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The contribution of inferior colliculus activity to the auditory brainstem response (ABR) in mice

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ABSTRACT

In mice, the auditory brainstem response (ABR) is frequently used to assess hearing status in transgenic hearing models. The diagnostic value of the ABR depends on knowledge about the anatomical sources of its characteristic waves. Here, we studied the contribution of the inferior colliculus (IC) to the click-evoked scalp ABR in mice. We demonstrate a non-invasive correlate of the IC response that can be measured in the scalp ABR as a slow positive wave P_0 with peak latency 7–8 ms when recorded with adequate band-pass filtering. Wave P_0 showed close correspondence in latency, magnitude and shape with the sustained part of evoked spiking activity and local field potentials (LFP) in the central nucleus of the IC. In addition, the onset peaks of the IC response were related temporally to ABR wave V and to some extent to wave IV. This relation was further supported by depth-dependent modulation of the shape of ABR wave IV and V within the IC suggesting generation within or in close vicinity to the IC. In conclusion, the slow ABR wave P_0 in the scalp ABR may represent a complementary non-invasive marker for IC activity in the mouse. Further, the latency of synchronized click-evoked activity in the IC supports the view that IC contributes to ABR wave V, and possibly also to ABR wave IV.

1. Introduction

Brainstem evoked response audiometry is a valuable non-invasive diagnostic tool of hearing status in humans and animals (Hecox and Galambos, 1974). The auditory brainstem response (ABR) reflects neuronal activity in the auditory pathway, which can be measured non-invasively from the scalp of the subject. The ABR consists of a series of short waves, where the individual waves reflect changes in the electrical fields, which are produced by the auditory brainstem structures of the auditory pathway (Biacabe et al., 2001; Henry and Haythorn, 1978; Melcher et al., 1996a; Picton et al., 1974).

The value of the ABR as a diagnostic tool improves with understanding of the origin of the individual ABR waves. Knowledge about the sources of ABR waves allows to make a link between the form and latency of the ABR to pathological changes in individual auditory brainstem structures. Although in mice, the ABR is frequently used to measure hearing status, interestingly, few studies have focused on the origin of the specific ABR waves in the mouse, with to our knowledge only one notable exception (Henry, 1979). Henry studied the sources of ABR waves in the mouse by a combination of lesions and recordings of local field potentials (LFP) in the brainstem. However, the study of Henry did not address the relation of ABR waves to local spiking activity, and further was not fully conclusive on the role of the IC in relation to the ABR waves. Because auditory phenotyping to identify mouse hearing models by means of the ABR has become a frequently used method (Hardisty-Hughes et al., 2010; Zheng et al., 1999), we here attempted to provide additional information on the contribution of the inferior colliculus to the mouse ABR.

We studied the relationship between click-evoked activity in the inferior colliculus (IC) and the ABR in mice, by comparing click-evoked local field potentials (LFP) and multiunit activity (MUA) in the IC to the click-evoked scalp ABR response. The IC is an obligatory relay for nearly all auditory input to the medial geniculate body and to the cortex and receives input from the cochlear nuclei, the olivary complex and the lateral lemniscus. Information on its signature in the ABR can help the interpretation of screening for mouse hearing models with respect to functional auditory impairments of the IC. For this study, we selected two commonly used strains, C57Bl/6 and CBA mice.

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We identified a marker for IC activity in form of a positive slow wave P_0 in the scalp ABR, which provides complementary information during non-invasive assessment of hearing in the auditory pathway of mice with the ABR. The study shows that IC spiking activity can be visualized in the scalp ABR as a slow wave P_0, when recorded with adequate wide band-pass filtering. We further found that wave IV and V are temporally related to onset spiking activity in the IC response.

2. Methods

2.1. Mice

We studied 12 female C57Bl/6 mice (Charles River, France) between age two and six months and 9 female CBA/J (Charles River, France) mice between age two and twelve months. CBA/J mice had click thresholds with a mean of 22 dB peSPL (3 SD (SD = standard deviation) (n = 9), which were slightly lower than for C57Bl/6 mice with 31 dB peSPL (4 SD) (n = 12). In the following, stimulus levels are provided relative to hearing level defined as the ABR click-threshold. All experiments were conducted in accordance with ethical standards for the care and use of animals in research and the German law for the protection of animals. All experiments were approved by the ethics committee of the state of Lower Saxony.

2.2. Anesthesia and surgical procedure

Animals were anesthetized by an intraperitoneal injection of Ketamine/Xylazine (100 mg/kg, Ketamin Gräub, Albrecht GmbH, Germany; and 4 mg/kg Xylazin 2% Albrecht GmbH, Germany). Animals were then placed on a temperature probe controlled heating pad (TC-1000 Temperature Controller, CWE Inc., USA). First, we determined scalp auditory brainstem response (ABR) thresholds with teflon coated subdermal monopolar needle electrodes (0.35 mm × 15 mm, GVB Gelimed, Germany). For details about recording position see below. Animals were then tracheotomized and a tracheal tube was inserted into the trachea. They were then mechanically ventilated (SAR-1000 Ventilator, CWE Inc., USA). General anesthesia was maintained with isoflurane (Isofluran Baxter, Baxter Deutschland GmbH, Germany) between 0.8 and 1.2 vol % in a mixture of O_2/N_20 (1:2). Animals were subsequently fixed in a head holder (Narishige, Japan) in combination with a custom made stereotactic frame. Then a small opening was drilled above the vertex, in order to insert a small silver-ball electrode, which served as a reference for the recordings in the IC. Last, an opening was drilled above the right IC, and the IC was covered with silicon oil. In the mouse, the IC is not covered by the occipital lobe, thus no aspiration of the visual cortex or manipulation of other brain structures is necessary to access the IC. The preparatory procedure from initial anesthesia until opening of the skull and the insertion of the electrodes lasted approximately 90 min.

2.3. Electrophysiological recordings

Auditory brainstem responses (ABRs) at the beginning of the experiment were recorded with teflon coated subdermal monoploar needle electrodes (0.35 mm × 15 mm, GVB Gelimed, Germany) positioned at the vertex against a reference behind the right ear and a ground in the neck or the other ear (Willott, 2006). ABR signals were amplified 10,000 times with a F1 amplifier (Otoconsult Co, Frankfur, Germany) and wide-band filtered 1–9000 Hz and subsequently recorded with a Neuralynx Digital Lynx SX at 32 kHz sampling rate.

Activity in the inferior colliculus was recorded with a linear 16 site multi-electrode array with site distance of 50 μm (1 × 16-50-177, NeuroNexus, USA, Impedance 2–3MOhm at 1 kHz) against a frontal epidural silver-ball electrode, which served as reference. The array was inserted into the inferior colliculus either at an angle of 40°–45° from a lateral position or inserted vertically in the middle of the visible portion of the IC (see Fig. 2B).

The multielectrode array was inserted until the tip reached a depth between 800 and 1000 μm relative to the surface. In one case we used a 32 site multi-electrode array (1 × 32-100-177 NeuroNexus, USA, Impedance 2–3MOhm at 1 kHz), which was introduced vertically until it spanned the full IC and covered a depth down to approximately 3200 μm (see Fig. 5B).

The electrode signals were band-pass filtered (1–9000 Hz) and digitized with 24 bit resolution at a 32 kHz sampling rate with a Neuralynx Digital Lynx SX (Neuralynx, USA). Electrodes were stained with DiI (Invitrogen, USA) and the electrode position within the inferior colliculus was determined in 8 mice with Nissl and Cytochrome oxidase staining. 3D-reconstruction of the electrode track within the IC was then performed with the use of Amira Software (Amira, FEI Visualization Sciences Group). For illustration of the mouse auditory brainstem we generated a 3D model using available data resources on the mouse brain from the Allen Mouse Brain Atlas (Lein et al., 2007) and extracted 3D models of brainstem structures of the mouse brain with the help of the Scalabale Brain Atlas at www.scalablebrainatlas.incf.org (Bakker et al., 2015; Majka et al., 2012) and then modified them further with Meshlab Software from www.meshlab.sourceforge.net (Cignoni et al., 2008).

2.4. Auditory stimulation

Clicks were applied using a tweeter speaker (Vifa/Peerless XT 300 K/4) positioned 30 cm in front of the animal at 5 cm elevation. Condensation clicks of 5 μs duration were presented at levels from 20 dB to 95 dB peSPL in 5 dB steps. The inter-click interval was 100 ms and clicks were repeated 300 to 600 times. Click level was defined as the sinusoidal relative level and calculated for the rectified amplitude of the response, as measured with a calibrated Bruel and Kjaer microphone (Free-field 1/4 Microphone Type 4939, Bruel and Kjaer, Denmark). To measure the click level, the microphone was placed at the same position as the mouse head during the experiment in the same distance to the speaker. The speaker distance from the animal resulted in a sound traveling time delay of ~950 ± 50 μs, and respective sound traveling delays were accounted for in the subsequent latency analyses.

2.5. Signal processing

The recorded signals were processed off-line with Matlab 2013a (The Mathworks, USA). Local field potentials (LFP) were derived from the raw signal by filtering between 1 and 300 Hz (digital 4th order Butterworth filter). The bipolar derived local field potentials were calculated by subtracting neighboring sites of the multi-electrode array and then filtering the signal as described above. Multiunit activity (MUA) was retrieved by band-pass filtering between 500–9000 Hz. MUA was then defined as all spike events exceeding a threshold of 3.5 SD of the band-pass-filtered signal. MUA was stored as discrete digital timestamps for the respective spike events. The mean standard deviation of the band-pass filtered signal was for C57Bl/6 mice: 23 μV (SD 2 μV) n = 12 *16 sites = 192 and for CBA/J mice: 19 μV (SD 1.5 μV) n = 9 *16 sites = 144. Continuous high-frequency potentials (HFP) were derived by high-pass filtering the rectified signal between 3 and 9 kHz (Xing et al., 2009). The HFP represents a continuous measure of local high-frequency population activity around the electrode site, which can serve as an alternative measure of MUA activity (Land et al., 2013; Xing et al., 2009). The ABR_{IC} was derived from the