Ephrin-A3 is Required for Tonotopic Map Precision and Auditory Functions in the Mouse Auditory Brainstem

Ephrin-A3 is required for tonotopic map precision and auditory functions in the mouse auditory brainstem. Briefly, mice were kept in silence in an anechoic chamber (ENV-022SD, Med Assocaites) for an hour, followed by calibration with the PCB 378C01 ¼ inchfree-field microphone. After tone exposure, animals were transcardially perfused. 30 μM AChE sections were collected and c-fos was detected by in situ hybridization. The cochlear nucleus (CN) is a ventral to dorsal gradient. Neurogenesis of spiral ganglion cells in the anterior Lentiare and Swayn cochlea. ANFs of the C1ANFs from basal and middle regions of the cochlea at 16 kHz were found to be significantly more sensitive to frequency than the average of the frequency sensitivities of the ANFs from the small percentage of ANFs from the ventral part of the CN, and all regions of the CN are sensitive to auditory stimuli. The cochlear nucleus (CN) is a ventral to dorsal gradient.

**Results**

**Figure 3** Broader tonotopic bands of c-fos activation in the AVCN upon pure tone stimulation in ephrin-A3−/− mutant. ANFs in the ephrin-A3−/− AVCN detected by RNAscope® in situ hybridization. A) Representative ABR recordings from a 6-week-old littermate control (blue traces) and ephrin-A3−/− mutant (magenta traces) exposed to 8 kHz, 11.3 kHz, 16 kHz, or 22.6 kHz pure tone stimuli at intensity of 90 dB SPL. No significant difference of Wave I latencies was observed between controls and ephrin-A3−/− mutants (magenta) in response to 8 kHz, 11.3 kHz, 16 kHz, or 22.6 kHz pure tone stimuli at intensity of 90 dB SPL. (B) Average Wave I and Wave II thresholds between controls and ephrin-A3−/− mutants (magenta) exposed to 8 kHz, 11.3 kHz, 16 kHz, or 22.6 kHz pure tone stimuli at intensity of 90 dB SPL. No significant difference of Wave I latencies was observed between controls and ephrin-A3−/− mutants (magenta). Representative ABR recordings from a 6-week-old littermate control (blue traces) and a 6-week-old littermate control (red traces) exposed to 8 kHz, 11.3 kHz, 16 kHz, or 22.6 kHz pure tone stimuli at intensity of 90 dB SPL. No significant difference of Wave I latencies was observed between controls and ephrin-A3−/− mutants (magenta) in response to 8 kHz, 11.3 kHz, 16 kHz, or 22.6 kHz pure tone stimuli at intensity of 90 dB SPL.

**Figure 4** Ephrin-A3−/− mutants show a delayed wave I in auditory brainstem response (ABRs).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Wave I Latency (μs)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>4.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Mutants</td>
<td>5.2 ± 0.3</td>
<td>*P &lt; 0.05</td>
</tr>
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**Figure 5** Broader tonotopic bands of c-fos activation in the AVCN upon pure tone stimulation in ephrin-A3−/− mutant. ANFs in the ephrin-A3−/− AVCN detected by RNAscope® in situ hybridization. A) Representative ABR recordings from a 6-week-old littermate control (blue traces) and ephrin-A3−/− mutant (magenta traces) exposed to 8 kHz, 11.3 kHz, 16 kHz, or 22.6 kHz pure tone stimuli at intensity of 90 dB SPL. No significant difference of Wave I latencies was observed between controls and ephrin-A3−/− mutants (magenta) in response to 8 kHz, 11.3 kHz, 16 kHz, or 22.6 kHz pure tone stimuli at intensity of 90 dB SPL. (B) Average Wave I and Wave II thresholds between controls and ephrin-A3−/− mutants (magenta) exposed to 8 kHz, 11.3 kHz, 16 kHz, or 22.6 kHz pure tone stimuli at intensity of 90 dB SPL. No significant difference of Wave I latencies was observed between controls and ephrin-A3−/− mutants (magenta). Representative ABR recordings from a 6-week-old littermate control (blue traces) and a 6-week-old littermate control (red traces) exposed to 8 kHz, 11.3 kHz, 16 kHz, or 22.6 kHz pure tone stimuli at intensity of 90 dB SPL. No significant difference of Wave I latencies was observed between controls and ephrin-A3−/− mutants (magenta) in response to 8 kHz, 11.3 kHz, 16 kHz, or 22.6 kHz pure tone stimuli at intensity of 90 dB SPL.

**Figure 6** Ephrin-A3−/− mutants show a delayed wave I in auditory brainstem response (ABRs).

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**Figure 7** Ephrin-A3−/− mutants show impaired prepulse inhibition (PPI) of avoidance behavior responses in 30° and 180° phase shifts of changes in sound frequencies.

<table>
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<tr>
<th>Condition</th>
<th>PPI Score</th>
<th>Significance</th>
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<tr>
<td>Controls</td>
<td>0.8 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Mutants</td>
<td>0.5 ± 0.2</td>
<td>*P &lt; 0.05</td>
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**Figure 8** Potential Ephrin-A3 signaling pathway in developing spiral ganglion cells (SGCs).

**Figure 9** Proposed model for ephrin-A3 role in tonotopic map formation and sound discrimination.

**References**


2. Clause A, Lauer AM, Kandler K. Mice Lacking the Alpha9 Nicotinic Acetylcholine Receptor Subunit Have Impaired concerts and impeded ability to detect small frequency bands. Conclusions

- Ephrin-A3 is expressed in the cochlear nucleus (CN) and is critical for auditory development.
- ANFs targeted to the ventral CN are more sensitive to frequency change than ANFs targeted to the dorsal CN.
- Ephrin-A3−/− mice show impaired frequency discrimination, indicating that they have an increased threshold for detecting small frequency changes.
- Ephrin-A3−/− mice also exhibit broader tonotopic bands of c-fos activation in the AVCN.

**Implications**

- Ephrin-A3 signaling is crucial for the development of auditory frequency selectivity.
- The role of Ephrin-A3 in auditory function highlights the importance of cell-cell interactions in the formation of tonotopic maps.

**Further Studies**

- Further investigation into the mechanisms underlying Ephrin-A3's role in frequency discrimination.
- Exploring the role of Ephrin-A3 in other auditory functions, such as sound localization.

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