The Auditory Brainstem Response in Hatchling Chicken

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Abstract

- The auditory brainstem response (ABR) is an invaluable assay in clinical audiology and animal/human research.
- The embryonic chicken is an extensively studied model system for auditory development, function, and brainstem microcircuitry.
- Despite modern ABR studies in altricial birds like the finch, budgerigar, and owl, recent ABR studies for precocious birds like the chicken are minimal.
- Recordings from 43 wildtype hatchling chickens (post-hatch age P1-P2) presented with 3 positive going peaks within 6 ms of a suprathreshold click stimulus. Peak-to-trough amplitudes ranged from 2-11 µV at high intensity levels and ABRs exhibited appropriate latency-intensity functions.
- Hatchlings had an average click threshold of 15 dBSPL and a U-shaped ABR gram for tone bursts. Best sensitivity was found between 750-2000 Hz.
- This study allows for comparison with *in-vitro and in-vivo* data across avian species, and the functional consequence following genetic manipulations.

Background

The auditory brainstem of birds is well correlated to that of mammals. Both species have an auditory nerve that provides excitatory, glutamatergic input to distinct cochlear nucleus structures. The avian cochlear nucleus magnocellularis (NM) is considered analogous to the mammalian anterior ventral cochlear nucleus (AVCN). NM sends an excitatory projection to nucleus laminaris (NL), which is analogous to the mammalian medial superior olive (MSO). This auditory microcircuitry is critical for sound localization and early auditory temporal processing. The composition of the ABR up to auditory midbrain is comparable among species and classes. The ABR in hatchling chickens is not only an objective measure of auditory neural synchrony and hearing sensitivity, but an invaluable *in-vivo* methodology to compare to *in-vitro* molecular and developmental research.

Method

- Animals were anesthetized with 50 mg/kg ketamine and xylazine. Once fully unconscious, feathers on the head were removed with a depilatory cream.
- Stainless steel silver chloride needle electrodes were placed in the following montage: A positive (inverting) electrode above the brainstem, a reference (non-inverting) electrode behind the right ear, and a ground electrode in the neck.
- Temperature was monitored and kept at 39 °C. Electrode impedances were below 5.0 kΩ and interelectrode impedances below 3.0 kΩ.
- Stimuli were generated and recorded using Intelligent Hearing Systems (IHS) Universal Smart Box and the ER3 insert earphone.
- Click stimuli had a 100 µs duration and ABRs were recorded across a 12 ms recording window. Tone burst stimuli had a 5 ms duration and 24 ms recording window. Thresholds were identified subjectively by agreement among two experimenters and a clinically trained audiologist.

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Click ABR Characterization

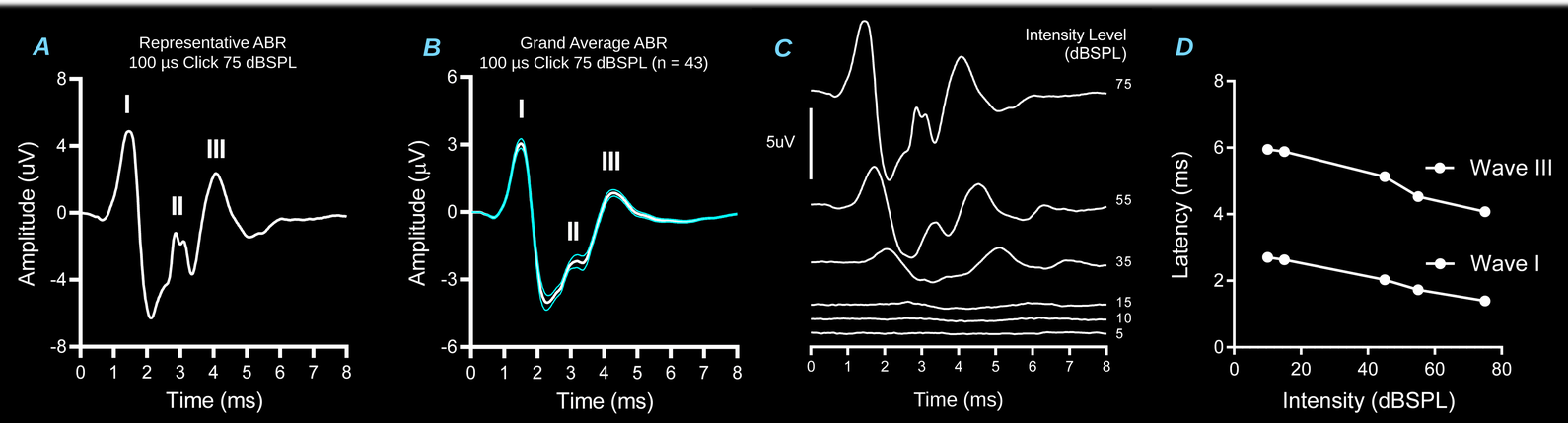

Figure 1. Characterization of Click ABR at a 75 dBSPL 100 µs Click. (A) Representative click ABR at 75 dBSPL. Roman numerals denote waves I, II, and III. (B) Grand averaged ABR (white trace) to a 75 dBSPL click stimulus across 43 animals. Cyan indicates the standard error of the mean (SEM). (C) Representative click ABR shown at decreasing stimulus intensity levels (75 to 5 dBSPL). (D) Latency intensity function for the two most prominent peaks (wave I and wave III) in Figure 1C.

Tone Burst ABR Characterization

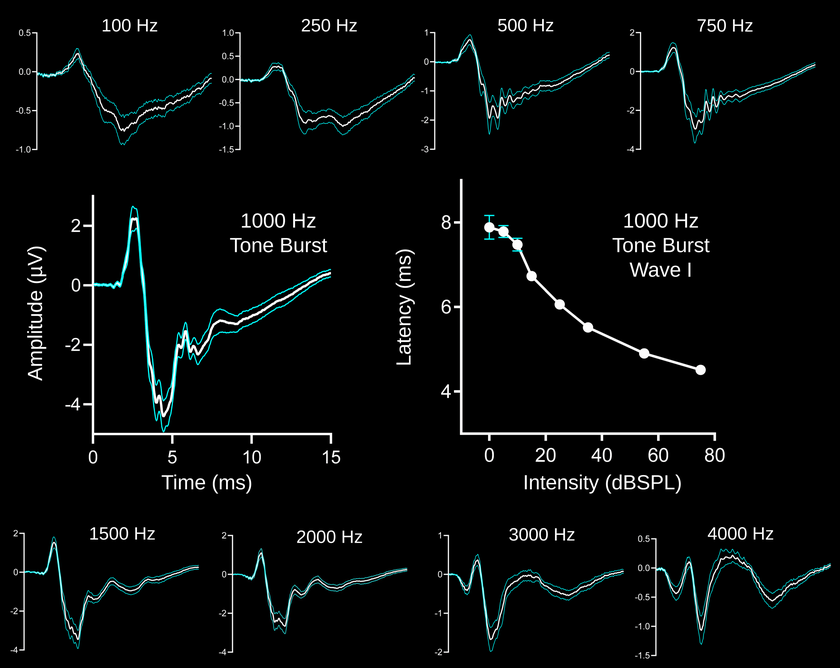

Figure 2: Characterization of Tone Burst ABRs at a Suprathreshold Intensity. Averaged ABRs for 100-4000 Hz tone bursts across 8 animals (white traces). Y-axis amplitude scales are different, but the x-axis time scale is 15 ms. Responses between 750-2000 Hz have the highest amplitude and mimic the waveform of the click ABR. Cyan indicates standard error of the mean (SEM).

ABR Thresholds

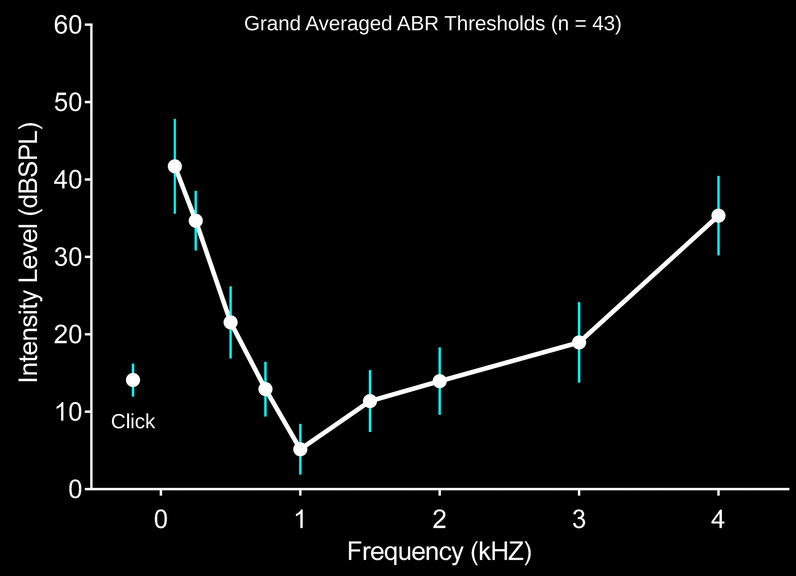

Figure 3. ABR Threshold to Click and Tone Burst Stimuli. Threshold of the ABR for P1-P2 hatchling chickens. Average click threshold shown as individual white dot. Cyan error bars denote 95% confidence intervals (CI).

Conclusions

1. The ABR methods outlined here permit accurate and reproducible recording of *in-vivo* auditory function in hatchling chicken.
2. These results are comparable to other avian ABRs, including altricial species like owls and songbirds.
3. The functional consequence of genetic embryonic manipulation via *in-ovo* electroporation can be assayed with normative ABR data.
4. Avian, mammalian, and human models of hearing loss, aging, or other manipulations can be applied to the hatchling chicken.