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Research paper

Application of frequency modulated chirp stimuli for rapid and sensitive ABR measurements in the rat

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ABSTRACT

Rodents have proven to be a useful model system to screen genes, ototoxic compounds and sound exposure protocols that may play a role in hearing loss. High-throughput screening depends upon a rapid and reliable functional assay for hearing loss. This study describes the use of a frequency modulated (FM) chirp stimulus as an alternative to the click to derive a rapid assessment of auditory brainstem response (ABR) threshold in the rodent. We designed a rising frequency A-chirp based upon the spatial mapping of preferred frequency along the rat basilar membrane to provide a more synchronous and equipotent input across the length of the cochlea. We observed that the ABR wave I and wave IV amplitudes evoked by the A-chirp were significantly greater than the click and that A-chirp minimum response thresholds were lower than the click. Subsequent analyses compared the efficacy of the A-chirp to linear, time-reversed and amplitude-reversed chirps and confirmed that the A-chirp was most effective chirp configuration. These data suggest that the A-chirp may be optimally suited as a single screening broad-frequency stimulus for rapid ABR threshold estimations in the rodent and could serve to complement more detailed frequency-specific physiologic and behavioral estimates of hearing threshold.

1. Introduction

The past decade has seen an increase in the number of researchers using rodent models for studies of auditory system function. Whether this trend is motivated by the advent of molecular tools to study the genetic basis for hearing or because rodents permit experimental designs in which larger numbers of drugs or sound exposure regimens can be screened for their peripheral or central effects, there is an impetus to develop valid and reliable tools to assay hearing thresholds in rodents. While the behavioral audiogram will remain the gold standard for precise measurements of hearing threshold, there is a continuing need to further optimize methods for rapid functional assays of hearing threshold to complement high-throughput screening for genes or biochemical compounds implicated in hearing loss. Rapid measurements based on unconditioned behaviors such as pre-pulse inhibition or electrophysiological measurements such as the auditory brainstem response (ABR) are well suited to this purpose.

The ABR offers several advantages as a rapid metric for assessing hearing loss in rodents: (i) it is not subject to potential motor deficits that may arise from genetic manipulations or exposure to noxious compounds; (ii) ABR affords researchers the opportunity to ascribe hearing deficits to deficiencies in distinct temporal components of the waveform. Small lesions positioned at different points along the central auditory neuroaxis have been demonstrated to have a differential impact on the ABR response; lesions in nuclei closer to the periphery have the greatest impact on earlier temporal components, whereas lesions in nuclei positioned further from the periphery relate most directly to later portions of the ABR waveform (Melcher and Kiang, 1996).

ABR estimates of hearing threshold are typically performed either with tone burst stimuli, which provide estimates of frequency-specific hearing thresholds, or a click stimulus which, by virtue of its rapid onset and broadband frequency composition, is thought to synchronously activate a broader region of the basilar membrane, thereby providing a rapid stand-alone estimate of hearing threshold. However, in the cochlea, the response to a click stimulus is not entirely synchronous; the peak of the response occurs several milliseconds later in low frequency bands relative to high frequency bands due to the time-dependent propagation of the traveling wave between the oval window to the helicotrema (von Bekesy, 1960). Moreover, the spectral composition of a click does not make it an ideal broadband stimulus; clicks have periodic nulls in their spectra at frequencies equal to integer multiples of 1

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divided by the duration (i.e. for a 50 μs click every 20 kHz) and typically feature high frequency roll-off on the order of 20 dB/octave for sound frequencies near the center of the rodent hearing range (Gorga and Thornton, 1989; Gorga and Neely, 1994). These observations reveal some of the potential shortcomings of a click stimulus for ABR estimates of hearing threshold and suggest potential points of improvement for other types of broadband stimuli.

Fortunately, alternatives to a click stimulus have been developed that provide a more synchronous and equipotent activation of the basilar membrane from apex to base (Dau et al., 2000; Elberling et al., 2007; Fobel and Dau, 2004; Shore and Cullen, 1984; Shore and Nuttall, 1985). These frequency modulated (FM) stimuli, often designated as "chirps", consist of a rising frequency stimulus that produces simultaneous displacement along the cochlear partition. By sweeping from low to high frequencies at a rate that synchronizes the arrival time of each frequency component with the corresponding position of maximal excitation along the basilar membrane, chirps can circumvent problems with phase cancellation and loss of synchronization that occur with click stimuli (for review see Elberling et al. (2007). Although originally developed in the guinea pig (Shore and Cullen, 1984; Shore and Nuttall, 1985), chirps have been used most extensively in studies of human ABR (Dau et al., 2000; Elberling et al., 2007; Fobel and Dau, 2004). Given the importance of developing rapid measurement methods for hearing threshold in the rodent, the present study was undertaken to adapt and test the chirp stimulus for ABR recording in the rodent. As a first step, we characterized the frequency-specific latency shifts in the ABR to guide the development of chirp stimuli optimized for the rat basilar membrane (Experiment 1). Next, we compared the efficacy of several different types of chirp stimuli with a click based on ABR amplitude (Experiment 2) and ABR threshold (Experiment 3).

2. Methods

2.1. Animal preparation

Fourteen healthy young adult *Rattus norvegicus* (age 8–12 weeks) were used in the study. In the majority of cases, two separate measurements were obtained from stimuli delivered to the left and right ears. All surgical procedures were approved by the Institutional Animal Care and Use Committee at Vanderbilt University. Rats were anesthetized with pentobarbital sodium (50 mg/kg followed by supplements at 20–40% of the initial dose as necessary) or a combination of ketamine and medetomidine (100 mg/kg and 0.3 mg/kg, respectively, followed by supplements at 20–40% of the initial dose as necessary). Respiratory rate, corneal and hind-paw reflexes were monitored routinely to maintain an aflexive state. Body temperature was maintained near 37.3 °C with a rectal probe and homeothermic blanket system (Fine Science Tools). Rats were placed in a head clamp manufactured in house that allowed unimpeded access to the external auditory meati. The external ear and surrounding temporal muscle were excised to allow direct access to the tympanic membrane (TM).

All experiments were conducted in a double-walled sound attenuating booth (Acoustic Systems). Auditory stimuli were delivered monaurally to each ear via an electrostatic headphone (Stax) coupled to custom designed hollow ear bars that fit snugly into the cranial opening of the ear canal, terminating approximately 0.5 mm from the TM. Acoustic calibration was performed in situ with a 1/4-in. microphone (Brüel & Kjær) sealed to the hollow ear bar to ensure a flat frequency response (±3 dB SPL) with minimal harmonic distortion (THD < 2%) over a range of frequencies spanning 0.5–60 kHz. All stimuli were calibrated at peak SPL to minimize duration-dependent effects on sound spectra (Burkard, 2006). ABRs were recorded with platinum sub-dermal needle electrodes (Grass) positioned at the vertex (positive), proximal to the bulla of the stimulated ear (negative), and in the neck musculature (ground). Auditory stimuli were repeated 1000 times each at rate of 27.7 Hz. Filters were set at 100 and 3000 Hz. ABR signals were acquired, filtered, amplified, and analyzed with equipment and software manufactured by Tucker–Davis Technologies.

2.2. Auditory stimuli

ABR measurements were obtained with three types of auditory stimuli: tone bursts, clicks and frequency modulated chirps.

2.2.1. Tone and click stimuli

Tone bursts (2 ms duration, Blackman window with 1 ms rise/fall) were used to characterize changes in wave IV latency for varying frequency and intensity (Fig. 1). Tone bursts were presented from 1 to 32 kHz (1/3 octave increments) at sound levels ranging from 5–65 dB peak SPL (10 dB increments; Fig. 1). Clicks were 50 μs positive polarity square wave pulses.

2.2.2. Chirp stimuli

As illustrated in Fig. 2A, four different chirp stimuli were used in this study: A latency-adjusted rising frequency chirp (A-chirp), a linearly increasing rising frequency chirp (L-chirp), a rising frequency chirp with the inverse slope of the A-chirp (Flip Chirp) and a time-reversed A-chirp (Reverse Chirp). Lastly, A-chirp and L-chirp stimuli were presented at various sound levels either by presenting an attenuated signal in which the rate of frequency change had been adjusted to account for level-dependent variations observed in tone burst ABR latencies (adjusted, Fig. 2B) or by simply attenuating a single stimulus derived from latency measurements at the highest intensity (65 dB peak SPL) without adjusting for level-dependent latency shifts (unadjusted).

The rat A-chirp stimulus was developed according to the methods used by Fobel and Dau (2004) for the A-chirp in humans with the exception that tone burst ABR latency measurements were based on wave IV rather than wave V. In small animals, the comparatively short length of the auditory nerve prevents identification of human wave II (Moller, 1983). Therefore, wave IV in the rat is approximately equivalent to human wave V (Zhou et al., 2006). The basilar membrane group delay was indirectly estimated by the following power-law relation

$$\tau_b = a + bc^{-i}(f^{-d})$$

where i is the tone burst intensity, f is the tone burst frequency, a reflects the frequency and level-independent neural component of

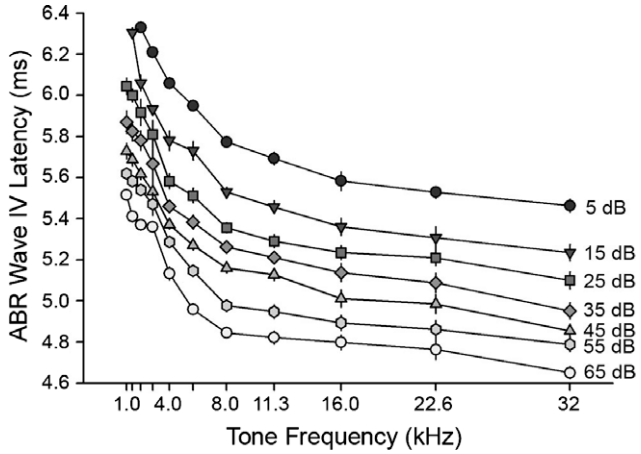

Fig. 1. Frequency- and intensity-dependent shifts in ABR latency. Tone bursts were used to characterize wave IV latency for frequencies ranging from 1 to 32 kHz and from 5 to 65 dB peak SPL.

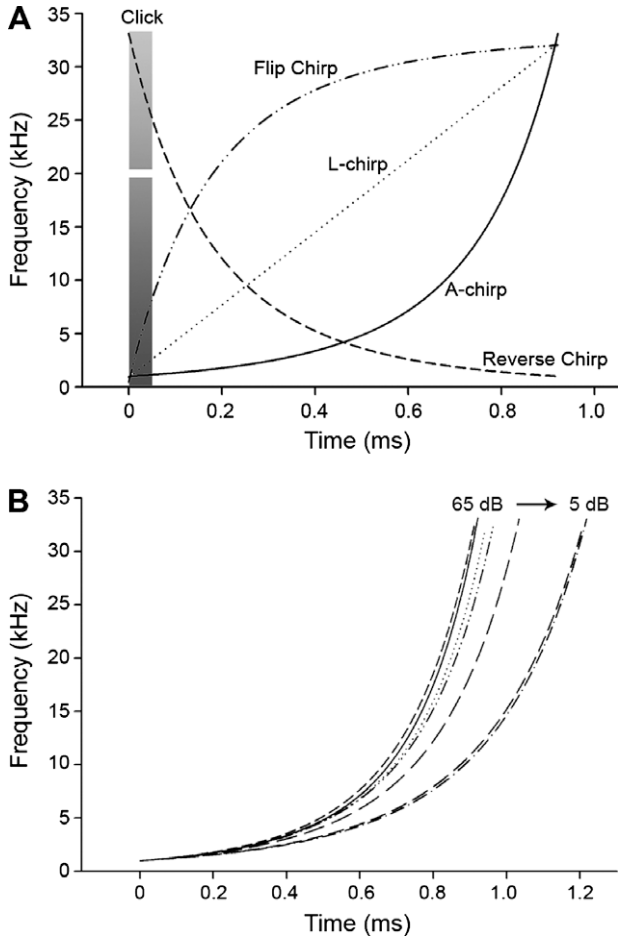

Fig. 2. Spectrotemporal profile of five stimuli used for ABR analysis. (A) The FM characteristics of the A-chirp is represented by the exponent of the power function fit applied to the ABR latency shift at 35 dB peak SPL. The L-chirp, flip chirp, and reverse chirp each provide a means for testing the efficacy of the specific FM characteristics present in the A-chirp. The spectrotemporal profile of a 50 ms click stimulus is also provided for comparison. Note the characteristic null at frequencies equal to integer multiples of 1 divided by the duration (i.e. 20 kHz for a 50 μs click). Also note the characteristic high frequency roll-off (approximately 8 dB/octave, represented by grayscale gradient). (B) Differences in the instantaneous frequency functions of A-chirps adjusted for tone burst ABR latency shifts for sound levels ranging from 5 to 65 dB SPL.

the latency, and bc^{-i} (f^{-d}) reflects the mechanical component of the latency due to the propagation in the cochlea, as described previously (Anderson et al., 1971; Fobel and Dau, 2004; Neely et al., 1988). The data from this equation were recast by subtracting "a" which reflects neural latency, therefore $\tau_{bm} = bc^{-i}(f^{-d})$ (rat a = 3.8 ms, comprised of 1 ms inner hair cell synaptic delay + 2.8 ms for difference between wave I and IV) representing the basilar membrane group delay. A curve was fit using a two parameter power equation, where bc^{-i} was considered as one parameter k. Thus, the equation was readjusted to

$$\tau_{bm} = k(f^{-d})$$

which was used to represent the basilar membrane group delay at each individual intensity from 5 to 65 dB peak SPL in 10 dB increments. Once k and d were established at each intensity, the inverse function and instantaneous phase $\varphi_A(i,t)$ were calculated

$$\varphi_A(i,t) = \frac{2\pi(k^{-i})^{\frac{1}{d}}}{\frac{1}{d}-1}\left[\frac{1}{(t_\sigma(1)-t)^{\frac{1}{d}-1}} - \frac{1}{t_0(1)^{\frac{1}{d}-1}}\right]$$

Next, the amplitude factor was calculated as:

$$A_A(i,t) = \sqrt{\frac{(K^{-i})^{\frac{1}{d}}}{d[t_0(i)-t]^{\frac{1}{d}-1}}}$$

to produce a stimulus with a flat magnitude spectrum. Finally, the A-chirp stimulus was given by

$$S_A(i,t) = A_A(i,t)\sin[\varphi_A(i,t) - \varphi_0]$$

The L-chirp was created by fitting the ABR tone burst data with a linear function rather than a power function but was otherwise identical to the A-chirp. The reverse chirp was created similar to the A-chirp, except now the stimulus is simply reversed, high frequency sweeping to low frequency. The temporal progression is reversed with the frequency parameter. Finally, the flipped chirp is a variation of the A-chirp in that the rising frequency nature of the chirp is maintained, however the temporal progression is reversed. The temporal change associated with low frequencies is applied to the high frequencies and the vice versa.

The envelopes of all chirp stimuli were shaped with raised cosine gating functions to reduce spectral splatter artifacts. In all cases, frequencies contained within the rising (0.3 ms) and falling (0.05 ms) portions of the chirp stimulus were outside of the 1–32 kHz frequency range of interest to these experiments. Lastly, the time-varying components of each stimulus were filtered by the inverse of the loudspeaker's impulse function to compensate for the frequency transfer function of the loudspeaker.

2.3. Experiments

The study was conducted in three sequential experiments. In Experiment 1, tone burst (1 kHz to 32 kHz in 1/3 octave increments) ABR measurements were made to determine the frequency- and intensity-dependent (65 down to 5 dB peak SPL in 10 dB increments) shifts in wave IV latency that would allow us to optimize the FM component of the chirp to best fit the frequency selectivity of the rat basilar membrane (N = 6). The overall length of the basilar membrane and the spatial mapping of preferred frequency from apex to base differ among species. Designing chirp stimulus that is most appropriate for a given animal model must take these factors into account. Since the time required for the traveling wave to reach the apex of the basilar membrane is greater than that required to reach the base, the latency of auditory potentials elicited by low frequency tone bursts is greater than high frequency tone bursts. The extent of this latency shift, therefore, can be used as an indirect estimate of the length and frequency mapping of the basilar membrane when direct post-mortem measurements of basilar membrane mechanics are not possible (Elberling et al., 2007; Fobel and Dau, 2004; Ruggero and Temchin, 2007; Shore and Nuttall, 1985).

In Experiment 2, wave I and wave IV amplitudes were compared between click and chirp stimuli and between two different anesthetic regimens (N = 12). By presenting a low to high frequency FM chirp in accordance with measurements from Experiment 1, we hypothesized that we could create an optimized acoustic stimulus for the rat that would provide a more temporally coincident and spatially coherent activation of the cochlea than a conventional broadband stimulus such as a click. We further hypothesized that this difference would produce a more synchronized volley of action potentials along the auditory nerve, resulting in a higher amplitude ABR. In order to test these hypotheses, frequency–latency functions were fit with a power function (the A-chirp) and compared to chirps based on a linear fit (L-chirp) as well as two chirps designed to explicitly contradict the frequency–latency measurements made in Experiment 1: an amplitude-reversed chirp (Flip Chirp) and a time-reversed chirp (Reverse Chirp) as shown in Fig. 2A. ABR amplitude measurements

were made from wave I, which has a peripheral origin likely generated by the spiral ganglion cells and wave IV, which has a central origin thought to predominantly emanate from the superior olivary complex (Melcher and Kiang, 1996).

Although measurements of ABR amplitude are arguably more sensitive metrics of stimulus efficacy, the minimum sound level eliciting a reliable ABR response (i.e. the threshold) is commonly used as an indirect estimate of hearing threshold. As a final step, we sought to compare the threshold sound levels for the A-chirp, L-chirp and click. Therefore, in Experiment 3 minimum threshold measurements for chirp and click stimuli were performed ($N = 5$).

2.4. Data analysis

ABR amplitudes were calculated by measuring the difference between the positive peak in waves I and IV and the subsequent local minima. ABR threshold was defined by identifying the lowest sound level that could reliably produce a stimulus-evoked peak that followed the progressive trend for decreasing amplitude and increasing latency observed over a range of sound levels ranging from 40 to −2 dB peak SPL (2 dB increments). Amplitude and threshold measurements were confirmed by two external and blinded expert reviewers. At least two of three reviewers agreed on amplitude and threshold for each response. When differences did exist they were no greater than 2 dB. Data were analyzed using mixed design ANOVA followed by linear contrasts with specific error terms and invoking Greenhouse–Geisser epsilon based correction. The Bonferronni correction factor was used in all post-hoc pairwise tests in which multiple comparisons were made against the same data sample. In these instances the reported p values reflect this adjustment. Descriptive statistics are reported as means ± standard error of the mean.

3. Results

3.1. Experiment 1

In Experiment 1, tone burst ABR measurements were made to determine the frequency- and intensity-dependent shifts in wave IV latency that would allow us to estimate the basilar membrane group delay of the chirp stimuli. We observed that wave IV latencies elicited by 65 dB peak SPL tone bursts decreased by a total 0.91 ms as the stimulus frequency increased over a five octave range from 1 to 32 kHz ($F = 163.1$; $p < 0.001$, Fig. 1). Moreover, the slope of this frequency–latency function can change according to sound level due to changes in the time constant for mechanical and/or synaptic summation at low sound intensities. As expected, we observed an increase in the overall latency of wave IV with decreasing sound level ($F = 407.5$; $p < 0.001$) in addition to a slight increase in the overall latency shift at lower sound levels (0.91 versus 1.2 ms for 65 and 5 dB peak SPL, respectively).

3.2. Experiment 2

In Experiment 2, we derived four different types of FM chirp stimuli based on the tone burst ABR measurements performed in Experiment 1 and compared them to each other and to a click stimulus (Fig. 2A). We observed that ABR wave I amplitude was significantly greater for A-chirp stimuli presented at 65 (Fig. 3A) or 35 (Fig. 3B) dB peak SPL than every other stimulus type ($F > 12.1$, $p < 0.01$ for each post-hoc pairwise comparison). The wave IV A-chirp-evoked amplitude at 65 dB peak SPL, by contrast, was not greater than other stimulus types (Fig 3C). In fact, the A-chirp wave IV amplitude was significantly weaker than the L-chirp-evoked response ($F = 18.2$, $p < 0.005$) but not significantly different

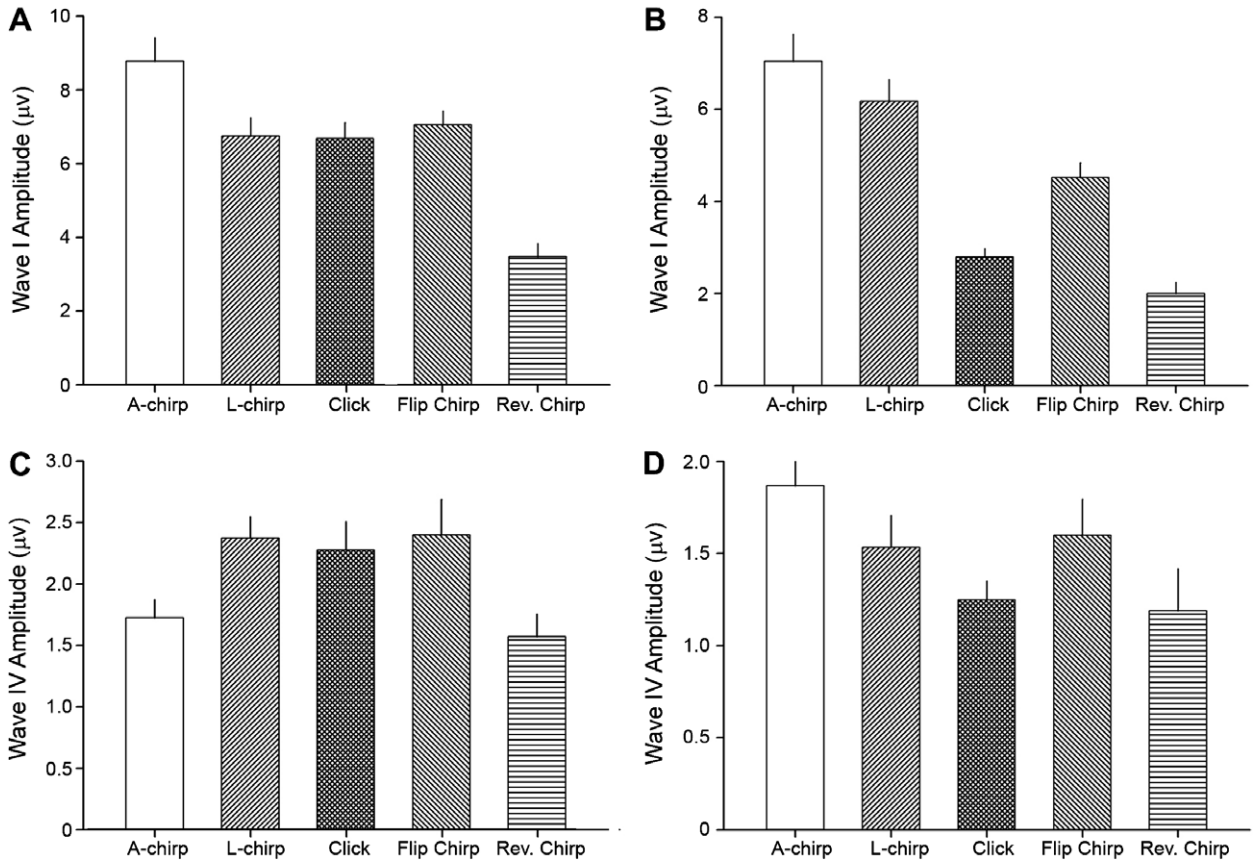

Fig. 3. Comparison of ABR amplitudes evoked by chirp and click stimuli. Wave I (A,B) and IV (C,D) amplitudes obtained from 65 (A,C) and 35 (B,D) dB peak SPL A-chirp, L-chirp, flip chirp, reverse (Rev.) chirp and click stimuli.

from any of the remaining stimulus types ($F < 4.4$, $p > 0.06$ for each remaining comparison). However, at 35 dB peak SPL the wave IV amplitude differences were more similar to that observed with wave I in that the A-chirp was significantly greater than all other stimulus types ($F = 7.67$, $p < 0.01$ for each pairwise comparison; Fig. 3D). Subsequent analyses revealed that the A-chirp amplitudes were not significantly different between pentobarbital versus ketamine anesthetic protocols ($F = .616$, $p > .547$), nor were any systematic differences observed between recording/stimulation from the left versus right ear ($F = 2.060$, $p > .167$). Accordingly, subsequent analyses combine data collected from both anesthetic regimens and hemispheres.

As a next step we compared ABR amplitudes between the two most promising chirp stimuli (A-chirp and L-chirp) with a click stimulus over a broader range of sound levels. As illustrated in Fig. 4A, we observed a significant main effect for wave I amplitude between stimulus types across all sound levels ($F = 169.6$, $p < 0.001$). The increased amplitude of wave I elicited by the A-chirp compared to the L-chirp and both chirps relative to the click was clearly evident at higher stimulus intensities but still preserved even at the lowest intensity (Fig. 4A inset, A-chirp versus L-chirp, $F > 12.0$, $p < 0.01$ for all pairwise comparisons). The poor performance of the A-chirp on wave IV amplitude described in Fig. 3C was not supported when our analysis was extended to a broader range of sound levels in agreement with the differences at 35 dB peak SPL presented in Fig. 3D. In fact, when a comparison of wave IV amplitudes was made excluding the highest sound level, we observed a significant main effect for stimulus types that followed the same trend as that observed for wave I ($F = 7.487$, $p < .005$). The superiority of the A-chirp wave IV amplitude over the L-chirp and the L-chirp wave IV amplitude, in turn, over the click was upheld even at the lowest sound level in keeping with the wave I data (Fig. 4B inset, $F > 8.9$, $p < 0.05$ for all pairwise comparisons). Therefore, the diminished wave IV response obtained with the A-chirp is specific only to the 65 dB SPL stimulus and likely reflects an artificially shortened latency from the tone burst ABR due to an upward spread of activation with higher levels of stimulation (Elberling et al., 2007).

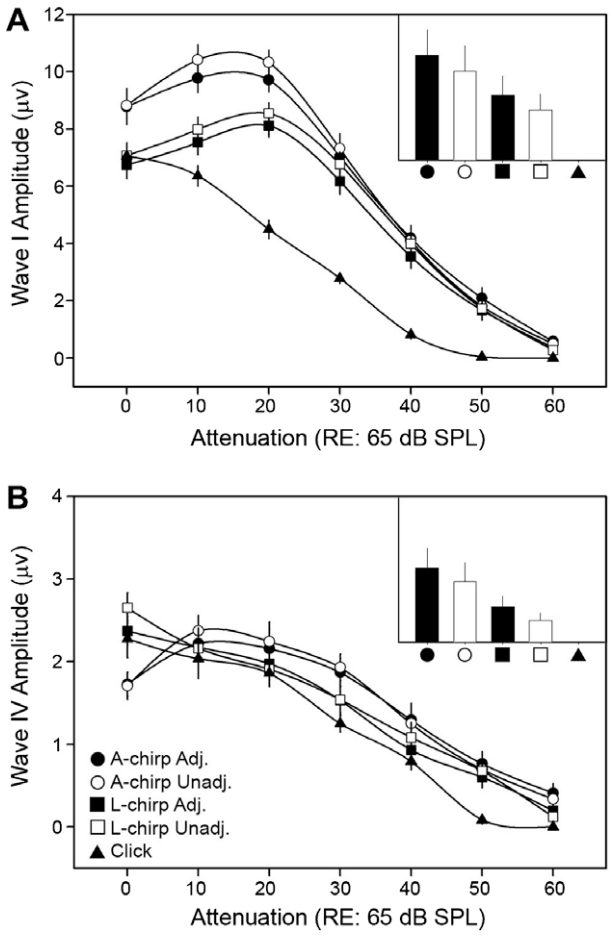

Fig. 4. Amplitude-Level function comparisons for chirp and click stimuli. Comparison of ABR wave I (A) and IV (B) amplitudes for the A-chirp (circles), L-chirp (squares) and click (triangle) for intensities ranging from 0 to 65 dB peak SPL. Responses for each sound level are provided for chirp stimuli adjusted (filled symbols) or unadjusted (open symbols) for the basilar membrane group delay documented at each sound level. Insets: ABR amplitudes documented at 5 dB peak SPL. Symbols correspond to those provided in legend. Ordinate scale = 0–0.8 μv for both graphs.

Recall from Experiment 1 that the shift in ABR latency across a five octave range of tone frequencies was slightly more pronounced when tones were presented at 5 dB peak SPL (1.2 ms) than 65 dB peak SPL (0.91 ms). This raises the possibility that optimizing chirp stimuli for the rat must not only take the latency-frequency mapping into account, but it must do so independently for each sound level. This idea was tested by comparing the wave I and wave IV amplitudes for the A-chirp and L-chirp across a range of sound levels, when the chirp stimuli derived from latency shifts observed at the highest intensity were simply attenuated (unadjusted) versus recalculated for the specific latency shift at every sound level and then attenuated (adjusted, Fig. 2B). As shown in Fig. 4, adjusting for this small shift in the estimated basilar membrane group delay across sound levels did not have a major impact on the ABR waveform amplitudes. Subsequent analysis demonstrated that the slightly enhanced efficacy of the unadjusted stimulus on wave I amplitude was attributable to differences at higher sound levels, where the latency adjustment is comparatively minor, and was attributable to a difference in the L-chirp ($F = 13.7$, $p < 0.005$), not for the A-chirp ($F = 0.6$, $p = 0.46$).

3.3. Experiment 3

The results from Experiment 2 indicated that rising frequency chirp stimuli (A-chirp, L-chirp and Flip chirp) were indeed capable of eliciting higher amplitude ABR responses than the conventional click stimulus. Moreover, they demonstrated that effective stimuli could be derived from ABR latency measurements obtained at a single sound level with subsequent signals simply attenuated rather than having to recreate the specific frequency–latency basilar membrane group delay estimates for each sound level. Since ABR threshold is commonly used for rapid measurements of hearing, as a final step we sought to compare the threshold sound levels for the A-chirp, L-chirp and click. Analysis of ABR thresholds revealed a significant difference between the three stimulus types wherein the A-chirp stimulus evoked the lowest threshold response (8.0 ± 2.45 dB peak SPL), followed by the L-chirp (12.0 ± 2.61 dB peak SPL) and lastly by the click (23.2 ± 1.02 dB peak SPL; $F = 17.5$, $p < 0.05$; Fig. 5). Post-hoc pairwise contrasts indicated a significantly lower threshold for the A-chirp compared to L-chirp $F = 20.0$, $p < 0.05$, while the L-chirp had a significantly lower threshold than the click ($F = 12.3$ $p < 0.05$).

4. Discussion

We have demonstrated that an ABR-based FM chirp stimulus designed for use in humans can be adapted to the rat to elicit higher amplitude ABR waveforms and a more sensitive estimate of hearing threshold than a click. In keeping with previous reports, we compared the relative efficacy of chirps derived from linear models of basilar membrane frequency mapping (L-chirp) to

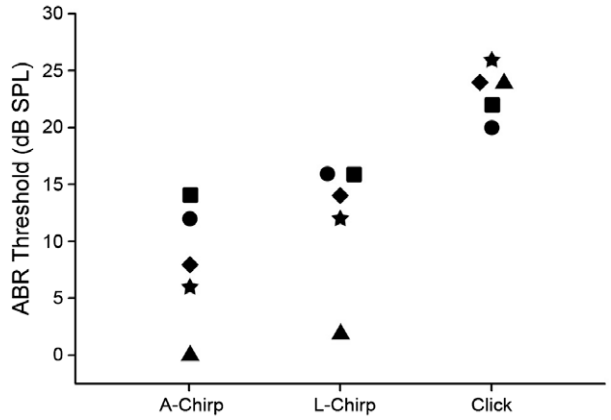

Fig. 5. ABR threshold estimates for chirp and click stimuli. Thresholds for the A-chirp, L-chirp and click were obtained from five separate subjects (each subject is represented as a unique symbol).

non-linear fits of ABR-based frequency mapping based on a power function (A-chirp) (Elberling et al., 2007; Fobel and Dau, 2004; Neely et al., 1988; Shore and Nuttall, 1985). Specificity to the optimized stimulus configuration was further examined by comparison to time-reversed and amplitude-reversed chirps (reverse chirp and flip chirp). Overall, our analysis revealed that the A-chirp was the optimal stimulus for ABR amplitude and threshold in accordance with comparisons made in humans (Fobel and Dau, 2004).

We also compared the effect of adjusting the FM sweep speed (and therefore the duration of the stimulus) to fit the level-dependent increases in ABR latency shifts between low and high frequency stimuli. We did not observe a significant difference between the adjusted and unadjusted versions of the chirp stimuli. This might be attributable to the observation that there was a strong interaction between sound level and the slope of the frequency–latency function in humans, whereas these functions were nearly parallel over a variety of sound levels in the rat (Fig. 1 versus Neely et al., 1988). In support of this point, adjusting the A-chirp for sound level had a far more significant impact on the overall stimulus duration and slope of the instantaneous frequency functions than it did on the overall shape of the A-chirp (Fig. 2B). In this sense, our failure to find a significant effect for the sound level adjustment underscores the limited contribution of extended duration stimuli on the ABR and reinforces the idea that ABR amplitude is primarily indicative of spectral composition of the stimulus and shape of the onset envelope rather than the duration of the envelope plateau (Dau et al., 2000; Hecox et al., 1976).

The importance of spectral composition was further supported by the relatively poor response to the reverse chirp and flipped chirp stimuli. Despite the similar rapid onset envelopes for each chirp stimulus, the highest amplitude responses were only observed for stimuli that modulated the rate of frequency transition in accordance with the ABR tone burst measurements. The contributions of sound spectra were particularly exaggerated at lower sound levels, where the shape of the amplitude envelope is likely to contribute less (Figs. 3B,D and 4). Similar level-dependent relationships have been observed in the human chirp literature and have been related to a loss of frequency specificity and cochlear upward spread of masking at higher sound intensities (Dau et al., 2000; Fobel and Dau, 2004; Elberling et al., 2007).

We also observed that minimum thresholds were significantly lower for A-chirp stimuli than L-chirp and click stimuli. In fact, thresholds were not only lower on average for A-chirp stimuli but in comparing each of the five data points collected for our threshold analysis, one can observe that every subject abides by the same ordinal relationship between the three stimuli. It is also interesting to note that the variance in threshold is greater for the A-chirp than the click. While at first glance this may seem disadvantageous, it raises the possibility that the A-chirp may be more sensitive to intersubject variation across frequency that is observed in frequency-specific measurements of hearing threshold such as the behavioral audiogram, tone burst ABR, or the auditory steady-state response that would otherwise be masked with an analysis restricted to click stimuli (Chiappa et al., 1979; Gorga et al., 1988; Picton et al., 2005; Stockard et al., 1979).

These data raise the possibility that the A-chirp, because it provides a more complete readout of the basilar membrane, is more sensitive to small differences to peripheral hearing status than the click. This possibility could be pursued in future studies that titrate the degree of mechanical or sensorineural damage to the cochlea and document the relative sensitivity of chirp versus click stimuli. Additionally, future research might also explore the use of masking paradigms and band-limited chirp stimuli to identify a middle ground between a broad spectrum chirp and single tone burst. This would afford researchers the opportunity to examine changes in hearing thresholds attributable to restricted regions cochlea or its efferent targets without the time commitment of frequency-specific tone burst ABR threshold mapping.

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