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.

Results

Ephs and ephrins are a family of signaling proteins that are known to be involved in contact-dependent axon guidance. Previous studies have shown the involvement of Eph receptors and ephrin ligands in the development of topographic gradients within other sensory systems. This study seeks to determine the role of ephrin-A3 in tonotopic map formation.

Fig. 3. Expression of ephrin-A's in the CN from E15.5 and E17.5 mouse embryos. (A-F) The expression levels of ephrin-A2, -A3, and -A5 in the CN were detected by RNAscope® in situ hybridization using a HRP-based chromogen (blue) or an AP-based chromogen (red). Cochlear nuclei are outlined with red dashed lines. For E15.5, the CN anlage is shown, and for E17.5, the VCN anlage is shown. 8n: eighth nerve root. LR4V: lateral recess of 4th ventricle.

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 cues 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.

Fig. 4. Ephrin-A3 stripe assays. In control cultures both stripes contained pre-clustered unconjugated human-IgG Fc but the first strip was pre-clustered with an Alexa-488-goat anti-human IgG-Fc (shown in pseudo-colored magenta). In ephrin-A3 cultures, the first stripe contained ephrin-A3-Fc pre-clustered with an Alexa-488-goat anti-human IgG-Fc (shown in pseudo-colored magenta) and the second stripe was the same as in the control cultures. A subset of SGN fibers are repelled by ephrin-A3-Fc stripes. (D) High magnification of the boxed region in C shows the hair cells labeled by Myosin-6 staining (green) and the SGN peripheral fibers labeled by neurofilament/GAP43 (red). Arrowheads (in C) indicate hair cells in the sensory epithelium labeled by Myosin-6 staining (shown in green). Neurites were labeled in red by neurofilament/GAP43 staining.

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.

Ephrin ligands interact with Eph receptors. Currently, we are studying the ephrins of interest within the CN, yet to determine the molecular mechanism of tonotopic map formation, we must also examine the presence and the significance of Eph receptors on the SGN. We posit that the Eph receptor expression varies with the frequency response of the neuron, and thus that Eph-ephrin signaling can mediate tonotopic map formation. To determine if Eph receptors are present on SGN, a RNAscope assay will be conducted using a whole mount cochlea.

References

