Spiral Ganglion Neurons with Distinct Preferred Frequency Response
Employ Different Strategies to Innervate the Cochlear Nucleus
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Department of Biology, Loyola University Chicago (*: equal contribution)

Results

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Results

Sound information begins from hair cells (HCs) in the inner ear and travels to the brain through spiral ganglion neurons (SGNs) (Figure 1). The peripheral processes of SGN auditory nerve fibers (AFNs) extend toward the HCs and the central processes of ANFs bifurcate and project into the cochlear nucleus. The ascending branch goes into the anteroventral cochlear nucleus (AVCN) and the descending branch extends toward the dorsal cochlear nucleus (DCN) via the posteroventral cochlear nucleus (PVCN). When SGN innervate neurons in the DCN, the endings of SGN AFNs form a small button-like synapse. In contrast to the DCN, the auditory nerve fibers connect to the bushy cells neurons in the AVCN with an extraordinary large synaptic ending called the Endbulb of Held.

Figure 1 Schematic of auditory circuits in the cochlea and the CN.

In the cochlea and CN, SGN cell bodies and their auditory nerve fibers, respectively, are also arranged in a tonotopic gradient according to frequency responses and each of three subdivisions of the CN is also tonotopically organized (Figure 2). SGNs responding to high frequency sounds are located at the basal portion of the cochlea and project their fibers to dorsal regions of the CN subdivisions, while SGNs that convey information of low frequency sounds are located at the cochlear apex and send their fibers to ventral portions of the CN subdivisions, forming isofrequency bands where nearby neurons in the CN have similar frequency responses. How SGNs that respond to different sound frequencies innervate the CN to form a tonotopic map have not yet fully understood. As defects of the tonotopic map in central auditory circuits often result in central auditory processing disorders (CAPDs).

Understanding the mechanism of how functionally distinct SGNs innervate the CN to establish a tonotopic organization will help us uncover the causes and pathogenesis of some CAPDs. In this study, we use genetic approaches to label functionally distinct SGNs and their auditory nerve fibers at different stages. We found that SGNs with distinct preferred frequency responses employ different strategies to target and innervate CN neurons during tonotopic map formation.

Methods

SGNs originate from a neurogenic domain of the otic vesicle by transiently expressing the transcription factor Neurog1/Ngn1 (in a basal to apical progression along the length of the cochlea between E9.5 and E12.5 in mice (Koundakjian et al., 2007). Therefore, a small subset of SGNs can be genetically labeled using the Ngn1-CreERT2 reporter mouse line and a Cre-dependent reporter upon Cre induction by a single low dose tamoxifen administration. This allows us to reproducibly label SGNs and their ANFs that respond to different sound frequencies by providing tamoxifen at a specific time point between E9.5 (start of neurogenesis, label high-frequency-responsive neurons and their fibers) and E12.5 (end of neurogenesis, label low-frequency-responsive neurons and their fibers) (Figure 2 and 3). Two Cre-dependent reporter mouse lines were used. R26RAP Cre reporter expresses alkaline phosphatase after Cre-mediated recombination and allows us to examine overall innervation patterns of ANFs in the CN. Ai14 tdTomato Cre reporter expresses tdTomato red fluorescence proteins after Cre-mediated recombination and allows us to visualize individual ANFs and their synaptic endings.

Figure 2 The tonotopic maps of the cochlea and the CN.

Figure 3 Genetic labeling of high- or low-frequency-responsive SGNs and their fibers. By treating Ngn1-CreERT2; R26RAP mice with a single dose of tamoxifen on either E9.5 or E12.5, we can respectively label the high- (tamoxifen on E9.5) or low-frequency (tamoxifen on E12.5) SGNs and their afferent fibers. Labeled neurons and fibers were revealed by staining of alkaline phosphatase (AP) activity in the cochlea. AP staining of apical or basal turns of P0 cochleae with tamoxifen on E9.5 (A,A') or E12.5 (B,B'). 9B is the angular spread of AP staining in the cochlear turn. Green arrowhead indicates that the labeled central fibers from the apical SGNs can be seen in the basal turn.

Figure 4 Tonotopic innervation of high- or low-frequency fibers in the CN. (A-F) Innervation of high- (tamoxifen on E9.5) or low-frequency (tamoxifen on E12.5) fibers in the CN on E15.5 and P0 or in three subdivisions of the CN on P20. Red dotted lines outline the CN or CN subdivisions (DCN, PVCN, or AVCN). AP staining of CN sections revealed that afferent inputs from the cochlea were tonotopically distinct by E15.5 (A,D), and high- or low-frequency fibers targeted dorsal or ventral portion respectively in the CN subdivisions on P20 (C, F). ChP: choroid plexus.

Figure 5 High- and low-frequency fibers show differential innervation patterns in the CN during development. (A-H) Innervation of high- (tamoxifen on E9.5) and low-frequency (tamoxifen on E12.5) fibers in the AVCN on P0, P5, P10, and P20. Red dotted lines outline the AVCN. High-frequency fibers initially innervate a widespread area but later confine to a small region. In contrast, low-frequency fibers are more accurate in initial targeting and stay in a small area from P0 to P20. ChP: choroid plexus.

Figure 6 Quantification of normalized surface fraction of AVCN innervated by AP-labeled fibers (Sµ/Sµµµ). To normalize the amount of SGN labeling between samples, we measured the angular spread of AP staining from the cochlear turn (θ) to be normalized (e.g. Fig. 3A and B) and normalized this value to the average angular spread of staining of all cochlear turns (θavl). High-frequency fibers initially innervated ~68% AVCN surface area (Sµµµ) on P0 but gradually confined to ~16% Sµµµ on P20. In contrast, low-frequency fibers underwent minimal target sampling, and the percentage of Sµµµ targeted by low-frequency fibers refined only slightly from ~24% to ~13% from P0 to P20. n= 3 animals per group per age. ***: P<0.005, ns not statistically significant. Means ± SEMs are shown.

Conclusions

Functionally distinct SGN populations employ different strategies to target and innervate CN neurons during tonotopic map formation.

1. High-frequency fibers initially overshoot and sample a large area of different targets before refining their connections to correct targets, while low-frequency SGNs are more accurate in initial targeting and undergo minimal target sampling.

2. The central fibers of SGNs also show a gradient of development along the tonotopic axis, with outgrowth and branching of high-frequency fibers initiate two days earlier than the processes of low-frequency fibers.

3. The processes of synaptogenesis are similar between high- and low-frequency fibers but a higher proportion of low-frequency fibers form smaller endbulb endings with multiple terminal branching.

References
