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Murine auditory brainstem evoked response: Putative two-channel differentiation of peripheral and central neural pathways

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Abstract

Standard noninvasive recordings of the auditory brainstem evoked response (ABR) from a single pair of obliquely oriented electrodes (typically midline vertex referenced to mastoid) confound inherently distinct signals propagating over peripheral and central neural pathways differing in location and spatial orientation. We describe here a technique for recording short-latency auditory evoked potentials that putatively differentiates peripheral and central neural activity in the mouse and rat. The technique involves recording from two orthogonally oriented electrode pairs using fast sample rates (100 k/s) to accurately measure differences in neural timing and waveform morphology. Electrodes oriented in a transverse plane (mastoid-to-mastoid) register an initial positive-going ABR peak ($P1_T$) earlier than a series of peaks recorded from electrodes oriented along the midline (anterior and posterior to the inter-aural line). The absolute $P1_T$ latency is consistent with an origin in the primary auditory nerve, while the delayed midline latencies implicate activity farther along central neural pathways. Differences between these latencies (midline minus transverse) provide new and precise measures of central conduction time (CCT), which in one case is as brief as 0.10 ms. Results in wild type (WT) and knockout (KO) mice, as well as rats, show significant differences in absolute latencies as well as CCT.

Keywords: Auditory; Brainstem; Short-latency; Evoked response; Orthogonal dipoles; Central conduction time; Knockout mouse models

1. Introduction

The sequence of waves in the noninvasive auditory brainstem evoked response (ABR), whether recorded in murine or human subjects, is known to reflect the synchronous short-latency synaptic activity of successive nuclei along the afferent auditory pathway. In the mouse the sequence of five positive ABR waves (P1–P5) has been localized to: cochlea and/or compound action potential of eighth nerve, cochlear nucleus, contralateral superior olivary complex, lateral lemniscus, and contralateral lateral inferior colliculus, respectively (Henry, 1979; Parham et al., 2001). However, as signals propagate farther along the sensory pathway the anatomical localization of a particular component becomes increasingly problematic due to such factors as varied central conduction velocities among different classes of auditory neurons, recurrent innervation, refractoriness, fast pathways that bypass some nuclei, etc. (Eggermont, 2001), as well as increasing variability of single unit firing latencies (Huang and Buchwald, 1977).

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A major consideration of the murine far-field ABR is the nonlineal location and orientation of auditory fibers as they course throughout the brainstem and midbrain. Thus, three-dimensional stereotaxic coordinates of inputs to the major brainstem auditory nuclei reveal a complicated, even circuitous, pathway (Paxinos and Franklin, 2001; Pellegrino et al., 1979). Depending upon the spatial geometry of the recording electrode dipole in relation to each stage of the pathway, individual ABR waves may be significantly altered. This suggests that standard single-channel ABR recordings may confound inherently distinct signals propagating over peripheral and central auditory pathways that differ in location and orientation.

We have previously shown in humans that horizontally oriented electrodes (e.g., mastoid-to-mastoid or meatus-to-meatus) record responses with a putative origin in the auditory nerve, while vertically oriented midline electrodes (vertex-to-inion or vertex-to-linked mastoids) register activity in rostral brainstem (Galbraith, 1994; Galbraith et al., 2000). Evidence supporting the distinction between these recording dipoles consists of shorter latencies and higher frequency-following in the horizontal recording channel, which is characteristic of signals generated in more distal segments of the auditory pathway (Trussell, 1999).

Using standard single-channel techniques we have previously reported reliable ABR differences as a function of pathology and age in NBC3 knockout (KO) mice (Bok et al., 2003). In the present study, we report a new approach recording murine ABRs from two orthogonally oriented electrode pairs using fast sample rates to accurately measure differences in neural timing as well as waveform morphology. We studied normal mice and rats, and two KO mouse models in which central neurogenesis and myelin formation is known to be affected, and where immunoreactivity has been observed in the cochlear nerve trunk and spiral ganglion (Kitanishi et al., 1998). Thus a priori we expect our assessments to show predictable differences in neural function (e.g., longer ABR latencies when myelin formation is disturbed). However, no attempt is made here to systematically map out such group or developmental differences. Rather, these animal models are used only to show that our two-channel recording method yields useful and novel data capable of differentiating pathological and developmental patterns of auditory function in future experimental studies. This new methodology will be beneficial in non-invasively monitoring auditory brainstem responses in murine models of human hearing disorders and central neural pathology.

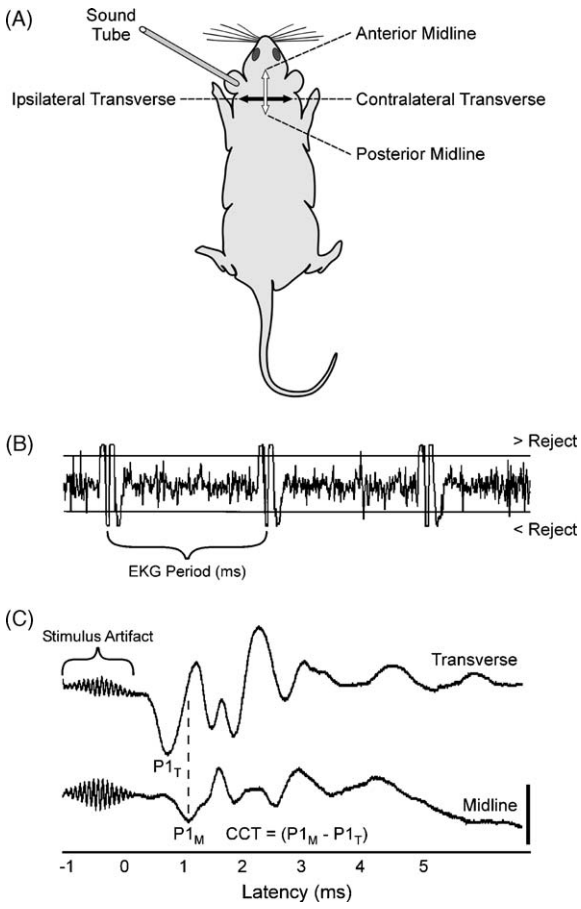

Fig. 1. (A) Illustration of orthogonally oriented bipolar needle electrodes and sound delivery tube. Transverse electrodes record from the base of each ear (mastoid) ipsilateral and contralateral to left ear receiving auditory input; midline electrodes are positioned anterior and posterior to the interaural line. (B) Transverse EEG sample showing electrocardiogram (EKG) artifact (sampled at 1 kHz). During actual data collection all epochs containing EEG values falling beyond the empirically adjusted threshold levels (horizontal lines) are rejected and the stimulus repeated. This figure also illustrates the computation of EKG period (in ms) from which heart rate (beats/min) is derived as the reciprocal of period. (C) Sample of wild type mouse auditory brainstem responses (ABRs) recorded from transverse and midline bipolar electrode pairs. Due to sound tube length the acoustic stimulus arrives at the ear canal after a 1 ms delay, thus 0 ms of the ABR begins exactly at termination of the stimulus electrical artifact. This figure also illustrates the consistent finding that the first positive-going deflection occurring in the transverse recording ($P1_T$) always precedes that in the midline recording ($P1_M$); and the latency difference between these components ($P1_M$–$P1_T$) is a measure of central conduction time (CCT). Positive voltage is graphed downward; vertical calibration is 2μV.

2. Materials and methods

2.1. Animal preparation

Wild type and KO mice ranging in age from 16–48 days, and rats ranging from 27 to 52 days, were anesthetized either with: (a) Avertin (2,2,2-tribromoethanol, Aldrich T4,840.2), i.p. 0.5 ml/25 gm; (b) a mixture of 20 mg ketamine and 2 mg xylazine in 0.9% saline (average 0.03 ml plus 0.01 ml booster as needed); or (c) Isoflurane gas (3% delivered with oxygen at 0.5 l/min). Grass Medical Instrument (Quincy, MA) needle electrodes were then positioned at the mastoid behind and at the base of each pinnae ("transverse" channel), and on the scalp midline symmetrically located anterior and posterior to the inter-aural line ("midline" channel) at a distance approximately equivalent to that of the transverse channel (Fig. 1A). The anesthetic and experimental protocol met all university requirements regarding the care and use of small animal subjects and was approved by the UCLA Chancellor's Animal Research Committee.