the morphological substrate for tonotopic organization described in electrophysiological studies (for review see Irvine, 1992; Malmierca, 2003). Laminar inputs originate from the cochlear nuclear complex, dorsal nucleus of the lateral lemniscus and lateral superior olive bilaterally, the ventral complex of the lateral lemniscus ipsilaterally, and the contralateral IC (Beyerl, 1978; Adams, 1979, 1983; Brunso-Bechtold et al., 1981; Coleman and Clerici, 1987; Oliver, 1987; Malmierca et al., 1995a, 1998; Merchan et al., 1996; Kelly et al., 1998; Oliver et al., 1999; Bajo et al., 1999). While it is known that these laminar inputs parallel the dendritic fields of the neurons (Oliver et al., 1991; Malmierca et al., 1993a, 1995a), the details of the organization and convergence of these different brainstem projections to the IC are not well known. While the dendritic component of the fibrodendritic laminae has been extensively studied using quantitative analysis both in cat and rat (Oliver et al., 1991; Malmierca et al., 1993), little is known about the axonal component of these laminae.

The cochlear nuclei are the first relay center in the auditory pathway and from their different morphological and physiological cells types emerge a number of parallel pathways (Osen, 1969; Brawer et al., 1974; Cant and Morest, 1979a; Hackney et al., 1990; Cant and Benson, 2003), most of which terminate directly or indirectly in the IC. The present study aims to address the composition of direct laminar inputs from the cochlear nucleus. Laminar inputs from the cochlear nuclei in both the cat and rat were investigated to determine whether morphologically heterogeneous populations of synaptic boutons display different distributions within the IC. Small injections of different anterograde tracers were made into the dorsal cochlear nucleus (DCN) and/or ventral cochlear nucleus (VCN) in the same animal to define the morphology, distribution, and the degree of convergence of terminal boutons in the central nucleus

of the IC. Image processing of large numbers of boutons permitted a quantitative analysis of their size and laminar distribution. These data suggest that there are several distinct populations of boutons that emerge from the cochlear nucleus. Preliminary reports of these findings have been presented elsewhere (Malmierca et al., 1999, 2003, 2004; Smith et al., 2005).

EXPERIMENTAL PROCEDURES

All experiments were carried out with methods in keeping with the standards and approval of the University of Salamanca Animal Care Committee and the National Institutes of Health animal care guidelines approved by the Animal Care Committee of the University of Connecticut Health Center. Every effort was made to minimize the suffering of animals used in these experiments, and whenever possible the data from these animals has been used for other projects to minimize the number of animals required for research. Fifteen rats (Table 1 in Malmierca et al., 2002) and nine cats with injections in the cochlear nuclei were used to examine the anterograde labeling of axons in the IC. Injections of biotinylated-dextran (BDA, D-1956, Molecular probes, Eugene, OR, USA), FITC-dextran (FD, D-1820, Molecular probes, Eugene, OR, USA), and TRITC-dextran (TRD, D-1817, Molecular probes, Eugene, OR, USA) were made in physiologically defined regions of the DCN and/or VCN in the same animal as published previously (Oliver et al., 1997; Malmierca et al., 2002). For rats, an areflexive, anesthetic state was induced by i.m. administration of ketamine (57 mg/kg) and xylazine (8.6 mg/kg) and maintained with the same compounds. For cats, general anesthesia was induced with ketamine (33 mg/kg) and xylazine (1 mg/kg), intubated, and then maintained with isoflurane delivered with medical grade oxygen. Animals were held in a stereotaxic frame and monitored by observing respiration rate and reflexes. A craniotomy was performed over the cerebellum, and a part of the lateral and floccular cerebellum was aspirated to expose the cochlear nucleus.

Extracellular recordings in response to acoustic stimulation allowed the determination of best frequency (the sound frequency to which the neurons respond with the lowest stimulus intensity) at the injection sites in the right DCN, right anteroventral cochlear nucleus (AVCN) or posteroventral cochlear nucleus (PVCN). Animals were placed in a double-wall sound-attenuation chamber. Earphones in a sealed enclosure were coupled to the ear bars of the stereotaxic frame, and pure tones were delivered by digital stimulus generators under the control of a computer system (Rhode, 1973; Rees et al., 1997). Recordings were made with glass micropipettes (tips 10–40 μm) filled with injection solutions for anterograde transport. The injection electrode contained either 10% TRD dissolved in saline, or a mixture of 10% BDA and 10% FD in saline. Once the desired site was found, the dextrans were injected by iontophoresis (2–6 μA for 5–24 min).

Seven to 10 days after the injections, the brains were perfusion-fixed and prepared for light microscopy. While under deep surgical anesthesia, the animals were perfused transcardially with a buffered washout solution (2% sucrose in 0.12 M phosphate buffer, pH 7.4, containing 0.05% lidocaine and 0.004% $CaCl_2$) followed by a buffered (0.12 M phosphate buffer, pH 7.4) 4% paraformaldehyde fixative solution. After fixation, decapitation, and dissection, the brain tissue was cryoprotected in 30% sucrose and sectioned in the transverse plane into 35 μm or 50 μm-thick slices on a freezing microtome. One cat was cut at 100 μm. Adjacent sections underwent avidin–biotin complex histochemistry for BDA (black reaction) followed by immunohistochemistry with antisera to rhodamine, biotinylated secondary antisera, and avidin–biotin histochemistry (red reaction). Every third or fourth section was used for Nissl counterstain.

Data analysis

Terminal fields in the IC were observed with a Leica DMRB microscope and digitized with Neurolucida software (Microbrightfield, Inc.; Colchester, VT, USA). In every third or fourth section, the labeled laminar axonal plexuses were plotted. Digitized terminal axonal plexus data were visualized in with the Neurolucida system, and the width of the plexus was measured at several sites in each section. Additional drawings were made with the aid of a drawing tube attached to a Leica DMRB microscope.

Digital images for quantitative analysis were acquired with a Zeiss Axiophot microscope and a Micromax cooled CCD camera (Princeton Instruments, Trenton, NJ, USA). Image processing methods are described elsewhere (Saint Marie and Oliver, 2004). Briefly, in each section through the IC, a rectangular area spanning a lamina was sampled with the long axis of the rectangle perpendicular to the lamina. Within each rectangular area, a Z-series of images separated by 2 μm was acquired and then collapsed to retain only in-focus objects. The composite image was filtered, converted to a binary image, and a series of erosions and dilations was used to separate boutons of different size classes from axons of different sizes. Boutons selected by this method were examined by the investigator, and objects determined not to be boutons were eliminated. Finally, the center of the lamina was determined by highest density of boutons, and a distance map was used to measure the distance of each bouton from the centerline of the lamina.

For illustrations, color digital images were taken with a Spot Insight color CCD camera (Diagnostic Instruments, Sterling Heights, MI, USA), and the contrast and brightness was adjusted with Adobe Photoshop.

RESULTS

Thick and thin axonal inputs to the IC from the cochlear nucleus in the rat

After injections in the DCN, separate thick and thin labeled axons can be seen entering the dorsal acoustic stria (DAS; Figs. 1 and 2A). It is unlikely that the thin axons in the DAS are collaterals of thicker fibers, since we did not see axonal bifurcations in the DAS. After such injections, thick and thin labeled axons can also be seen in the lateral lemniscus as they enter the IC. The presence of both thick and thin axons traveling from the DCN to the IC suggests that these two types of axons emerge from different types of neurons.

At the level of the IC, the axons from the cochlear nucleus terminate in a laminar pattern. The plot of the IC in Fig. 1 shows a typical projection from DCN in the rat. The labeled axons from small injections terminate in a '*narrow (axonal) lamina*' (Fig. 1, dark gray) that has two wings when cut in transverse sections. The prominent, medial wing (e.g. arrow, Fig. 1) of the lamina is located in the central nucleus of the IC and extends into the dorsal cortex (e.g. asterisk, Fig. 1), while the lateral wing of the lamina extends (e.g. arrowhead, Fig. 1) into layer 3 of the lateral cortex. Surrounding and parallel to the relatively dense, narrow lamina there is a broad zone composed of sparse, small fibers, hereinafter referred to as a '*wide (axonal) lamina*' (Fig. 1, light gray).