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DEVELOPMENT OF BANDED AFFERENT COMPARTMENTS IN THE INFERIOR COLLICULUS BEFORE ONSET OF HEARING IN FERRETS

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Abstract—Axonal projections from the lateral superior olivary nuclei (LSO), as well as from the dorsal cochlear nucleus (DCN) and dorsal nucleus of the lateral lemniscus (DNLL), converge in frequency-ordered layers in the central nucleus of the inferior colliculus (IC) where they distribute among different synaptic compartments. A carbocyanine dye, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), was used as a tracer to study the postnatal development of axonal projections in the ferret IC. The results indicated that projections from all three nuclei are present at birth, but are not segregated into bands. During the postnatal week between approximately postnatal days 4 and 12 (P4–P12), axons from LSO proliferate in IC, become more branched, and segregate into a series of bands composed of densely packed fibers and endings. LSO projections in these afferent bands course parallel to IC layers and are separated by intervening regions with few endings. A modest fit of a sine curve (R^2>0.15) to the pattern of spacing of LSO projections in IC indicated that regularly spaced bands are forming by P7. Similarly, banded patterns of DCN and DNLL projections to IC have developed by the end of the first postnatal week. Thus, well before hearing onset in ferret (P28–30), three different afferent projections have segregated into banded compartments along layers in the central nucleus of the ferret IC. Possible mechanisms in circuit development are discussed.

Key words: dorsal nucleus of the lateral lemniscus, lateral superior olivary nucleus, dorsal cochlear nucleus, fibrodendritic layers, postnatal, carbocyanine dye.

Fibrodendritic layers are characteristic of the architecture of input–output circuits in the central nucleus of the inferior colliculus (IC) (e.g. Rockel and Jones, 1973; Oliver and Morest, 1984). Multiple ascending inputs to these layers from frequency-matched regions of lower brainstem auditory nuclei establish a cochleotopic order (e.g. Merzenich and Reid, 1974; Fitzpatrick, 1975; Roth et al., 1978; Kelly et al., 1998). It is clear from the diverse response characteristics of cells receiving this layered input (Semple and Aitkin, 1979; Wenstrup et al., 1986; Schreiner and Langner, 1988; Brückner and Rübsamen, 1995) that different synaptic compartments exist within a layer and integrate specific sets of inputs (Oliver and Shneiderman, 1989). In particular, axonal projections from the lateral superior olivary nuclei (LSO), the dorsal cochlear nucleus (DCN), and the dorsal nucleus of the lateral lemniscus (DNLL) all have been described as ending in a pattern of complementary bands and patches along layers of the IC (Shneiderman and Henkel, 1987; Shneiderman et al., 1988; Oliver et al., 1997) providing opportunities for synaptic interaction.

Initially response patterns of IC cells suggest that some components of this complex circuitry are relatively mature at hearing onset in kittens while others continue to be refined after hearing onset (Aitkin and Moore, 1975; Moore and Irvine, 1980, 1981). However, relatively little detailed information is known about the spatiotemporal sequence for assembly and refinement of complex synaptic circuits in IC layers. Previously, it has been shown that LSO bands in IC already are present and well refined by birth in the kittens (Brunso-Bechtold and Henkel, 2005; Henkel et al., 2005). Axonal growth, proliferation, and branching also have been carefully described for development of DCN projections to the rat IC (Kandler and Friauf, 1993), but patterns of banded afferent compartments in IC layers were not described in that study. On the other hand, a similar sequence of developmental events has been shown for DNLL projections to IC in rat (Gabriele et al., 2000a) and in that circuit axons are present at birth, but do not segregate into distinct bands in IC layers until postnatal day 8 (P8), 4 days before hearing onset (Jewett and Romano, 1972).

In the present study, we describe the development of axonal projections from LSO to IC and compare the developing LSO projection pattern with that from another lower brainstem auditory nucleus, DCN, and a higher auditory nucleus, DNLL. The goal was to determine when afferent projections segregate into banded compartments in IC in the ferret, an altricial carnivore with a more protracted postnatal period before hearing onset at P28–P30 (Moore, 1982). A carbocyanine dye, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), was placed in the nuclei or in their output tracts in postnatal ferrets to label their projections to the IC. We examined labeled projections from these nuclei at semi-weekly or weekly time points between P0 (birth) and P28. The results show that axonal projections from LSO, DCN and DNLL are present in ferret IC at birth and that banded projections from all three nuclei are present in IC 2 weeks prior to hearing onset. Thus, the timing of segregation of afferent

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Abbreviations: DCN, dorsal cochlear nucleus; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DNLL, dorsal nucleus of the lateral lemniscus; IC, inferior colliculus; LSO, lateral superior olivary nucleus; P, postnatal day.

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bands from multiple sources is similar and precedes hearing onset.

Preliminary data on development of LSO projections to the ferret IC have been reported previously in abstract form (Keiger et al., 2005).

EXPERIMENTAL PROCEDURES

Axonal projections from brainstem auditory nuclei to the IC were studied in a collection of postnatal brains from 45 ferret kits labeled with a carbocyanine dye, DiI(Invitrogen–Molecular Probes, Eugene, OR, USA, #22885). DiI is lipophilic and diffuses retrogradely in membranes back to the somata of labeled axons as well as anterogradely through the axolemma to label their branches and endings. All procedures complied with the Guide for the Care and Use of Laboratory Animals. Animals were housed and cared for in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). Every effort was made to minimize the total number of animals used in this study as well as to alleviate any pain or suffering. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest University School of Medicine.

DiI labeling and histology

Ferret kits were culled from the litter for study at postnatal ages P0, P4, P7–8, P13–14, P21–22, and P26–30. The kits were given an overdose of ketamine and xylazine and perfused through the heart with 4% paraformaldehyde. Afterward, the brain was removed, postfixed overnight in 4% paraformaldehyde, and blocked coronally to include the region from IC to DCN. A glass pin coated with DiI was inserted in one of the three brainstem target structures or in the IC. For pin placement in LSO or the DNLL commissure (Gabriele et al., 2000a), the tissue was embedded in egg yolk-gelatin and vibratome sectioned (Technical Products International, Inc., St. Louis, MO, USA) from either the caudal or rostral face of the embedded block until the target for DiI placement was visualized. After the DiI-pin was secure, the blocks were rinsed and returned to 4% paraformaldehyde for the duration of the incubation. DiI-labeled blocks were incubated in the dark at 37 °C for 6 weeks to allow diffusion of the dye.

In some cases, DiI labeling of LSO projections to the contralateral IC was less extensive than labeling in the ipsilateral IC. Extending incubation times beyond 6 weeks appeared to have no consistent effect on the degree of labeling although this parameter can be important for visualizing projections (Lukas et al., 1998; Sparks et al., 2000). Perhaps myelination is more advanced for projections to the contralateral IC than for ipsilaterally projecting axons (e.g. sequential myelination of corpus callosum, Looney and Elberger, 1986; differential myelination of spinal tracts, Hamano et al., 1998) and, thus, interferes with DiI confusion or detection. The earlier birth dates of contralaterally projecting LSO cells in comparison with ipsilaterally projecting cells (Kudo et al., 1996) suggest that contralateral axon development could precede that for ipsilateral projections. Nevertheless, in those cases with well-labeled contralateral and ipsilateral LSO fibers the patterns of distribution were not different and conclusions are based on those cases.

After incubation, 75 μm thick coronal vibratome sections were cut through the brainstem. Sections were collected in phosphate buffer (0.1 M, pH 7.2), mounted onto glass slides, and coverslipped with Gel/Mount (Biomeda, Foster City, CA, USA, catalogue #M01).

Image acquisition and processing

Sections were viewed with an Olympus BX2 epifluorescent photomicroscope (Olympus, Melville, NY, USA) equipped with a Cy5 filter set (Chroma Technologies, Brattleboro, VT, USA) to visualize DiI-labeled cells and axons. Digital images (1600×1200 dpi and 0–255 gray levels) were captured from the microscope using an Optronics Magnafire camera (Optronics, Goleta, CA, USA). Images were collected in each case for the DiI pin site, labeled regions in brainstem auditory nuclei, and labeling in IC. Image processing software was used only to subtract background and to balance brightness and contrast. The final plates were composed and edited in Photoshop (Adobe Systems, San Jose, CA, USA).

To analyze the banded organization of projections in selected cases, a rectangular region of interest was selected to encompass the dense axonal labeling in the central nucleus of the IC with the major axis of the rectangle oriented orthogonal to axonal layers in IC. The average line plot profile of image density along scan lines across this rectangle (orthogonal to the labeled axons) was then determined with ImageJ software (NIH, Bethesda, MD, USA). Nonlinear regression (GraphPad Prism, San Diego, CA, USA) was used to fit the density profile from labeled axonal plexuses in IC to a sine curve. A sine curve was chosen to model regular spacing of dense afferent bands across a series of layers (Gabriele et al., 2000b; Henkel et al., 2003, 2005). At the outset, the center-to-center spacing (or period) in afferent banding patterns was measured directly from several cases. The median period subsequently was used in all cases to set the initial frequency value for curve fitting. Fit of the density data to a sine curve with an R^2 value greater than 0.33 was considered to be a good fit and strong evidence of segregation into regularly spaced banded compartments.

RESULTS

Retrograde labeling sources of IC afferent projections

DiI-pins were placed in IC (Fig. 1A) to label retrogradely axonal projections to IC and their cells of origin. The labeling pattern of one P7 case (Fig. 1) that was illustrative of both the trajectory and origin of afferent projections to the IC at this time point will be described. Retrogradely labeled cells were most prominent in the contralateral DNLL, the contralateral and ipsilateral LSO, and the contralateral DCN (Fig. 1B, C, and D). Although there were some labeled cells in other brainstem regions, (e.g. medial superior olivary nucleus, ventral nucleus of the lateral lemniscus, and ventral cochlear nucleus) these are not illustrated here.

Cells in the contralateral DNLL gave rise to a major projection to the IC and were heavily labeled (Fig. 1B). Axons decussating in the dorsal tegmental commissure (of Probst) were traced from the DiI-pin site to their cells of origin in the DNLL. Although a few labeled cells could be detected in the ipsilateral DNLL, for the most part they were lightly labeled (arrow, Fig. 1A) and obscured by labeling of the lateral lemniscal fibers.

In LSO, bilateral clusters of labeled cells were most dense medially, becoming sparser in the lateral portions of the nucleus (Fig. 1C). Similarly, DiIlabel was denser in the ventral part of the medial superior olivary nucleus. Labeled cells were present in the periolivary cell groups but will not be considered here. Retrogradely labeled fibers could be followed to the LSO from the decussation in the same region as the dorsal acoustic stria (arrow in Fig. 1C).

In the cochlear nuclear complex, labeled cells were located in the contralateral DCN (Fig. 1D) and the posteroven-