Published in final edited form as:

Ear Hear. 2016 ; 37(5): e291–e301. doi:10.1097/AUD.0000000000000315.

Test-Retest Reliability of the Binaural Interaction Component of the Auditory Brainstem Response

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Abstract

The binaural interaction component (BIC) is the residual auditory brainstem response (ABR) obtained after subtracting the sum of monaurally-evoked from binaurally-evoked ABRs. The DN1 peak — the first negative peak of the BIC — has been postulated to have diagnostic value as a biomarker for binaural hearing abilities. Indeed, not only do DN1 amplitudes depend systematically upon binaural cues to location (interaural time and level differences) but they are also predictive of central hearing deficits in humans. A prominent issue in using BIC measures as a diagnostic biomarker is that DN1 amplitudes not only exhibit considerable variability across subjects, but also within subjects across different measurement sessions. Here we investigate the DN1 amplitude measurement reliability by conducting repeated measurements on different days in eight adult guinea pigs. Despite consistent ABR thresholds, ABR and DN1 amplitudes varied between and within subjects across recording sessions. However, our analysis reveals that DN1 amplitudes varied proportionally with parent monaural ABR amplitudes, suggesting that common experimental factors likely account for the variability in both waveforms. Despite this variability, we show that the shape of the dependence between DN1 amplitude and ITD is preserved. We then provide a BIC normalization strategy using monaural ABR amplitude that reduces the variability of DN1 peak measurements. Finally, we evaluate this normalization strategy in the context of detecting changes of the DN1 amplitude-to-ITD relationship. Our results indicate that the BIC measurement variability can be reduced by a factor of two by performing a simple and objective normalization operation. We discuss the potential for this normalized BIC measure as a biomarker for binaural hearing.

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Conflicts of Interest

The authors have no conflict of interest to declare.

Introduction

One of the primary functions of the auditory system is to determine the location of objects in the environment (Tollin 2003). To achieve this, the auditory system processes binaural *acoustical cues* to sound location (interaural time and level differences, ITD and ILD), which result from the fact that the ears sample sound waves at locations on opposite sides of the head (Greene et al. 2014). Several nuclei in the auditory brainstem are known to represent those cues, based on the spectral and temporal characteristics of sounds arriving at the two ears (Tollin & Yin 2009). A non-invasive measure of the electrical activity of those nuclei is the binaural interaction component (BIC) of sound-evoked auditory brainstem responses (ABR). The BIC is an important topic of interest as, for example, a potential clinical diagnostic tool, yet the development of such techniques has been hampered by the unreliability of BIC measures for a given subject (Kelly-Ballweber & Dobie 1984; Wilson et al. 1985; Stollman et al. 1996). In this study, we seek to systematically quantify this variability, as well as potential methods to mitigate it, by performing repeated measures of the BIC in the same animal subject over a course of weeks.

The auditory brainstem response (ABR) is an electroencephalographic measure of the activity of the brainstem when a subject is presented with auditory stimuli. In response to monaural clicks, one generally observes a sequence of peaks (Figure 1A), which is thought to reflect the activity of neural structures along the auditory pathway. While there are species differences in which neural structures are responsible for the peaks of the ABRs (e.g. due to differences in head size (Møller, 1994)), in the guinea pig, early peaks (peaks I-III, Figure 1A) reflect the activity of peripheral monaural structures (auditory nerve, cochlear nucleus). Because of this monaural origin, the magnitude of early peaks observed in response to binaural stimuli is the same as the summed magnitudes observed in response to monaural stimuli (Figure 1A, black trace). Later peaks, such as the binaurally-evoked fourth peak (the Wave IV/V complex) are not equal to the sum of their monaural constituents, which is indicative of binaural-specific processing performed by the brainstem (Jewett 1970). This difference, usually termed the binaural interaction component (the difference between the sum of monaurally-evoked ABRs and the binaurally evoked ABR, BIC), has a prominent negative peak called DN1 (also known as the β -peak, Figure 1A). Both the amplitude and latency of this DN1 peak are modulated by ITD and ILD (Furst et al. 1985; McPherson & Starr 1995; Riedel & Kollmeier 2002a; Riedel & Kollmeier 2006, Figure 1C). Specifically, BIC DN1 peak exhibits maximal amplitude and minimal latency for ITDs and ILDs of zero (corresponding to sound sources on the midline), while typically remaining detectable outside the range of ecological cues for the species under study (e.g. Dobie & Berlin 1979; McPherson & Starr 1995; Riedel & Kollmeier, 2006; Ungan et al. 1997).

DN1 is robustly evoked in nearly all normal-hearing subjects (Dobie & Norton 1980; Kelly-Ballweber & Dobie 1984; Levine 1981; Riedel & Kollmeier 2002a; Riedel & Kollmeier 2002b; Riedel & Kollmeier 2006) but altered in various disease states that affect binaural hearing. For example, altered BIC has been shown to reflect long-term behavioral binaural processing deficits (Gunnarson & Finitzo 1991; Delb et al. 2003). Other applications include the assessment of developmental and functional brainstem integrity (Hosford-Dunn et al. 1981; Jiang & Tierney 1996), optimization of bilateral cochlear implants (Smith & Delgutte

2007; Gordon et al. 2012) or bilateral hearing aids (Purdy et al. 2004) as well as hearing dysfunction related to multiple sclerosis (a deficit which is binaural in nature and often not apparent from standard audiometric testing) (Hausler & Levine 1980; Furst et al. 1990; Fowler & Swanson 1988). However, the BIC waveform is highly variable both within- and between-subjects (Kelly-Ballweber & Dobie 1984; Wilson et al. 1985; Stollman et al. 1996), and therefore is often used as a binary criterion: the BIC is either present or absent. While the latency of the DN1 peak is quite robust, the absolute magnitude of the DN1 peak (BIC amplitude) is quite variable across subjects and recordings sessions, which makes it hard to detect potential alterations that could be attributed to binaural processing deficits in individual subjects (Wilson et al. 1985). We investigate here methods to reduce the variability in the amplitude of the DN1 peak of the BIC.

Some studies have reported better BIC amplitude reproducibility when experiments are conducted in the same session, particularly keeping earphones and electrodes in place between recordings (Dobie & Berlin 1979). We thus hypothesize that the amplitude of the BIC is not *inherently* variable, and rather experimental factors account for amplitude variability in ABR waveforms and therefore also the BIC (e.g. earphone placement, electrode impedance, contact quality, location, etc.). While the influence of these factors can be minimized by improving the experimental protocol (e.g., a more controlled electrode placement, or acoustical input calibration; see Beutelmann et al. 2015), some within and across-subject test-retest variability inevitably persists. We propose here to use *normalization* to mitigate experimental variability factors in BIC recordings. We address the detection of *changes* in the relationship between BIC amplitude and ITD (Figure 1C), as opposed to previous work focusing on the mere detection of significant BIC peaks (Elberling 1979; Kileny 1987; Brantberg et al. 1999; Furst et al. 1985; Gunnarson & Finitzo 1991; Levine & Davis 1991; Riedel & Kollmeier 2002a; Riedel & Kollmeier 2002b; Riedel & Kollmeier 2006; Stollman et al. 1996).

From a clinical perspective, some studies suggest that the DN1 amplitude at 0 ITD is reduced due to long-term behavioral binaural processing deficits, as depicted on Fig 2A (blue and green dots) (Gunnarson & Finitzo 1991). The detection of such changes in absolute BIC amplitude is difficult because of the high variability of the BIC amplitude. In fact, when one considers the complete BIC-to-ITD relationship, at least two transformations could explain the reduction observed: a global shrinking (*scaling*, Figure 2A) or a shift along the ITD axis (*ITD shift*, Figure 2B). We argue that normalization methods may enable experimenters to detect smaller *scalings* or *shifts* of the BIC amplitude-to-ITD relationship, and seek to provide a quantitative analysis of the benefits of normalization in this context.

Here, we first record BIC waveforms from a population of eight guinea pig subjects, repeating measures on different days. As expected, we observe that the relationship between BIC DN1 amplitude and ITD can be accurately described as a bell-shaped curve (e.g., Fig. 1C). Inspecting the parameters of those curves, we show that the absolute values of the DN1 peak, but not its relationship to ITD, strongly correlates with a measure of the ABR signal-to-noise ratio. Using a normalization strategy suggested by these results, we show that the within- and across- subject variability is drastically reduced. Finally, we quantify our ability to detect abnormal BIC-to-ITD relationships in different normalization conditions.

Methods

ABR Recordings

All surgical and experimental procedures complied with the guidelines of the University of Colorado Anschutz Medical Campus Animal Care and Use Committee and the National Institutes of Health. ABR recordings were conducted in eight normal-hearing adult pigmented guinea pigs (*Cavia porcellus*, Elm Hill Labs, Chelmsford, MA; weight 520-953g) on different days over the course of 4-8 weeks. Prior to recording sessions, animals underwent otoscopic examination using a Zeiss surgical microscope to confirm that tympanic membranes and external ear canals were intact and free of pathology. ABR recordings were collected under ketamine/xylazine anesthesia (70mg/kg ketamine + 7mg/kg xylazine) delivered intraperitoneally. During recording sessions, physiological temperature was maintained using a heating pad. Heart rate, respiration rate and blood-oxygen levels (SpO₂) were monitored throughout each procedure. At the beginning of each individual recording session, click-evoked monaural ABR thresholds were assessed in each animal using 5dB steps. In all cases, interaural ABR threshold asymmetries were less than 10 dB and inter-session ABR thresholds were consistent (within 10 dB for all sessions in each animal). Between 3 and 5 experimental runs were successfully performed for all animals.

The BIC acquisition system used for this study replicates that of Beutelmann et al. (2015). Presentation of stimuli and acquisition of evoked potentials was performed via an RME (Haimhausen, Germany) Hammerfall Multiface II sound card (sample rate 44.1 kHz) controlled by a PC running custom MATLAB (The Mathworks, Inc., Natick, MA) software in a Linux-based environment (OpenSUSE 13.1). Click stimuli were presented at an average interstimulus interval of 30 ms, with a standard deviation of 10 ms, at 80 dB peSPL (baseline-to-peak, referenced to 1kHz tone, see Beutelmann et al. (2015)). 500 artifact-free repetitions were presented for each monaural and binaural condition, which is sufficient to achieve a good signal-to-noise ratio. BIC was recorded across an ITD range spanning ± 2000 μ s (± 2000 , 1000, 750, 500, 375, 250, 125, 0 μ s), all conditions were randomized.

Stimuli were presented through stainless steel insert earpieces using TDT System 3 (Tucker Davis Technologies, Inc., Alachua, FL, USA) CF1 speakers powered by a TDT SA1 amplifier. Sound level and phase were calibrated for each session via Etymotic Research (Elk Grove Village, IL, USA) ER-7C microphones, with probe tubes positioned ~2 mm into the ear canal. The calibration was applied using a 129 tap minimum phase filter, which ensures good delivery of a temporally precise click (see Beutelmann et al. 2015 for more details). The microphones were kept in place during the experiment and their output was recorded simultaneously with the ABR signal to ensure signal fidelity throughout the duration of each experiment. The absolute sound pressure level was referenced to a 1 kHz tone. Recordings were made with platinum subdermal needle electrodes (F-E2-12 electrodes, Grass Technologies, West Warwick, RI, USA) at the apex (active) and nape of the neck (reference) with a hind leg ground (see Figure 1B). Prior to acquisition, sound-evoked ABR signals were pre-amplified (10,000x) and bandpass filtered (cutoffs 100Hz and 3kHz) by a WPI ISO-80 amplifier. An automated artifact rejection threshold was set prior to each recording session and was typically approximately ~10 μ V.

Analysis—Recorded signals were averaged by condition, and processed through a digital Butterworth filter (3rd order, cutoffs at 100Hz and 3kHz). The BIC waveform was then calculated for each ITD ($BIC = ABR_{Binaural} - [ABR_{LeftMonaural} + ABR_{RightMonaural}]$), with the monaural ABR traces time-shifted before summation according to the timing of click presentation in the corresponding binaural condition. For example, if a 250 μ s ITD was present in the binaural condition stimulus presentation, BIC calculation would be performed by time-shifting the monaurally-recorded ABRs in the corresponding direction by 250 μ s prior to summation, for comparison with the recording evoked by the binaural stimulus with that ITD. The amplitude of DN1, the peak of interest in the BIC waveform, was then measured for each condition as the maximum of the signal over a 10 ms window starting at stimulus onset.

BIC amplitude vs ITD function curve fitting procedure

We approximate the relationship between DN1 peak (BIC) and ITD as Gaussian, and thus fit a four-parameter curve separately to all DN1 peak measurements (N=29 over 8 animals). The four parameters are the ITD of maximal BIC, ITD_0 , the width of the relationship, σ , as well as the modulation of the curve, A, and the baseline BIC value, B (see Figure 4B). The fitted function can be expressed as

$$BIC_{model}(ITD) = B + A\phi\left(\frac{ITD - ITD_0}{\sigma}\right)$$

where ϕ is a Gaussian kernel describing the bell shape of the curve. Specifically

$$\phi(x) = e^{-\frac{x^2}{2}}$$

For a given set of measurements, we set $BIC_{measured}(ITD)$ to the values of measured DN1 amplitude, then the coefficients are found by minimizing the fit mean squared error:

$$\min_{A, B, ITD_0, \sigma} \sum_{ITD} (BIC_{measured}(ITD) - BIC_{model}(ITD))^2$$

which is achieved using a standard gradient-descent algorithm (Levenberg-Marquardt algorithm).

Reported fit r^2 are computed using the standard sum-of-squares approach:

$$r^2 = 1 - \frac{SS_{reg}}{SS_{tot}}$$

Where SS stands for “sum of squares” and thus:

$$SS_{reg} = \sum_{ITD} (BIC_{measured}(ITD) - BIC_{model}(ITD))^2$$

And

$$SS_{reg} = \sum_{ITD} (BIC_{measured}(ITD) - \langle BIC_{measured}(ITD) \rangle)^2$$

BIC amplitude normalization procedure

We define the RMS as the average root mean square measure of the two monaural ABRs. Letting ABR_L and ABR_R be the ABRs measured in response to monaural stimulation and N_t the number of timepoints in the ABR recording, we define:

$$RMS = \frac{1}{2} \left(\sqrt{\frac{1}{N_t} \sum_t (ABR_L(t))^2} + \sqrt{\frac{1}{N_t} \sum_t (ABR_R(t))^2} \right)$$

We find (see text) that the two scale parameters of the Gaussian fits, A and B (magnitude and baseline, respectively), are very well correlated with this RMS, which is the basis of the normalization procedure. In fact we can reasonably assume that the relationships are linear (with zero intercept):

$$\begin{aligned} A(RMS) &\approx \beta_A RMS \\ B(RMS) &\approx \beta_B RMS \end{aligned}$$

Where β_A (resp. β_B) is the slope of the linear regressions of A (resp. B) against the RMS. Furthermore, we show that the other parameters (the position and width of the Gaussian dependence) do not depend on RMS. Therefore the BIC component can be expressed as:

$$BIC(RMS, ITD) \approx \beta_B RMS + \beta_A RMS \phi \left(\frac{ITD - ITD_0}{\sigma} \right)$$

From this expression, it is apparent that BIC is proportional to RMS. Therefore a *normalized* BIC measure, BIC_n , can be obtained by dividing by the value of the RMS:

$$BIC_n(ITD) = \beta_B + \beta_A \phi \left(\frac{ITD - ITD_0}{\sigma} \right)$$

All other parameters (σ , ITD_0) do not depend on the RMS, and in fact vary only little on the individual or experiment (see text), and thus do not require normalization.

Testing for abnormal BIC amplitude vs ITD functions

We seek to quantify the ability to distinguish an “altered” BIC vs. ITD relationship, with alteration taking one of three forms: multiplicative scaling (the whole curve amplitude is multiplied by a certain factor, e.g., Fig. 2A), ITD shift (the position of the curve peak shifts to a nonzero ITD, e.g., Fig. 2B), or ITD scaling (the width of the curve narrows or widens). Specifically:

- *Multiplicative scaling*: the altered BIC BIC_{alt} is defined as: $BIC_{alt}(ITD) = \alpha * BIC_{original}(ITD)$ where α is the scaling factor.
- *ITD shift*: the altered BIC is defined as $BIC_{alt}(ITD) = BIC_{original}(ITD - \tau)$ where τ is the value of the ITD shift.
- *ITD scaling*: the altered BID is defined as $BIC_{alt}(ITD) = BIC_{original}(\alpha * ITD)$ where α is the ITD scaling factor.

We adopt a classical leave-one-out approach (Kohavi 1995) as depicted on Figure 7A. For any given alteration parameter, we run the procedure $N=29$ times. At every run, we pick a different BIC-ITD function from the population, which will be altered in one of the ways described above. We then take the average over the rest of the population, to which we fit a Gaussian template. Finally, the altered BIC-ITD function is compared to this Gaussian template, using a classical RMS error measure:

$$Fit\ quality = \sqrt{\frac{1}{N} \sum_{ITD} (BIC_{alt}(ITD) - BIC_{template}(ITD))^2}$$

After $N=29$ runs, we record the average and standard deviation of the fit qualities for the alteration type and alteration parameter value. The procedure is then replicated for each different alteration type as well as different parameter values. Note that in the case of alterations of the ITD scale, the alterations are applied on the analytically expressed Gaussian template rather than the altered BIC, which avoids having to interpolate.

On Figure 7, we also represent baseline variability (gray areas), which we measure by performing the exact same procedure, except without altering the chosen BIC at all. This is equivalent to estimating the mean and standard deviation of the fit quality to the average template over the whole population by bootstrapping.

Results

Variability of BIC DN1 peak recordings

We performed recordings of the BIC DN1 peak in 8 animals for 3 to 5 different experimental runs (Figure 3A), adding up to a total of 29 BIC recordings. There was no significant difference between the amplitude of ABRs evoked by left and right ear presentation (as measured by the RMS, Wilcoxon signed-ranked test, $Z = 141$, $p = 0.04$), and the mean absolute difference was small ($0.014 \mu V$, about 10% of the mean ABR RMS). Consistent with previous reports (see Introduction), within-subject BIC DN1 peak measurements can vary by $\sim 0.08 \mu V$ (average standard deviation, s.d.) over different recording sessions, when the maximal BIC amplitude is of about $0.8 \mu V$ on average (Figure 3A). Although small in absolute magnitude, this variability is actually quite sizeable, in that it represents up to 25% of the total modulation of the BIC amplitude vs. ITD curve. Similarly, when the animals are compared on the basis of their average DN1-to-ITD trace across sessions (Figure 3B), the inter-subject variability is even greater, with DN1 values at midline ITD ranging from 0.4 to $1.2 \mu V$: a three-fold increase between the most extreme animals. As a result of these two types of variability, the standard deviation over the whole

population (0.17 μ V, Figure 3C) is about 30% of the total modulation of the BIC DN1 peak across ITDs (0.6 μ V).

To investigate the source of BIC DN1 peak variability, we attempted to tease apart the influence of ITD and the variability due to experimental factors. We therefore fitted every individual curve using a bell-shaped curve (Gaussian kernel, see Figure 4B and Methods). The bell-shapedness of the kernel describes the influence of ITD (ITD_0 , the position of the peak, and σ , the width of the curve), while the baseline and modulation values of the curve do not depend on ITD (A, modulation of the curve, and B, baseline value – i.e. at large ITDs). For all animals and experimental runs, we find that this model is a very good fit to the data, with r^2 on average 0.9 ($\pm$ 0.05 s.d.) over the population (minimum r^2 = 0.73).

We hypothesized that experimental factors influence the amplitude of the DN1 peak (parameters A and B), while only marginally affecting the relationship between ITD and DN1 peak (ITD_0 and σ), as the latter represents a fundamental property of binaural brainstem processing. We reason that the same experimental factors will affect the SNR of monaurally evoked ABRs, which can be indirectly measured by the RMS of the monaural ABR signals. To test this hypothesis, we perform linear regressions between the parameters of the Gaussian fits and the RMS of monaurally evoked ABRs (the average of left and right monaural ABR RMS, Figure 4C-F). As expected, the baseline and modulation of the fitted Gaussian kernels are very well correlated with the monaural ABR RMS (baseline B, r^2 = 0.65, modulation A, r^2 = 0.83, Figure 4 C-D). Regression slopes are significantly different from zero (both $p < 10^{-5}$), while the intercept is not (A, p = 0.71, B, p = 0.18), suggesting a linear relationship between amplitude parameters and RMS. There was no significant subject-specific effect on the slopes or intercept of the fits, which may however be due to the low number of repeated experiments per subject (all individual subject slopes within a 95% confidence interval bound of their population counterparts). On the other hand, shape parameters (ITD_0 and σ , affecting only the relation of the BIC to ITD) are not systematically affected by the ABR RMS (ITD_0 , r^2 = 0.04, and σ , r^2 = 0.16, Figure 4 E-F). None of these regression slopes were different from zero (ITD_0 , p = 0.29, and σ , p = 0.03). Consistent with previous reports, ITD_0 parameters were not significantly different from zero (intercept of linear regression p = 0.2), which indicates that for all tested animals the DN1 amplitude was indeed maximal for zero ITD (mean ITD_0 was -12μ s $\pm$ 63 s.d.). Similarly, σ (the width of the curve) was distributed around 412 μ s ($\pm$ 100 μ s s.d.), close to twice the physiological range for guinea pigs (about 250 μ s, Greene et al, 2014). In other words, DN1 amplitude continues to be modulated by ITDs well outside the range that the animal would be expected to normally experience.

BIC amplitude normalization strategies

The linear relationship between the ABR RMS and amplitude parameters of the DN1 peak to ITD relationship (Figure 4 C-D) suggests that it is possible to nearly completely remove the effect of ABR RMS on the BIC. Indeed, simply dividing BIC DN1 amplitude by the ABR RMS should account for most of the DN1 amplitude variability. This procedure yields a unitless DN1 value, which we represent on Figure 5 for all subjects and experimental days. Within and across subjects (Figure 5A and B respectively), the variability is visibly reduced

compared to raw data alone. By construction, the resulting BIC values should be correctly fitted by a bell shaped curve with baseline and modulation equal to the slopes of the linear regressions obtained on Figure 4C and D (see Methods). Finally, the population average represented on Figure 5C shows that the standard deviation across the population is reduced when compared to the modulation of the average curve across ITDs.

It can be hard to compare the raw and normalized data, because they are expressed in different units. A direct comparison of the numerical values of the population-wide standard deviations of Fig 3C and 4C therefore does not make sense. Our goal, however, is to maximize the modulation of the BIC by ITD. A possible method for comparison is thus to express the value of the BIC amplitude at each ITD in units of the number of standard deviations above the lowest BIC amplitude (found at large ITDs). This is represented on Figure 6A for the raw data (black line) and data normalized to the ABR RMS (blue line). In this representation, we can see that the normalization procedure provides roughly a two-fold gain in the modulation of the BIC-vs-ITD relationship. In other words, if we were hypothetically given BIC recordings at two unknown ITDs, we would be able to resolve ITD twice as precisely in the normalized data as opposed to the raw data.

Normalizing BIC amplitude to ABR Wave III amplitude

Another strategy that has been put forward and perhaps most often used in previous studies (e.g. Cone-Wesson et al. 1997) is to normalize the BIC value using component/peak amplitudes derived from monaurally recorded ABRs (Figure 1). Wave III is the most prominent peak in guinea pig ABRs and is thus easy to detect and measure. This is especially true in comparison to Waves I and II, and particularly given the present electrode montage (Figure 1B) which is typically used to record the BIC in animal subjects. Furthermore, Wave III is thought to have its origin in a monaural structure (the medial nucleus of the trapezoid body, MNTB, Tsuchitani 1994; Jalabi et al. 2013; Koka & Tollin 2014) and thus does not contribute to the BIC waveform (i.e. it is equal to Wave III calculated from the sum of the two appropriately time-shifted monaural waveforms), in contrast with Wave IV/V (Figure 1).

We evaluated this strategy by computing the Wave III amplitude for each recording session from the binaural ABRs at 0 ITD, and dividing the BIC DN1 peak amplitude recordings accordingly for normalization. The obtained normalization method also yields a great increase in the resolution of the BIC vs. ITD relationship (Figure 6A, green line), of the same order of magnitude as the previous RMS based method. The measure of a Wave III amplitude should be affected by many factors common to the measure of the ABR RMS. That is, we expect that if the overall energy in a monaural ABR is increased (i.e., the RMS), then so should be the Wave III peak. It is unsurprising, then, that the Wave III amplitude is well correlated with the ABR RMS amplitude (Figure 6B). The good fit between those values, as well as the similar normalization performance may indicate that they are generally equivalent. However, we argue that RMS is a more direct measure of the overall signal-to-noise ratio to be used for normalization. Furthermore Wave III in some species is reduced or absent (Jalabi et al. 2013; Kulesza & Grothe 2015), and peak magnitude measurements often require a manual identification. On the other hand, ABR RMS is well-defined regardless of

the precise shape and peak morphology of ABRs for that species, and can be objectively computed.

Testing for abnormal BIC amplitudes

An important motivation for the study of the DN1 peak is its potential application as a diagnostic tool for binaural hearing impairment. That is, it is important to be able to detect when a given DN1-to-ITD relationship curve is “abnormal” when compared to a population of normal-hearing subjects or when compared in repeated measurements in an individual. Binaural processing impairments have been suggested to be reflected in alterations of the BIC of the ABR. A potential avenue we seek to address here is alterations of the BIC-to-ITD relationship. Such alterations could be based upon the two transformations depicted in Figure 2: a reduction in BIC amplitude or a lateral shift of the DN1 to ITD relationship. In addition, the width of the relationship is linked to the underlying processing of ITDs and ILDs by neurons in the auditory brainstem (Ungan et al 1997, Riedel and Kollmeier, 2002); we therefore also decide to investigate the detection of changes in width. We study how one can detect a BIC signature altered in any of those three ways.

To do so, we pick a given DN1 to ITD relationship from a single measurement (Figure 7A), apply some alteration to it, and then compare it to a Gaussian template, extracted from the average of the other DN1 to ITD relationships in our population ($N-1 = 28$). We use the root mean squared error over ITDs as a fit quality measure (higher values are indicative of a poorer fit). This procedure is then iterated for the N possible partitions of the initial measurement set, and for different values of the alteration parameter and type. Figure 7B-G represents the distributions of RMS error values as a function of alteration parameter value, for different alteration types (columns), and both normalized (top row, blue) and raw BIC values (bottom row, red). The “baseline” fit quality represents the average RMS error between a non-altered DN1 to ITD relationship to all others in the data set (gray areas on Figure 7B-G).

The panels on Figure 7 can be read as follows: when for a given parameter the colored and gray areas overlap, the altered BIC has a fit quality value within the range of the expected noise in our normal population. That is, it is no more different from the average of the population of normal BICs than any unaltered BIC. When, on the other hand, the shaded areas do not overlap, the fit quality obtained is two standard deviations away from results expected in our normal population. The altered BIC is therefore more different to the average BIC of the population than unaltered BICs: it is then possible to decide that the altered BIC relationship is “abnormal”. This is not a proper test *per se*, and it may make sense to ask for more separation from the noise level — or enforce certain false positive/etc. rates — but these questions are beyond the scope of the present study. Rather, here we wanted to qualitatively demonstrate the improved resolution in the DN1-vs-ITD relationship after normalization.

For the three alterations tested in this study, we find that the normalized data allows for a much better resolution to detect a difference than the raw data. For *multiplicative scaling*, on normalized data we are able to distinguish a reduction or increase of 20% of the BIC vs ITD relation (Figure 7B, insert). While a reduction of more than 50% (or a two-fold increase) is

necessary to detect abnormality on the raw data (Figure 7E, insert), the results are even more striking when the alteration is a lateral (peak BIC-to-ITD) shift of the BIC vs ITD curve. Using the raw data, no shift of this relationship, even as large as 1 or 2 ms can be distinguished, while in contrast the normalization allows for the detection of a lateral shift in this relationship curve as low as 250 μ s (Figure 7 C,F). Similarly, whereas scaling of the width of the curve is undetectable using the raw data even for very large changes, a doubling or halving of curve width becomes detectable after normalization (Figure 7 D,G).

Discussion

General conclusions

We performed repeated recordings of the BIC DN1 amplitude in guinea pigs while varying ITD. Consistent with previous studies, we find BIC DN1 amplitudes varied considerably both between- and within- subjects across recording sessions, despite consistent ABR thresholds. That is, ABRs and BIC varied although there was no indication of any change in the hearing capabilities of the animals across test session. As a result of this variability, a corrective strategy must be applied to BIC data so as to allow for the detection of changes to the BIC waveform. We provide such a strategy in the context of the BIC DN1 to ITD amplitude relationship.

Investigating the source of BIC DN1 peak variability, we find that the relationship between BIC and ITD is well described by a Gaussian model. Furthermore, we find that BIC DN1 amplitudes correlate highly with the RMS of the parent ABR waveforms, while the shape of the dependence on ITD does not. Together, these results confirm our hypothesis that common experimental factors (e.g. electrode depth, contact quality, small variations in electrode placement and earphone location, etc.) are likely responsible for the variability in the amplitude of the recorded ABR waveform (i.e. affecting SNR), and in turn the BIC DN1 peak amplitude. While monaural and binaural amplitudes changed concordantly between tests, the overall shape of DN1 amplitude dependence on ITD did not. This suggests that the BIC — and the DN1 potential in particular — reliably probes the brainstem mechanisms of binaural hearing, even under recording conditions that vary slightly from test to test. As a result, normalization is indeed an appropriate strategy to compensate for across measurements variability.

Strategies for comparing DN1 within and across subjects and recording sessions: RMS vs prominent peak component

In order to be compared to one another, raw BIC DN1 amplitudes should be normalized so as to correct for amplitude variations that can be wholly attributed to the parent ABR waveforms. As demonstrated in Figure 7, such a strategy allows for finer comparison and assessment of changes to the BIC waveform. Any measure of the amplitude of parent ABR waveforms could in principle be appropriate for use in normalizing BIC amplitudes. And indeed we find that both the RMS amplitude of monaural ABR waveforms (Figure 4), as well as wave component amplitudes (Wave III, Figure 6B), predict maximal BIC DN1 peak amplitudes. As a consequence, we find that normalizing to a component (e.g., Wave III) or to the RMS provides an equivalent gain in resolution (Figure 6A).

This is generally consistent with some earlier studies that have applied normalization strategies in attempts to compensate for inter-subject variability of the ABR and BIC (e.g. Cone-Wesson et al. 1997; Fowler & Leonards 1985; Fowler & Broadard 1988; McPherson et al 1989). In those studies, normalization relative to the corresponding component (i.e. peak) in the [Left + Right] sum (i.e. sum of monaural waveforms) was performed. Cone-Wesson et al. and Fowler et al. (1988) both similarly applied this strategy, normalizing the equivalent of the DN1 peak to the summed monaural Wave V peak amplitudes, measured peak-to-peak. McPherson et al. applied a similar strategy using group means of waveforms to derive DN1 and wave component amplitudes, setting the summed monaural Wave V peak amplitude equal to “100%”. In theory, since ABR waveform components scale together, it should be equivalent to normalize BIC DN1 to any ABR component that is reliably present without being sensitive to binaural cues. This is supported by Edwards et al. (1982) (human subjects) who found no significant difference in Wave I to Wave V amplitude ratio either within subjects (2 recording sessions in one sitting or separated by several months) or between subjects, since relative peak amplitudes meeting these criteria remain constant under non-pathological conditions.

In summary, strategies of normalization to prominent ABR wave peak components are indeed appropriate for facilitating comparisons and, as we have shown, provide a similar advantage as normalization to RMS amplitude of the ABR under normal-hearing conditions. However, species differences in ABR waveform morphology and relative peak amplitudes can make wave-based normalization more labor-intensive and subject to inter-rater reliability issues. Ultimately, the use of peak amplitudes is impractical, as they cannot always be reliably automatically recovered with hardware or software analyses. Use of an RMS-amplitude-based normalization strategy bypasses these issues; it can be fully automated and is completely objective. Furthermore, some pathology-related changes in ABR waves (such as broadened wave) may be accounted for by RMS but not by peak amplitude based measures. In the end, the relevant quantity with which the BIC should be normalized is the signal-to-noise ratio of the monaural ABR, adequately measured by the RMS amplitude (after averaging) that captures the energy in the ABR waveform. As shown above, this strategy achieves excellent definition in the DN1-ITD amplitude relationship (Figure 4) and brings variable recordings into alignment for comparison both within and across subjects. In conclusion, since it does not rely on the prominence, presence or identifiability of specific peaks or components for comparisons, we argue that RMS normalization is better across species, and in general when the ABR waveform morphology is a concern.

Importantly, normalizing by any effective method, including RMS normalization, enhances the ability to detect changes to the BIC. As exemplified by RMS normalization in Figure 7, normalization provides a critical advantage when assessing ABR recordings for differences from a normalized population. Thus, it is important to account for sources of variability between recordings by normalizing before making comparisons. When a strategy is implemented that employs use of both monaural waveforms, it does not depend on the binaurally-evoked signal and thus cannot contain variability attributable to the origin of the BIC and thus cannot confound the computation of the BIC. Also, such a strategy takes variability attributable to the signal from either left or right ear inputs into account. Small

changes to the BIC that are not attributable to the ABR evoked by either the left or right ear will be evident and easily identified.

Use of the BIC DN1 peak amplitude vs ITD function as a diagnostic tool

Clinical and diagnostic use of the BIC has been previously suggested, since DN1 is robustly evoked in nearly all normal-hearing subjects (Dobie & Norton 1980; Kelly-Ballweber & Dobie 1984; Levine 1981; Riedel & Kollmeier 2002a), but is altered in various disease states that affect binaural hearing (Gunnarson & Finitzo 1991; Delb et al. 2003; Fowler & Swanson 1988). This possibility has, however, been hampered by a documented unreliability of BIC amplitudes both for a given subject as well as between groups (Kelly-Ballweber & Dobie 1984; Wilson et al. 1985; Stollman et al. 1996). We have shown here that it is possible to account for and remove this common but systematic variability due to common factors in ABR recording. This variability removed, the BIC holds promise as a test for the detection of abnormal or altered DN1 amplitudes or abnormal DN1 modulation by ITD and ILD (Furst et al. 1985; Furst et al. 1990; McPherson & Starr 1995; Riedel & Kollmeier 2002a). This capability could be employed for detection of long-term behavioral binaural processing deficits (Gunnarson & Finitzo 1991; Delb et al. 2003), hearing dysfunction related to multiple sclerosis (Hausler & Levine 1980; Furst et al. 1990; Fowler & Swanson 1988), assessment of developmental and functional brainstem integrity (Hosford-Dunn et al. 1981; Jiang & Tierney 1996), and optimization of bilateral cochlear implants (Gordon et al. 2012) or bilateral hearing aids (Purdy et al. 2004).

To conclude, for the purpose of comparing BIC component amplitudes between or within subjects in different recording sessions, the optimal strategy for facilitating accurate comparisons and detecting changes to the BIC waveform is the objective method of normalizing amplitudes to the RMS amplitudes of left and right-evoked monaural ABR waveforms. If this strategy is not feasible due to equipment or other concerns, a more subjective strategy based on identification and measurement of a prominent wave peak is an alternative option, so long as the selected peak does not vary with binaural hearing.

Acknowledgments

Sources of funding:

This work was supported by NIH Grants R01-DC011555 (DJT, ATF, VB), F30-DC013932 (ATF) and P30NS041854 (custom hardware design).

We thank Kelsey L. Anbuhl for contributing illustrations for Figure 1. We thank Dr. Michael Hall for preparing custom hardware (P30NS041854).

ATF designed and performed experiments. VB and ATF analyzed the data. ATF and VB wrote the manuscript. DJT designed the research and provided guidance and critical revisions. All authors discussed the results and implications and commented on the manuscript.

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Figure 1.

BIC recordings and reproducibility. A) Definition of the BIC: ABR waveforms obtained from monaural stimulations (blue: left, red: right) are summed to obtain the “Sum” waveform. The BIC is obtained by computing the difference between the waveform obtained by binaural stimulation (green) and the “Sum” waveform. When ITD is present in binaural stimulation, monaural waveforms are appropriately time-shifted prior to summation to match the binaurally presented ITD. The BIC amplitude is the amplitude of the DN1 peak. B) Electrode montage: The vertex-nape electrode montage employs an active electrode at the vertex and a reference electrode at the nape of the neck, with a third ground electrode placed at a distal site (here, at the hind leg). Both electrodes are midline, receiving symmetric contributions to the ABR signal and emphasizing central generators of the ABR waveform. C) Amplitude of the BIC DN1 peak measured as a function of ITD in one animal, over a range of ITD cues spanning ± 2 ms. Different curves represent the same experiment conducted on 4 different days.

Figure 2.

BIC amplitude at 0 ITD is affected by long term hearing loss (see text, blue and green dots), which could be the result of two transformations of the BIC amplitude to ITD relationship (blue curve): a global *scaling* of BIC DN1 amplitude across ITDs (A, green curve), or a lateral shift of the relationship curve toward nonzero ITDs (B, green curve).

Figure 3.

Reproducibility of the relationship between BIC and ITD. A) BIC DN1 peak amplitude plotted as a function of ITD for eight animals, with 3-5 repeated measurements per animal. Shaded regions represent ± 1 standard deviation of DN1 amplitude from the mean. B) Mean DN1 peak amplitude versus ITD cue is plotted for each of eight animals above. C) Population mean for DN1 peak amplitude is plotted versus ITD for eight animals, with ± 1 standard deviation for the population mean depicted in gray.

Figure 4.

Model of the relationship between BIC and ITD. A) Gaussian fits to DN1 amplitude against ITD for each day for each animal (different days are represented by different shades); B) Schematics of the four parameters resulting from the Gaussian fit. A and B are magnitude parameters expressed in μV , ITD_0 and σ are expressed in ms. C) Scatter plot of the value of A for each measured BIC curve (all animals, all repetitions) plotted against the average RMS of the response to monaural stimulations (see text). D) Same as panel C, for the values of B. E) Same as panel C for the values of ITD_0 , F) Same as panel C for the values of σ . The mean of monaural ABR RMS waveform amplitudes = $(L_{RMS} + R_{RMS})/2$.

Figure 5.

DN1 amplitudes normalized to the average monaural ABR RMS are consistent and have ample amplitude range across physiologic ITDs. A) Normalized DN1 amplitude from individual experiments is plotted versus ITD per animal with ± 1 standard deviation shown in gray; Theoretical range of BIC amplitude (see Text) is depicted with dashed lines. B) Mean DN1 peak amplitude within subjects normalized to $[L_{RMS} + R_{RMS}]$ is plotted versus ITD cue for each of eight animals above; C) Normalized population mean for DN1 peak amplitude is plotted versus ITD for eight animals, with ± 1 standard deviation for the population mean depicted in gray. Systematic variability is reduced, enough that subtle changes to DN1 amplitude in the physiologic range of ITD cues are more easily detected.

Figure 6.

Wave III amplitude normalization. A) Comparison of different normalization. Each curve represents the BIC amplitude measured in number of standard deviations across the population (see Text). Blue curve is normalization to monaural ABR RMS, green curve to Wave III amplitude, and black curve is raw data. Normalization provides a greater dynamic range across ITDs. B) Wave III amplitude at 0 ITD correlates strongly with the monaural ABR RMS defined above.

Figure 7.

Testing for abnormal BIC. A) Schematics of the simulation procedure. Top row: A given BIC vs ITD recording is picked out of all the available ones ($N=29$), and altered in one of three ways (additive or multiplicative scaling, ITD shifting). Bottom row: The rest of the population's BIC recordings are averaged, and a Gaussian fit is applied to the average BIC to obtain the "template". Bottom-right corner: the altered BIC is compared against the "template", and the RMS error recorded. This procedure is then replicated by picking another BIC to alter, and/or varying the parameter of the alteration. See Methods for details. B-G) Each panel displays the fit quality as a function of the alteration parameter (colored shaded area: line is mean, area is ± 1 s.d.). In each panel the baseline variability (see Text) is represented (gray area). For parameter values where the colored and gray areas do not

overlap it is easy to distinguish the altered BIC from the population. B-D uses normalized BIC values, E-G uses the raw data.

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