Spiral Ganglion Neurons with Distinct Preferred Frequency Response
Employ Different Strategies to Innervate the Cochlear Nucleus

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Introduction

Sound information enters the cochlea through 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 hair cells 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 SGNs 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 in the AVCN with an extraordinary large synaptic ending called the Endbulb of Held.

Methods

SGNs originate from a neurogenic domain of the otic vesicle by transiently expressing the transcription factor Neurogenin1 (Ngn1) in a basal to apical progression along the length of the cochlea between E9.5 and E12.5 in mice (Koundakjian et al., 2002). Therefore, a small subset of SGNs can be genetically labeled using the Ngn1-CreER² 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. R26AP Cre reporter expresses alkaline phosphatase after Cre-mediated recombination and allows us to examine overall innervation patterns of ANFs in the CN. Ai14 tdTomato mice were used. R26AP Cre reporter expresses alkaline phosphatase 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-CreER²/R26AP 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 cochlea with tamoxifen on E9.5 (A,A') or E12.5 (B,B'). 9 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 target dorsal or ventral portion respectively in the CN subdivisions on P20 (C, F; F'). Chp²: choroïdal 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. Chp²: choroïdal plexus.

Figure 6 Quantification of normalized surface fraction of AVCN innervated by AP-labeled fibers (Ssurf/A100). 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 (θavg). High-frequency fibers initially innervated ~68% AVCN surface area (Ssurf/A100) on P0 but gradually confined to ~16% Ssurf/A100 by P20. In contrast, low-frequency fibers underwent minimal target sampling, and the percentage of Ssurf/A100 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 processes of SGNs also show a gradient of development along the tonotopic axis, with outgrowth and branching of high-frequency fibers initiating 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