Investigate the role of ephrin-A3 in Tonotopic Map Formation of the Mouse Cochlear Nucleus

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Introduction

Sound information is transmitted from cochlea, a structure within the inner ear, to the cochlear nucleus (CN) via spiral ganglion neurons (SGN). Both structures feature a tonotopic gradient as the neurons therein are organized by frequency response from high to low frequencies. SGN innervate the CN in a tonotopic fashion, preserving the tonotopic gradient as sound information flows from the inner ear to the brain (Fig. 1) (Yu, 2014). The purpose of this study is to determine what molecules regulate SGN axon guidance and thereby create a tonotopic gradient of information processing within the auditory system.

Methods

The first step determining if Ephs and ephrins are involved in the tonotopic map formation of the cochlear nucleus, is detecting if these proteins are present during neural development. To identify which Ephs and ephrins are present during development, RNAscope technology, a modification of in situ hybridization, was conducted on E15.5 and E17.5 embryos. To obtain cochlear nucleus samples, the head of the embryo was fixed; subsequently, 20 μm coronal sections of whole heads are prepared. mRNA signals were detected using RNAscope Detection Reagents. Finally, the chromogenic signals of mRNA were visualized under a microscope.

Once the Ephs present within the CN during tonotopic map formation were identified, stripe assays were conducted to determine the effect of the guidance cue on the SGN. A silicone matrix was used to apply a solution of the guidance cue to a cover glass in a striped pattern. The stripes containing the guidance cue were fluorescently labeled. Between these stripes, a control stripe was created with solution lacking the Eph molecule. Expiants of the CN were placed on the coverglass and subsequent outgrowth was measured.

Ephrin-A3 mutant mice were also imported and tested for hearing functions using recordings of auditory brainstem responses (ABRs). ABRs are electrophysiological responses of neurons in the cochlea and auditory brainstem after providing sound stimuli to mice. It provides an objective quantification of hearing and can detect even subtle changes of sound detection in the cochlea and sound processing in the brainstem.

Results

Ephrin-A2, Ephrin-A3, and Ephrin-A5 are present in the CN (Fig. 3). The RNAscope data indicates that ephrin A1 and A4 are not present in the developing CN and ephrins A2 and A5 present at low levels. Expression of ephrin-A3 is particularly interesting in that it is expressed at high levels with a regional difference within the cochlear nucleus. This graded expression of the contact-dependent guidance cue allows for the possibility that ephrin A3 is involved in tonotopic map creation. Thus, stripe assays were conducted using this transmembrane guidance cue. In these assays, the developing cochlea was dissected and placed on the coverglass containing the stripes.

Using the explant culture system, we found that a subset of auditory nerve fibers were repelled by stripes containing ephrin-A3-Fc (40 μg/ml, shown in magenta stripes in Fig. 4B, C). These observations lead us to suggest that ephrin-A3 forward signaling plays a role in controlling the growth response of auditory nerve fibers in tonotopic map formation in the CN and the populations of auditory nerve fibers are differentially sensitive to ephrin molecules.

Future Directions

We will assess sound discrimination ability of ephrin-A3 mutant mice using an acoustic startle response-based assay.

We will also evaluate tonotopic map precision of the auditory brainstem in ephrin-A3 mutants by c-fos induction after auditory stimulation.

Ephrins ligands interact with Eph receptors. Currently, we are studying Eph receptors and ephrin ligands. To determine if Eph receptors are present on SGN, a RNAscope assay will be conducted using a whole mount cochlea.

References

