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DISTRIBUTION OF GLIAL CELLS IN THE AUDITORY BRAINSTEM: NORMAL DEVELOPMENT AND EFFECTS OF UNILATERAL LESION

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Abstract—Auditory brainstem networks facilitate sound source localization through binaural integration. A key component of this circuitry is the projection from the ventral cochlear nucleus (VCN) to the medial nucleus of the trapezoid body (MNTB), a relay nucleus that provides inhibition to the superior olivary complex. This strictly contralateral projection terminates in the large calyx of Held synapse. The formation of this pathway requires spatiotemporal coordination of cues that promote cell maturation, axon growth, and synaptogenesis. Here we have examined the emergence of distinct classes of glial cells, which are known to function in development and in response to injury. Immunofluorescence for several astrocyte markers revealed unique expression patterns. Aldehyde dehydrogenase 1 family member L1 (ALDH1L1) was expressed earliest in both nuclei, followed by S100ß, during the first postnatal week. Glial fibrillary acidic protein (GFAP) expression was seen in the second postnatal week. GFAP-positive cell bodies remained outside the boundaries of VCN and MNTB, with a limited number of labeled fibers penetrating into the margins of the nuclei. Oligodendrocyte transcription factor 2 (OLIG2) expression revealed the presence of oligodendrocytes in VCN and MNTB from birth until after hearing onset. In addition, ionized calcium binding adaptor molecule 1 (IBA1)-positive microglia were observed after the first postnatal week. Following hearing onset, all glial populations were found in MNTB. We then determined the distribution of glial cells following early (P2) unilateral cochlear removal, which results in formation of ectopic projections from the intact VCN to ipsilateral MNTB. We found that following perturbation, astrocytic markers showed expression near the ectopic ipsilateral calyx. Taken together, the developmental expression patterns are consistent with a role for glial cells in the maturation of the calyx of Held and suggest that these cells may have a similar role in maturation of lesion-induced connections. **© 2014 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: deafferentation, brainstem, auditory system, astrocyte, microglia, oligodendrocyte.

INTRODUCTION

Precise neural circuits in the auditory brainstem compute binaural timing and intensity disparities that are used to localize sound sources. In mammals, auditory information is carried by the VIIIth cranial nerve into the central nervous system (CNS), where branches of VIIIth nerve fibers terminate onto targets in the ventral cochlear nucleus (VCN). VCN globular bushy cells project to the contralateral medial nucleus of the trapezoid body (MNTB) where their large reticulated terminations, the calyces of Held, synapse onto principal neurons (Kuwabara and Zook, 1991; Kuwabara et al., 1991; Kandler and Friauf, 1993; Kil et al., 1995). MNTB neurons in turn provide glycinergic inhibition to the medial superior olive (MSO) and the lateral superior olive (LSO), which integrate excitation and inhibition to compute interaural time differences and interaural level differences, respectively.

This unique projection matures over a protracted period of development (Nakamura and Cramer, 2011). Axons reach contralateral MNTB and form immature connections by embryonic day (E) 17 (Borst and Soria van Hoeve, 2012). At postnatal day (P) 0, rudimentary calyces are seen with several inputs on each MNTB neuron. As the terminations expand, the number of VCN inputs is reduced until a single input encapsulates each MNTB neuron by P4 (Hoffpauir et al., 2006; Holcomb et al., 2013). Synapse formation and pruning have been shown to involve several forms of cell–cell communication. Notably, glial-secreted factors play a role in synaptic maturation (Mauch et al., 2001; Christopherson et al., 2005; Hughes et al., 2010; Kucukdereli et al., 2011; Allen et al., 2012; Korn et al., 2012). In addition, several types of glial cells have been shown to be important for synaptic refinement both in development and in response to injury (Chung and Barres, 2012; Karimi-Abdolrezaee and Billakanti, 2012; Schafer et al., 2012; Wake et al., 2013).

While the contribution of glial cells to the maturation of the central auditory circuitry is not known, recent studies have reported that astrocytes contact both the pre- and

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Abbreviations: aCSF, artificial cerebrospinal fluid; ALDH1L1, aldehyde dehydrogenase 1 family member L1; CR, cochlear removal; DAPI, 4′,6-diamidino-2-phenylindole; DCN, dorsal cochlear nucleus; GFAP, glial fibrillary acidic protein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IBA1, ionized calcium binding adaptor molecule 1; MNTB, medial nucleus of the trapezoid body; OLIG2, oligodendrocyte transcription factor 2; PBS, phosphate-buffered saline; PFA, paraformaldehyde; RDA, rhodamine dextran amine; SEM, standard error of the mean; VCN, ventral cochlear nucleus.

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postsynaptic membranes of the calyx of Held (Elezgarai et al., 2001). These astrocytes elicit slow inward currents in the postsynaptic MNTB neuron via gliotransmission in the mature animal (Reyes-Haro et al., 2010). The close apposition of astrocytes to the calyx suggests a potential role for astrocytes in the development and function of this pathway.

To explore the role(s) of glial cells in the maturation of auditory circuits, we characterized the spatiotemporal emergence of glial subtypes in the VCN and MNTB. We used several markers to identify multiple astrocyte-specific proteins, including the intermediate filament glial fibrillary acidic protein (GFAP), the calcium binding protein S100β, and aldehyde dehydrogenase 1 family member L1 (ALDH1L1) (Cahoy et al., 2008). Oligodendrocytes were identified by expression of oligodendrocyte transcription factor 2 (OLIG2). The emergence of microglia was assessed by expression of the ionized calcium binding adaptor molecule 1 (IBA1).

Additional clues to mechanisms of neural circuit formation may be obtained from experimentally induced reorganization of synapses. Following early postnatal unilateral cochlear removal, the cochlear nucleus on the deafferented side undergoes substantial cell death (Trune, 1982; Hashisaki and Rubel, 1989; Mostafapour et al., 2000). Axons from the intact VCN subsequently branch and contact the ipsilateral, denervated MNTB, in addition to their normal contralateral target (Moore and Kowalchuk, 1988; Kitzes et al., 1995; Hsieh and Cramer, 2006; Hsieh et al., 2007). Here we examined the expression of glial markers in denervated and intact MNTB after cochlear removal.

During normal development we found a diversity of patterns for development of glial cell types in VCN and MNTB from birth to the time of hearing onset. We found that expression of astrocyte and oligodendrocyte markers following cochlear removal was similar to the distribution of these glial markers during normal development. In addition, glial cells and their processes were seen in close proximity to the emerging ipsilateral calyx; as in normal development, these populations were primarily astrocytes. Together with our developmental expression data, we posit that glial cells may be important for the development and early plasticity of the mammalian auditory circuit, and that different glial cell types may serve distinct functions in this pathway.

EXPERIMENTAL PROCEDURES

Animals

Wild-type mice on CD-1 background were used for these studies. Expression studies included animals at several developmental ages, including postnatal day (P)0 (n = 6); P6 (n = 9); P14 (n = 10); and P23 (n = 7). Cochlear removal (CR) or sham operation was performed at P2 and animals survived until P4 (n = 9 for CR and n = 9 for sham) or P9 (17 CR and 11 sham). All procedures were approved by the University of California, Irvine Institutional Animal Care and Use Committee.

Immunohistochemistry

Pups were euthanized with isoflurane and perfused with 0.9% saline in phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Brainstems were fixed for 2 h in PFA and cryoprotected in 30% sucrose in PBS overnight at 4 °C. Tissue was sectioned coronally on a cryostat (Leica Microsystems, Buffalo Grove, IL, USA) at 18–20 μm thickness onto chrome-alum-coated slides. Sections were outlined in PAP pen (Binding Site, San Diego, CA, USA) to provide a hydrophobic layer and heated on a slide warmer at 37 °C for 30 min. Slides were rinsed in PBS and blocked with 4% bovine serum albumin (BSA) in 0.1% Triton-X100 in PBS for 1 h at room temperature. Slides were incubated in primary antibodies diluted in blocking solution overnight at room temperature in a humid chamber. The following day, slides were rinsed with PBS and incubated with goat anti-rabbit, goat anti-mouse, or donkey anti-chicken secondary antibody (1:300, Alexa Fluor, Invitrogen, Carlsbad, CA, USA) at room temperature for 1 h. Slides were then rinsed with PBS and cover slipped with Prolong Gold Anti-Fade mounting medium with 4′,6-diamidino-2-phenylindole (DAPI) to label cell nuclei (Invitrogen).

Antibodies

For immunofluorescence we used primary antibodies to identify five different glial markers for astrocytes, oligodendrocytes and microglia. For astrocytes, we used three antibodies: S100β, GFAP, and ALDH1L1. Rabbit S100β (1:500, Abcam, Cambridge, MA, USA) is a monoclonal antibody generated from a synthetic peptide corresponding to C-terminus human S100β. S100β is an 11-kDa calcium binding protein found in mature CNS astrocytes. Anti-GFAP chicken polyclonal antibody (1:1000, Abcam) was generated against the full length native bovine protein (50 kDa). GFAP antibody was purified from a Triton-X100 extract of myelin-associated material, and purified by centrifugation and ion exchange chromatography. The GFAP antibody stains for both reactive and resting state astrocytes. Anti-ALDH1L1 rabbit polyclonal antibody (1:500, Abcam), was generated from a synthetic peptide conjugated to KLH derived from a peptide sequence within amino acid residues 300–400 of mouse ALDH1L1. Anti-OLIG2 was used as an oligodendrocyte-specific marker (1:500, Millipore, Temecula, CA, USA). The polyclonal rabbit antibody was generated against the recombinant mouse OLIG2. Microglia-specific populations were stained using rabbit anti-IBA1 polyclonal antibody (1:1000, Wako). The antibody was generated from a synthetic peptide (PTGPPAKKAISELP) corresponding to the C-terminus fraction of the protein and purified by antigen affinity chromatography from rabbit antisera.

Cochlear removal

CR was performed at P2 using previously published methods (Hsieh and Cramer, 2006; Hsieh et al., 2007; Nakamura and Cramer, 2011). Animals were

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