Spiral Ganglion Neurons with Distinct Preferred Frequency Response
Employ Different Strategies to Innervate the Cochlear Nucleus
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Introduction
Sound input from the periphery travels to the brain via synaptic pathways that involve spiral ganglion neurons (SGNs) (Figure 1). The peripheral processes of SG neuronal nerve fibers (ANFs) 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 posterovertebral cochlear nucleus (PVCN). When SGN innervates neurons in the DCN, the endings of SGN ANFs form a small button-like synapse. In contrast to the DCN, the auditory nerve fibers connect to the bushy cell 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.

Methods
SGNs originate from a neurogenic domain of the otic vesicle by transiently expressing the transcription factor Neuronin1 (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 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. R26iAP Cre reporter expresses alkaline phosphatase after Cre-mediated recombination and allows us to examine overall innervation patterns of ANFs in the CN. AIt4 tdTomato Cre reporter expresses tdTomato red fluorescence proteins after Cre-mediated recombination and allows us to visualize individual ANFs and their synaptic endings.

Results

Figure 3 Genetic labeling of high- or low-frequency-responsive SGNs and their fibers. By treating Ngn1-CreERT2/R26iAP 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. (A,B) AP staining of apical or basal turns of P0 cochleae with tamoxifen on E9.5 (A,A') or E12.5 (B,B'). 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 target dorsal or ventral portion respectively in the CN subdivisions on P20 (C,C', F, and F'). ChP: choroid plexus.

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

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 CN subdivisions on E15.5 (A,D), and high- or low-frequency fibers target dorsal or ventral portion respectively in the CN subdivisions on P20 (C,C', F, and F'). ChP: choroid plexus.

Figure 7 Bifurcation and outgrowth of high- or low-frequency fibers in the CN. (A-H') Genetic labeling of high- or low-frequency responsive SGNs and their fibers in the CN. (A-H') Genetic labeling of high- or low-frequency responsive SGNs and their fibers in the CN.

Figure 8 Synaptogenesis of high- or low-frequency fibers in the CN subdivision. (A-H') Descending branches of high- and low-frequency fibers make standard boutons synaptic endings in the DCN.

Figure 9 Endbulb synaptic terminals of high- or low-frequency fibers in the AVCN. (A-H') Descending branches of high- and low-frequency fibers make standard boutons synaptic endings in the DCN.

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 a higher number of boutons synaptic endings (Figure 9).

References