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

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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 P0 with peak latency 7–8 ms when recorded with adequate band-pass filtering. Wave P0 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 P0 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.

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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 = 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 teton 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 recordings position see below. Animals were then tracheotomized and a small silver-ball electrode, thus no aspiration of the visual cortex or manipulation of other structures of the mouse brain with the help of the Scalable 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 K4) 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 of-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. Multitunit 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
recording within the IC by band-pass filtering the multi-electrode array signal between 30 and 3000 Hz and then averaging over the repetitions. It should be noted that individual spike waveforms present within the passband of 30–3000 Hz in the signal were not discernible anymore due to the averaging process.

2.6. Data analysis

Click evoked responses were calculated by averaging activity over the 300–600 repetitions. ABR, field potential and MUA latencies were defined by measuring peak latencies of the respective waves or MUA peaks. MUA peaks were selected by identifying peaks with a peak height of 10% of the maximum response. The similarity in ABR waveform shapes in relation to position within the IC was quantified by determining the standard deviation of the signals over time across all 16 channels of the electrode. The underlying rationale is that an increase in variation (i.e. standard deviation) indicates an increase of position dependent near field effects. If not stated otherwise all errorbars are ± standard deviation across the animal sample.

2.7. Nomenclature

We denoted positive peaks of the ABR waves by roman numbers I-V. The ABR recorded from the scalp is denoted as ABRscalp, the ABR derived from the recording with IC as ABRIC. Filter settings are reported at the specific parts in the text. The positive wave recorded in the scalp is denoted here as P0. This follows the nomenclature for auditory evoked components by depicting the first positive component following the fast ABR waves and preceding the middle latency response Pa as P0 (Picton et al., 1974). It might be added that the N0/P0 is presumably the same wave as the SN10 slow negative wave (Hashimoto, 1982).

3. Results

3.1. Click-evoked slow wave P0 in the scalp ABR

We recorded the free-field click-evoked binaural ABRs of C57Bl/6 and CBA/J mice with a scalp vertex electrode against a reference behind the right ear (Fig. 1A, B). The ABR waves expressed the typical increase in amplitudes and decrease in peak latencies with increasing click intensity (Fig. 1C).

To analyze both high- and low-passed filtered waveforms (Fullerton et al., 1987; Ozdamar and Kraus, 1983) an off-line band-pass filter (30–3000 Hz) was used to additionally visualize low-passed waveforms. Using this approach, a positive slow wave P0 could be identified following the initial five waves in the scalp ABR (Fig. 1D). This slow wave P0 increased in amplitude with increasing click intensity (Fig. 1E, D). The mean amplitude at 30 dB above hearing threshold was 3.6 μV (SD 0.6) for CBAJ and 3.5 μV (SD 1.1) for C57Bl/6 mice. The P0 latency was 7.4 ms (SD 0.3) for CBAJ and 7.8 ms (SD 0.6) for C57Bl/6 at 30 dB above threshold (Fig. 1F). Latencies decreased with increasing click level initially, however, at higher levels they did not show a monotonic linear decrease with increasing click level (Fig. 1F). This was explained by the fact that the slow wave increased in duration/width for higher click levels, and thus measuring latency by determining the peak latency shifted the peak latency back towards slower latencies for higher click intensities.

3.2. Click-evoked local field potentials and multunit responses in the inferior colliculus

To test whether the slow wave P0 reflects activity in the IC, we recorded the depth profile of activity in the IC with a linear multisite electrode array, spanning the IC from the surface to an approximate depth of 800–1000 μm (Fig. 2A-C). We then characterized the depth profile of click-evoked responses of different signals in the inferior colliculus (Fig. 2D-G).

Click-evoked responses had a typical spatial pattern across the depth of the IC. It was possible to differentiate the surface region from the central region (Fig. 2D). Close to the surface (<200 μm) the LFP response was biphasic with two positive peaks relative to the vertex electrode, and we did not observe evoked spike responses there (Fig. 2E). Deeper in the IC, the LFP response showed an initial positive peak followed by a negative peak relative to the vertex electrode. The negative peak amplitude of the LFP reflected the strength and duration of the MUA spiking response (Fig. 2D, E). The maximum of the evoked LFP and the maximum MUA response were located within one or two neighboring electrode sites separated by 50–100 μm. The MUA spiking response increased with depth in the IC towards a maximum at the depth of the greatest sensitivity (Fig. 2E). Importantly, the multunit spiking response in the IC to the short transient click stimulus (<0.2 ms, Fig. 1B) consisted of several subsequent peaks in millisecond intervals, within a
We differentiated the volume-conducted far-field components in the LFP from localized activity in the IC by bipolar derivation of the LFP (Fig. 2F). The first positive LFP component did not change with the depth of the IC recording, and was removed by bipolar derivation of the LFP signal demonstrating a far-field origin (Fig. 2D, F). There was also no activity during the positive component of the LFP in the local high-frequency potential (HFP) response, which mirrored the temporal and spatial response distribution of the multiunit activity along the depth of the IC (Fig. 2G). HFP and MUA activity accompanied the onset of the negative component of the LFP response. With regard to spatial distribution, HFP signals could be used in combination with the LFP and MUA for a spatial differentiation and characterization of the IC by their response characteristics (Fig. 2G).

Notably, the click-evoked IC response to a single click lasted up to 10 ms, depending on the click level, with multiple peaks in the spiking response. This duration of the spike response was observed both in the LFP and the HFP, with the positive peak of the LFP in the inferior colliculus preceding the onset of spiking activity. The latency and duration of the signals in the IC correlated with the sustained spike response (Fig. 2E).

3.3. Slow wave P0 in the scalp ABR correlates with inferior colliculus activity

The slow wave P0 in the scalp ABR reflected the click-evoked LFP response within the IC (Fig. 3A). Fig. 3A shows a recording with a 32-site electrode array spanning the inferior colliculus and the deeper brainstem showing a strong negative evoked response localized to the IC. The negative component of the IC LFP response corresponded in shape and latency to the slow wave P0 in the scalp recording. ABR waves IV-V occurred during the positive peak of the LFP response (Fig. 3B).

Both P0 and the evoked LFP in the IC changed similarly with stimulus intensity, indicating that they reflect the same electrical field recorded from different positions (Fig. 3C). Latencies were similar for the negative peak of the LFP and P0 for C57Bl/6 and CBA/J mice (Fig. 3E) when comparing the peak latency of the maximum response of the LFP in the IC to P0. The latency of P0 was 7.4 ms (SD 0.3) for CBA/J and 7.8 ms (SD 0.6) for C57Bl/6 at 30 dB above threshold and with the latency of the LFP with 8.4 ms (SD 1) for CBA/J and 8.2 ms (SD 1) for C57Bl/6 mice (Fig. 3E). The slow wave P0 thus reflected the latency and amplitude of the IC LFP and MUA response, and may represent a marker of the main IC activity.
3.4. The relation of IC spiking activity to ABR wave IV and V

The negative peak of the evoked LFP and the ABRscalp component P0 reflected the sustained multiunit spiking activity in response to the click stimulus (Figs. 2D, E and 3A). The duration of the click response increased in relation to the click intensity. Importantly, MUA spiking activity showed one or two sharp peaks at the onset of the response, in intervals of approximately 1 ms, which were followed by subsequent additional peaks (Fig. 4A). The first two of the peaks had the following latencies for peak 1: C57Bl/6 3.78 ms (SD 0.10) and CBA 3.8 ms (SD 0.16) and for peak 2: C57Bl/6 4.76 ms (SD 0.16) and CBA/J 4.6 ms (SD 0.18) at 30 dB above HL. These latencies corresponded to latencies of wave IV and V of the ABRscalp at the corresponding hearing level (Fig. 4B, D, and Table 1). However, the first peak was only observed in around 50% of the recorded electrode penetrations, whereas the second peak was present in nearly all recorded electrode penetrations (Fig. 4D). Then, subsequent activity > 5 ms was present in all IC recordings in all mice (Fig. 4A).

3.5. Position dependence of ABR waves recorded in the IC

To further test the relation of IC activity to the ABR waves, we derived the ABR recorded from within the IC by band-pass filtering the recorded signal in the IC between 30 and 3000 Hz (see Methods). After averaging, this resulted in the ABRIC, the combination of fast far-field brainstem evoked signals and slow near-field components generated within the IC (Fig. 4D). The fast ABRIC waves were superimposed on the near-field LFP components in the IC. Peaks of wave I and II of the ABRIC were little modulated by recording depth within the IC, whereas the wave shapes and amplitudes of peaks IV and V were position dependent. This was consistent in all mice. Quantifying this modulation by calculating the variation of the ABRIC peak amplitudes along the depth of recording position shows an increase in position dependence from wave III to wave V (Fig. 5B).

The peak of wave III was less pronounced and occurred during the up-flank of the positive peak of the evoked LFP. Peaks of wave IV and V were also paired and superimposed on the positive peak component of the evoked LFP. The ABRIC waves had peak amplitudes in average around 5–10 times larger in magnitude than the ABRscalp. Fig. 5B shows a comparison of responses recorded with an electrode for filtering with band-pass filter 10–3000 Hz and filtering 300–3000 Hz (Fig. 5A). When using a bipolar derivation of the signals, ABR waves I-III disappeared, demonstrating a far-field origin outside of the IC (Fig. 5C, lower panel).

The ABR waves from near-field sources that are generated within or close to the IC will change in dependence on recording position of the electrode within the IC as the electrodes pass near their sources of origin. To investigate the position dependence of the waves we used the depth recording with the multi-electrode array of the signals described above, shown in the butterfly plot in Fig. 5A with the superimposed signals of the sixteen signals along the electrode shank. Waves I-III were little modulated by recording depth within the IC, whereas the wave shapes and amplitudes of peaks IV and V were position dependent. This was consistent in all mice. Quantifying this modulation by calculating the variation of the ABRIC peak amplitudes along the depth of recording position shows an increase in position dependence from wave III to wave V (Fig. 5B).

This conclusion was supported by the bipolar derived signal of the LFP (Fig. 5C, lower trace). Bipolar spatial derivation removed wave I to III from the signal. Calculating the variance between the signals of the 16 electrode contacts in the IC shows that the signal within the IC increases in amplitude after ~3 ms post stimulus, which indicates the onset of localized activity within the IC.
following these latencies (Fig. 5C, D).

Last, we recorded the modulation throughout the full depth in the IC with a long electrode that spanned the full IC and the brainstem below and recorded activity from different positions within and tangential to the IC (Fig. 6A,B). In the center of the IC, waves IV and V were strongly modulated in amplitude by spatial position, whereas ABR waves I and II were not position dependent. Along the midline and rostral to the IC, the ABR waves were not modulated by recording depth (Fig. 6C, left panel and lower panel). Only within the IC there was a modulation of wave IV and V, and not at positions below the anatomical borders of the IC (Fig. 6C, upper panel and middle panel). Electrode penetrations lateral to the IC reached proximity to the cochlear nucleus, and this was reflected in an increase of amplitude of wave I and II (Fig. 6C right panel). Wave IV and V were exclusively modulated within the IC.

4. Discussion

We studied the click-evoked IC response in mice and its relation to the ABR response recorded from the scalp. IC activity in the mouse shows close temporal correspondence to a slow positive component P0 in the scalp ABR that can be visualized as a potential marker for IC activity by adequate band-pass filtering. Additionally, the onset of the click-evoked response in the IC is temporally related to mouse ABR wave IV and V, however to a lesser extent to wave IV. Further, we show that a significant part of activity within the IC occurs following the ABR waves IV/V.

4.1. The slow wave P0 as a correlate of the click-evoked IC response

Table 1

<table>
<thead>
<tr>
<th>Latencies (30 dB HL)</th>
<th>Wave I (ms)</th>
<th>Wave II (ms)</th>
<th>Wave III (ms)</th>
<th>Wave IV (ms)</th>
<th>Wave V (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57Bl/6 ABR</td>
<td>1.39 (9)</td>
<td>2.03 (12)</td>
<td>2.84 (8)</td>
<td>3.80 (16)</td>
<td>4.78 (13)</td>
</tr>
<tr>
<td>C57Bl/6 MUA</td>
<td>3.8 (10)</td>
<td>4.76 (16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBA/J ABR</td>
<td>1.26 (4)</td>
<td>2.01 (11)</td>
<td>2.66 (6)</td>
<td>3.63 (16)</td>
<td>4.58 (17)</td>
</tr>
<tr>
<td>CBA/J MUA</td>
<td>3.45 (10)</td>
<td>4.6 (18)</td>
<td></td>
<td></td>
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Whereas the onset of the IC response was temporally related to wave IV and V of the ABR, the major part of the IC multiunit response occurred after ABR wave V. Notably, the IC response to the brief click was temporally stretched over several milliseconds (Figs. 2E and 4A), producing an elongated response, with median spike latencies ~ 8 ms similar to previous studies (Bibikov et al., 2008; Ehret and Romand, 1992; Qiu et al., 2007; Tan and Borst, 2007). This click-evoked spiking response was reflected in duration and strength by the negative LFP component, and is similar in shape and peak latency to the LFP response in the IC of the guinea pig, rat and human (Funai and Funasaka, 1983; Harrison and Palmer, 1984; Möller and Jannetta, 1982a, 1982b).

Our data indicate that in the mouse, the electric field generated by the click-evoked IC response is reflected as the positive slow wave component P0 in the non-invasive scalp ABR, and can be visualized with wide band-pass filtering. This slow wave is usually filtered out in ABR recordings by the use of band-pass filters with cut-off frequencies between 100 and 300 Hz. We suggest recording of the ABR with open filters and applying digital filtering off-line, which allows isolation of P0 if necessary as complementary...
information on the IC component in the ABR. However, it should be added that additional lesion experiments need to establish a causal relation between IC activity and the P0 component. So far our results can only indicate a close temporal correspondence between both, although the evidence seems to be compelling, suggesting a correlation of IC activity and P0.

In contrast to larger species, the measurement of the IC response in the mouse scalp ABR is likely facilitated by the size of the IC relative to the rest of the brain, the thin skull, and that the IC is not covered by the occipital cortical lobe, which reduces the distance between source and the scalp recording electrode. However, the IC response in form of the P0 component is also present in the ABR/MLR in larger animals and man, however considerably smaller (Hashimoto, 1982). But given the similarity of the IC response between species (Funai and Funasaka, 1983; Harrison and Palmer, 1984; Møller and Jannetta, 1982a, 1982b), and the similarity in auditory brainstem anatomy and brainstem response latencies across species (Funai and Funasaka, 1983; Meininger et al., 1986), the IC electric response is possibly represented as the slow component P0 following the fast ABR waves in the scalp ABR also in other species, depending on the conditions that facilitate its presence in the scalp signal. For example, in humans, the N0/P0 component of the scalp MLR wave has been suggested to relate to the upper brainstem and the IC response (Battmer and Lehnhardt, 1981; Davis and Hirsh, 1979; Eggermont and Ponton, 2002; C.M. Huang, 1980; Hunter and Willott, 1987; Kraus et al., 1985; Melcher et al., 1996b; Picton et al., 1974; Ridgway et al., 1981). Further, the IC onset latencies are similar across species of different sizes, with minimal IC onset latencies of 4–6 ms being similar in the mouse, guinea pig, bat and cat (Harrison and Palmer, 1984; Langner et al., 1987; Shepherd et al., 1999; Voytenko and Galazyuk, 2008; Walton et al., 1998). With P0 occurring around 7–8 ms in the mice and <13 ms in man (Picton et al., 1974), it might be a common cross-species candidate for the IC response described here. Although auditory evoked potentials and ABR waves of different species should be compared with caution, the limiting factor of IC latency is the number of synaptic steps, which is similar across species, whereas the absolute axonal distance between brainstem nuclei plays a lesser role (Ridgway et al., 1981).

4.2. Onset spiking in the mouse IC is temporally related to ABR waves IV and V

The IC onset peaks of the MUA response indicated a contribution to wave V and to some amount to wave IV of the ABR. The MUA

![Fig. 5. Position dependence of ABRIC waves within the IC distinguishes far- and near-fields. (A) The evoked response of the wide-band filtered signal at the 16 sites at different depths in the inferior colliculus. ABRIC waves are superimposed on the evoked response. Lower panel shows the same recording with decreased filter bandwidth of 300–3000 Hz. Again individual traces depict recording at different depths of the IC from the same electrode. Variability in the shape of ABRIC waves I–V show different far and near field origins. (B) Variance of wave amplitude shows the influence on position within the IC on the respective ABRIC waves. (C) Bipolar derivation of the LFP signal in (A) removes activity during wave I–III, demonstrating their far-field origin not located within the IC. (D) The variation over time of the signals along the shank of the response of the bipolar derived LFP for C57Bl/6 and CBAJ mice (solid lines: mean; dotted lines: ±SD, CBA n = 9, C57 n = 12). Localized activity in the IC starts around 3 ms (dotted black line), and peaks around 7–8 ms.](image-url)
spike response consisted of multiple peaks in roughly 1 ms intervals, beginning > 3 ms, with onset latencies of IC stimulus-locked spiking in the range of ABR wave IV and V. Similar first-spike latencies for the mouse IC has been measured previously (Walton et al., 1998; Zhou et al., 2006). A similar study performed in the guinea pig (Harrison and Palmer, 1984) showed that only high-frequency cells in the ventro-lateral IC have fast enough responses to contribute to wave V. Most sites we recorded from had characteristic frequencies above 10 kHz, and these showed fast click-evoked onset responses. Interestingly, these responses were often double-peaked with additional early MUA responses in the latency range of wave IV. These earliest responses might be species-specific, and their origin and nature need to be further addressed. In the guinea pig, similar click-evoked double-peaked onset responses in the IC has been reported for single unit responses (Harrison and Palmer, 1984).

The synchronized MUA onset peaks occurred in close relation to the ABR waves, especially with the intervals between MUA peaks reflecting the ABR wave intervals. It is tempting to assume that here the ABR waves may simply reflect at least partly the temporal summation of the sum of the spike waveforms, that is the synchronous extracellular action potentials (Ozdamar and Kraus, 1983). Especially, because the positive component P0 reflected the spiking response in the IC, the fast positive ABR waves may similarly represent the summed temporal synchronized spike waveforms in the IC. However, this view might reflect an oversimplification, and would neglect the considerable effort that has gone into identifying the generators of the various ABR components over the years.

The consensus view attributes auditory brainstem generation to parallel pathways (Melcher and Kiang, 1996; Ponton et al., 1996). The ABR generation can be attributed to the axonal fiber tracts that give rise to the potentials seen by remote electrodes as their consistent orientation allow summation resulting in a dipole source, whereas nuclei are not so ordered and hence their activity does not sum in the same way. The appearing and disappearing dipoles generated during the activation of different brainstem nuclei cause the summed compound vector of the overall electrical field of the brainstem to flip from one side to the other generating the waves of the ABR in the recording, thus representing a convolution of the signals from different dipole sources. Interestingly, the occurrence of early onsets was related to click intensity, with early responses more likely to occur with higher intensities, which suggest a contribution of the IC to ABR waves depending on stimulus intensity.

A contribution of the IC to ABR waves IV and V was supported by the fact that amplitudes of wave IV and V were influenced by recording position within in the IC indicating an origin within or in close proximity of the IC. This relies on the assumption that near-fields are more location dependent than far-fields (Luders, 1989), and assumes linear additive properties of the brainstem electrical fields (Bullock, 1997; Buzsáki et al., 2012). Previous lesion experiments suggested an origin of wave V in mice in the vicinity of the lateralmost portion of the IC in the mouse (Henry, 1979). In contrast, waves I and II were independent of recording position within the IC, and were only modulated when the recording sites were close to the cochlear nucleus (Fig. 5C right panel), reflecting an origin from the auditory nerve and cochlear nucleus (Buchwald and Huang, 1975; Henry, 1979; Legatt et al., 1988; Melcher and Kiang, 1996; Melcher et al., 1996a; Möller and Jannetta, 1982a).

Generally, the attempt to determine a one-to-one correlation of one specific ABR wave with an individual brainstem structure does not lead to a one-to-one correlation. The ABR waves represent the temporal summation of extracellular potentials at the recording site, and the waveforms reflect the electrical field of the brainstem, which is the result of the activity of many brainstem neurons. Therefore, the ABR waves are not solely generated in the IC, but also in other brainstem nuclei such as the lateral lemniscus and the inferior colliculus. Consequently, the ABR waves cannot be attributed to a specific brainstem structure, but rather represent the electrical field of the brainstem as a whole.
not match the nature of ABR generation (Achor and Starr, 1980). An unambiguous one-to-one correlation between click-evoked activity in the IC and a specific ABR wave in the mouse was not straightforward, given the temporal relation to both wave V and to wave IV of the ABR. The ABR response reflects separate parallel direct and indirect pathways to the central nucleus of the IC with different delays producing responses with varying latencies (Moore, 1987; Ponton et al., 1996; Ryugo et al., 1981; Willott et al., 1985). ABR waves can arise from multiple distributed sources, linked by their temporal onset as well as spatial origin within the same structure. The heterogeneity in projections to the IC with different response latencies might then lead to a sequential contribution of the IC to ABR wave IV and V, and possible subsequent waves present in the mouse ABR (Scimemi et al., 2014), which were also present as peaks in the MUA response (Fig. 4A). The later ABR waves then originate more likely from parallel activation at several distributed brainstem sites, and represent synchronized temporal activation of successive neuron populations independent of brainstem location (Achor and Starr, 1980; Kaga et al., 1997). This may explain the absence of an established unambiguous relation of the IC response with a single ABR wave (Buchwald and Huang, 1975; Funai and Funasaka, 1983; Harrison and Palmer, 1984; C.M. Huang, 1980; Kaga et al., 1997; Liu and Mark, 2001; Melcher and Kiang, 1986; Mellor and Jannetta, 1982b; Parkkonen et al., 2009). Here we suggest P0 as an alternative marker for IC activity in a non-invasive ABR measurement in the mouse.

4.3. Methodological considerations in measuring the ABR and the P0 component in the mouse

Last, it might be useful to address some practical issues with the ABR recording in mice. First, here we used a free-field binaural stimulation, as we were interested in the general characteristics of the response. This approach did not allow further characterization of the monaural response differences and binaural interactions, which can provide further information on the characteristics of P0 and ABR and auditory middle latency responses in general (Dykstra et al., 2016; Ozdamar et al., 1986; Van Yper et al., 2015).

The measure of the IC slow wave component P0 depends on recording quality. Unfortunately, the use of a wider band-pass filter with a lower high-pass cut-off frequency does not eliminate effectively additional low frequency noise in the signal, mostly from breathing movements, ECG and similar artifacts, which introduce noise to the recordings. This makes the measurement of the P0 with the wider passband prone to noise artifacts.

We used a recording configuration with a vertex electrode against one of the ears to measure the ABR. The shape of the waveforms in the ABR depends on the recording position, thus waveforms are sensitive to the recording configuration with respect to the location of the auditory pathway structures. In many studies, the observed variability in ABR waveforms may be explained by the difficulty of ensuring an exact and consistent recording position on the small skull of the mouse. This is less of an issue in larger animals with larger brains, where an absolute change in recording position has less effect on its relation to the auditory pathway structures. In the mouse with the small head, absolute changes in electrode position have larger relative effects on the ABR waveforms than in animals with larger heads and brains. We also encountered this problem, and tried to minimize this issue by putting emphasis on ensuring exact recording positions.

The identification of ABR waves is clearest for intensities closer to hearing threshold, because here usually the five waves were always best visible for intensities close to hearing threshold. With higher intensities, at least for our binaural free-field stimulus, the first five ABR waves split into multiple subcomponents waves (Tillein et al., 2012), often making it difficult to clearly define peak latencies for higher intensities. Further, with higher click intensities, we could distinguish more waves following wave V, similar to other studies in the mouse (Scimemi et al., 2014). These were reflected in the spike peaks for higher intensities, as well in the LFP intra IC (Figs. 4A and 5A, C).

4.4. Conclusion

By recording with an open-band-pass filter, the slow ABR wave component P0 represents a marker for IC activity that can easily be measured to increase the information content of the mouse ABR. With the mouse as a widely used model, this additional information may be valuable in phenotyping and the identification of different auditory pathologies. Further more given the general similarity of the auditory brainstem pathway in related species, the result might contribute to a better understanding of the relation of IC activity and the ABR in a wider context.

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References
