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Objective auditory threshold estimation using sine-wave derived responses

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A derived response method of acquiring frequency specific auditory evoked potentials that utilizes a pure tone in combination with a toneburst is applied to the measurement of hearing sensitivity in guinea pigs, chinchillas and pocket gophers. Two experiments which demonstrate that thresholds acquired via tone-derived responses are 10 to 15 dB more sensitive than thresholds to solitary tonebursts are described. The derived potentials approximate behaviorally acquired thresholds at frequencies of 0.5 kHz and above. This technique may provide a more rapid means of assessing hearing sensitivity in laboratory animals than by behavioral means.

Auditory brainstem response; Action potential; Threshold; Tonebursts; Sinusoids; Guinea pig; Chinchilla; Pocket gopher; Animal hearing

Introduction

Acquiring behavioral thresholds to sine waves in laboratory animals can be a time-consuming procedure. Although electrophysiological recording methods provide an objective measure and may be less time consuming, these procedures can be invasive and lack frequency specificity. Continuous sine waves cannot elicit synchronous discharges sufficient to measure action potentials and tonebursts with fast rise times usually fail to generate responses closer to tonal threshold than 15 to 20 dB. In addition, the energy of fast-rise tonebursts is spread across a considerable region of the cochlea, thus limiting frequency specificity (e.g. Davis et al., 1984; Gorga et al., 1988).

Various methods have been suggested to overcome some of the spectral problems. These methods include high-pass masking of clicks (Don and Eggermont, 1978; Don et al., 1979) and presentation of tonebursts in notched noise (Picton et al., 1979; Stapells et al., 1985). While such methods have been shown to effectively eliminate participation from certain frequency regions, considerably greater spread of energy remains in comparison to pure-tone stimuli.

We recently reported a derived response method of acquiring frequency specific action potential and auditory brainstem response data that utilizes a pure tone in combination with a toneburst (Berlin et al., 1990). Briefly, derived responses are obtained by subtracting an evoked action potential acquired to a low-intensity toneburst from a combination of a toneburst plus a low-level sine wave centered at the same frequency

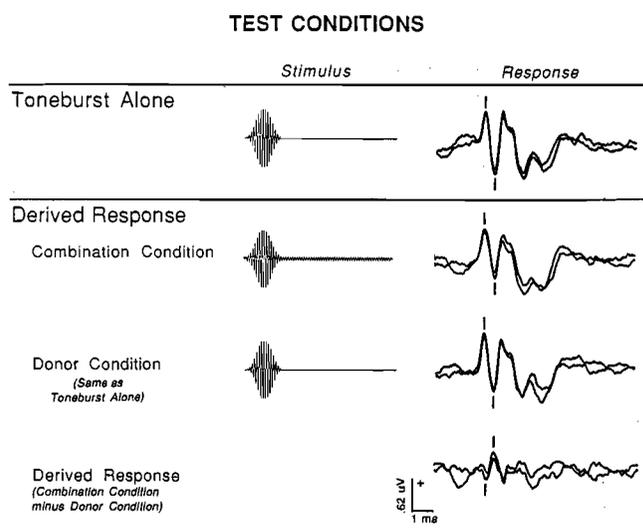


Fig. 1. Test stimuli and responses for the toneburst and derived response evoked potential test conditions described in this study. Stimuli are at 8 kHz and responses shown were obtained from one of the guinea pig subjects. Vertex positive is upward in this and all subsequent figures.

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(see Fig. 1). Consistent with the nomenclature suggested by Berlin et al. (1991), the two conditions contributing to the derived responses are noted as the donor (toneburst) and combination (toneburst plus continuous tone) conditions. Subtracting the donor response from the combination response yields the derived response. The utility of this technique in deriving frequency-specific action potentials at the round window has been reported recently (Salt and Vora, 1990).

Our purpose here was to investigate the feasibility of applying the non-invasive derived response technique to surface-recorded frequency-specific auditory brainstem responses. Such a technique would provide a baseline measure for longitudinal threshold studies as well as a method for quickly acquiring threshold data in animals who would then participate in other auditory studies. In this paper we report two experiments which demonstrate that derived responses provide a more sensitive measure of hearing than tonebursts alone and that they approximate behaviorally acquired thresholds for the frequencies between 0.5 and 32 kHz.

Experiment 1

Surface-recorded auditory brainstem responses were obtained from guinea pigs for solitary tonebursts and for tonebursts interleaved with continuous tone combinations. Thresholds obtained by the toneburst and derived response methods were then compared to behavioral hearing thresholds reported in the literature for guinea pigs (Heffner et al., 1971; Prosen et al., 1978).

Subjects

Eleven adult pigmented guinea pigs weighing between 200 and 300 g were subjects for this study. Each animal was sedated with chloral hydrate (up to 0.0076 cc/g of 5% chloral hydrate) for the duration of the testing and body temperature was maintained near 37°C using a rectal probe and an isothermic heating pad.

Methods

Two procedures were compared for their ability to produce thresholds approximating those obtained behaviorally. The first involved acquisition of intensity series for tonebursts alone centered at 13 frequencies from 0.25 to 18 kHz. Tonebursts had cosine-squared envelopes with 1 ms rise and fall times and a minimal plateau at 1 kHz and above; one cycle rise and fall times were used for 0.25 and 0.5 kHz. Tonebursts were generated by a SM700 Multi-Signal Generator component of a Nicolet Pathfinder II Evoked Potential Sys-

tem and were presented at levels well above threshold (approximately 70 dB peak sound pressure: SP) to just below threshold in 10 dB steps. The second method involved the derived response, which is the focus of this report. To obtain the derived responses two stimulus types were used: (1) a toneburst presented at a constant intensity of either 55 dB peak sound pressure or a level that was at least 20 dB above toneburst threshold (donor condition) and (2) a continuous tone presented at intensities that varied from below threshold to approximately 20 dB above derived response threshold (combination condition). Toneburst stimuli were centered at the frequency of the pure tones. The tonal stimuli used in the second stimulus condition were generated by a separate waveform generator (Hewlett-Packard Model 200CD Wide Range Oscillator) to avoid phase-locking between the pure tones and tonebursts. These tones were fed into a separate channel of the Nicolet SM700 system, mixed and attenuated individually. Stimuli were presented through an Etymotic Research ER-2 broadband insert earphone. Both stimulus conditions are illustrated in Fig. 1.

Stimuli were calibrated using a B&K 1/2" microphone and B&K Model 2610 Measuring Amplifier. An EAR foam plug with a narrow tube through it (of the type supplied with the insert earphones for clinical testing) was connected to the ER-2 earphone. The EAR plug was situated 1/4" from the face of the microphone in a 1/2" inner diameter polyethylene tube to simulate the guinea pig meatus. Stimuli were checked acoustically prior to testing and monitored electrically during testing using a Tektronix Model 2215 oscilloscope and a Dynascan Model 1803 frequency counter.

Surface recordings were obtained using a single-channel electrode montage with subdermal placement of needle electrodes at the vertex and at the dorsal midline between the bullae. Physiological activity picked up by the electrodes attached to the subject was monitored visually throughout testing using a Tektronix Model 2215 oscilloscope and auditorily with a loudspeaker. Stimuli were presented at 27.7 per second and recorded using the Nicolet Pathfinder II evoked potential system through a 0.1–3 kHz filter band across a 10-ms time window which was preceded by a 5-ms pre-stimulus baseline (15 ms total). Four replicate sets of 1250 sweeps for a total of 5000 sweeps were obtained for each of four test conditions and stored on disk for later analysis. The four conditions included one donor (toneburst alone) condition and three sets of combination conditions with different intensities of pure tones. Replicates of donor and combination conditions were interleaved with other test conditions. Interleaving of responses has been found to reduce artifacts resulting from changes in subject state over time (Berlin et al., 1991).

Results and Discussion

A toneburst intensity series for 8 kHz at intensities of 0 to 30 dB peak sound pressure is shown in Fig. 2 (left tracings). As expected, the toneburst latency-intensity function shows an increase in latency with decreasing intensity. For comparison, an example of an intensity series for the derived response technique is shown in Fig. 2 on the right. In this series the donor toneburst was presented alone at 55 dB peak sound pressure and in combination with tones from -10 to 20 dB SP. The donor toneburst response was subtracted from the combination response to obtain the derived response. As the intensity of the tone decreases, the amplitude of the difference trace decreases. In contrast to the toneburst alone latency-intensity series, no shift in latency is observed as the intensity of the continuous tone decreases (refer to Fig. 2).

The lowest intensities eliciting responses were determined for each method and plotted as a hearing threshold curve. Criteria for acceptance of a response were replicability, appropriate latency region, decreasing amplitude with decreasing intensity, peak-to-peak amplitude of at least $0.05 \mu\text{V}$, and response amplitude that exceeded the peak-to-peak amplitude of the pre-stimulus baseline. In addition, the latency of the toneburst waveforms had to increase in latency at progressively lower intensity levels to be acceptable. This latency criterion was not necessary for the derived responses since latency does not change as a function of intensity with the derived responses (Berlin et al., 1991). In fact, derived responses were generally easier to identify because the latency of the response was predictable.

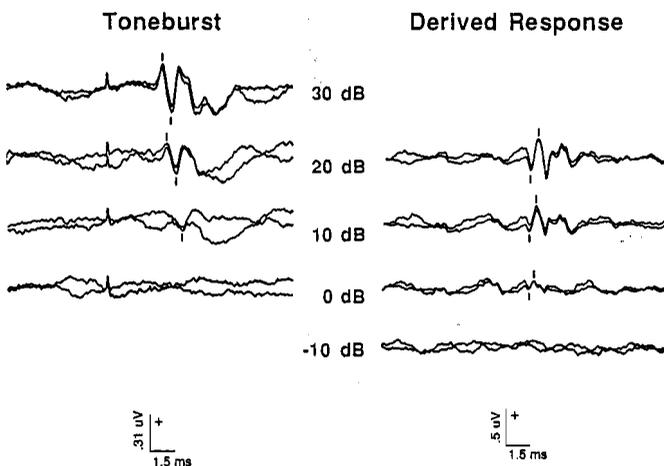


Fig. 2. Toneburst and derived response intensity series at 8 kHz for a guinea pig. Responses are shown by the vertical markers and tracings include a 5 ms pre-stimulus baseline. Thresholds are identified at 10 dB peak SP for toneburst stimuli and at 0 dB peak SP for derived responses.

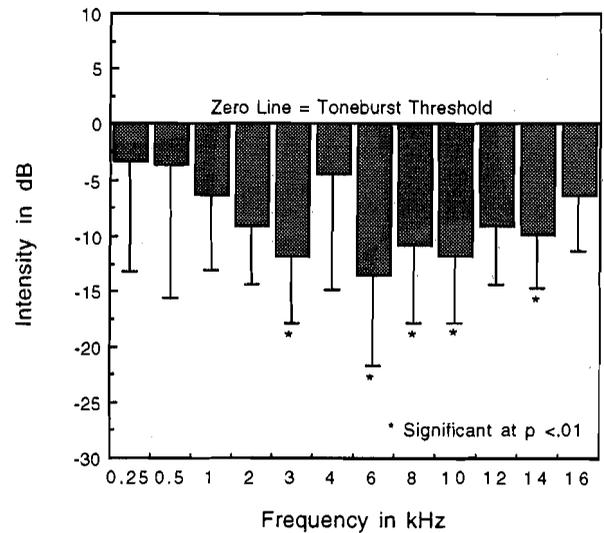


Fig. 3. Comparison of evoked potential thresholds from 0.25 to 16 kHz shows that derived response thresholds were more sensitive than toneburst thresholds. The zero line represents toneburst threshold for each frequency and the filled bars indicate the difference between toneburst and derived response thresholds (one standard deviation is shown by the line). $N = 11$ guinea pigs.

Fig. 3 shows a comparison of thresholds obtained using the toneburst and derived response methods. Toneburst thresholds ranged from 4.5 to 35 dB for frequencies between 0.25 and 16 kHz with the best thresholds observed at 4 kHz. Derived response thresholds ranged from 0.9 dB to 33.3 dB for the frequencies from 0.25 to 16 kHz with the best thresholds observed at 8 and 10 kHz. The average difference between thresholds obtained using tonebursts versus derived responses across the frequency range from 0.25 through 16 kHz was 8.2 dB. With the exception of 4 kHz, greater differences were noted in the mid frequencies than in the lowest or highest frequencies tested.

Repeated measures analysis of variance indicated that tone-toneburst thresholds were significantly better than toneburst thresholds ($F = 88.65$; $df = 1,10$; $P < 0.00001$). Thresholds at various frequencies also differed significantly ($F = 39.14$; $df = 11,110$; $P < 0.00001$) with low-frequency thresholds (0.25, 0.5 and 1 kHz) generally poorer than thresholds for the higher frequencies. A significant interaction was also observed for stimulus type (toneburst, tone-toneburst) and frequency ($F = 2.83$, $df = 11,110$; $P = 0.003$). Range tests indicated that thresholds obtained with the tone-toneburst method were significantly better ($P < 0.01$) than with the toneburst alone method for the frequencies of 3, 6, 8, 10 and 14 kHz. Threshold differences at 2 and 12 kHz nearly reached the same significance criterion ($P = 0.013$). Thus, with the exception of 4 kHz, greater differences between methods were noted in the mid frequencies than in the lowest or highest frequencies tested.

Electrophysiological thresholds were compared to behavioral data reported in the literature for adult pigmented guinea pigs. Heffner et al. (1971) determined behavioral thresholds for frequencies between 54 Hz and 50 kHz and reported thresholds ranging from -10 to 52 dB SPL with best sensitivity at 8 kHz. Prosen et al. (1978) tested the range from 125 Hz to 52 kHz and reported behavioral thresholds ranging from -11 to 42 dB SPL with best sensitivity in the 8 to 12 kHz region. While differences exist between the behavioral threshold studies, particularly below 8 kHz, the overall configurations of the two audibility curves are similar.

Behavioral and toneburst thresholds are compared in Fig. 4. Toneburst thresholds for most subjects are poorer than behavioral thresholds below 2 kHz and in good agreement from 2 through 4 kHz. However, for 8 through 16 kHz the toneburst thresholds are again less sensitive and miss the area of best sensitivity in the guinea pigs' audibility curve. The derived response thresholds are compared to behavioral thresholds in Fig. 5. These thresholds are poorer than the behavioral thresholds below 1 kHz in some subjects. The derived response thresholds fell between the behavioral threshold curves of Heffner et al. (1971) and Prosen et al. (1978) through 4 kHz and followed the behavioral audibility curve more closely than did the tonebursts for the frequencies up to 16 kHz. To summarize, derived responses were more sensitive than simple responses to tonebursts for all frequencies tested. Both toneburst and derived response thresholds agreed with behavioral thresholds from 0.25 through 4 kHz. De-

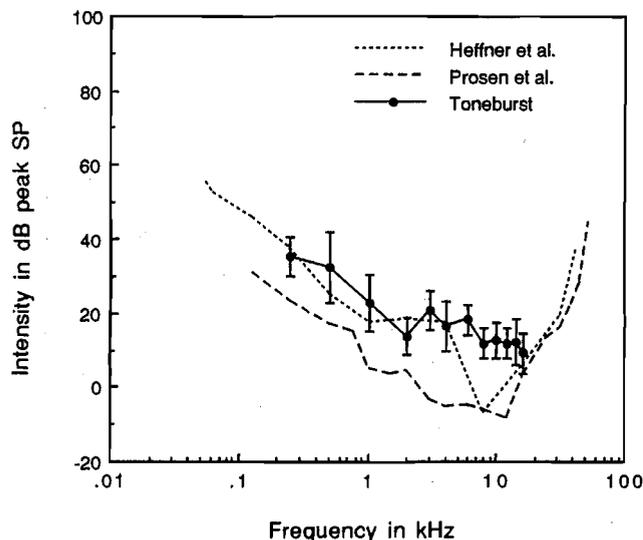


Fig. 4. Comparison of toneburst thresholds obtained in the present study with behavioral thresholds reported by Heffner et al. (1971) and Prosen et al. (1978) for guinea pigs. Mean and one standard deviation are shown for the tonebursts ($N = 11$).

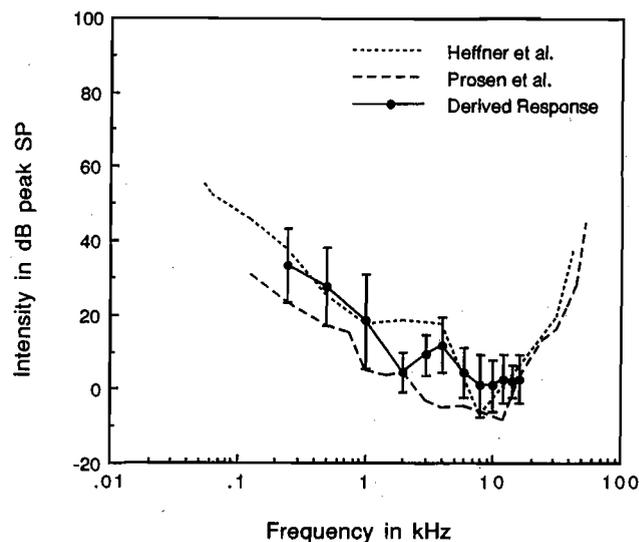


Fig. 5. Comparison of derived response thresholds obtained in the present study with behavioral thresholds reported by Heffner et al. (1971) and Prosen et al. (1978) for guinea pigs. Mean and one standard deviation are shown for the derived responses ($N = 11$).

rived responses proved more sensitive and followed behavioral thresholds more closely than tonebursts between 6 and 16 kHz.

Procedural differences between the behavioral measures and the current study existed in three areas that may have contributed to some of the observed differences. First, stimuli were presented via earphones in the current study while sound field presentation via a loudspeaker was used in both of the behavioral studies cited. Second, earphone stimulation was through a single earphone yielding a monaural mode whereas sound field testing used binaural presentation with the concomitant resonances of the external canal and pinna affecting sound levels delivered to the tympanic membrane. Finally, the toneburst stimuli were approximately 2 ms in duration while the tones used in the behavioral studies were up to 10 s in duration.

Experiment 2

In this experiment, both behavioral and evoked potential data were obtained from chinchillas and pocket gophers. To overcome some of the procedural differences listed above, we tested a series of animals using stimulus conditions as similar as possible to those used in the behavioral tests. Specifically, stimuli were presented via a loudspeaker to both ears of the animal. In the chinchillas, we also took the opportunity to obtain both behavioral and evoked potential measures in the same individuals.

Subjects

Two chinchillas, whose behavioral audiograms had been determined (Heffner and Heffner, 1991), were brought to Kresge Laboratory to permit acquisition of evoked potential data on the same animals. In addition, two pocket gophers, which have an unusually narrow region of hearing sensitivity were studied. Although behavioral thresholds were established on two female pocket gophers, two male pocket gophers were brought to Kresge Laboratory for testing because we did not want to risk losing trained individuals during transport or sedation. Threshold sensitivity curves for chinchillas have been known for some time (Miller, 1970) whereas auditory thresholds for pocket gophers were only recently reported (Heffner and Heffner, 1990).

Methods

Behavioral thresholds were previously established (Heffner and Heffner, 1991) using a conditioned avoidance response to stimuli presented through a loudspeaker. Surface-recorded auditory brainstem responses were acquired at Kresge Laboratory for tonebursts and for tone-toneburst combinations also presented in sound field through a loudspeaker. The behavioral audiograms were not revealed to the persons carrying out the electrophysiological tests until the tests were completed.

For the evoked potential recordings, the animals were sedated and monitored using the same techniques as in Experiment 1, and tested with stimuli ranging in frequency from 62.5 Hz through 45 kHz. Tonebursts with cosine-squared envelopes of 1 cycle rise and fall times below 1 kHz and 1 ms rise and fall times at 1 kHz and above were used. The stimulus envelope was set to the smallest plateau allowed by the stimulus generating equipment. An Mi^2 system generated the signals above 16 kHz. For the tonebursts alone, intensity varied from well above threshold to 10 dB below visual detection threshold of the response with 10 dB as the narrowest step. Tone-toneburst combinations were composed of a toneburst presented at 20 dB above threshold for the toneburst alone and continuous sinusoids that varied around threshold in 10 dB steps. For one-half of the test conditions, continuous sine waves were presented simultaneously with the tonebursts (combination condition); tonebursts were presented alone for the other conditions (donor condition). Stimuli were presented binaurally in sound field using a Sansui Network loudspeaker driven by an SAE amplifier for the frequency range of 0.062 through 8 kHz and an LTV EST-2 capacitance speaker driven by a Krohn-Hite amplifier that produced a 300 V DC offset for frequencies from 16 through 45 kHz. The

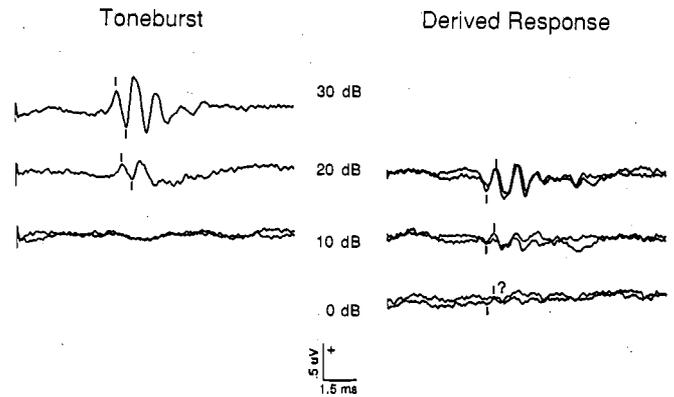


Fig. 6. Intensity series obtained from a chinchilla for tonebursts centered at 4 kHz at 10, 20 and 30 dB SP (left) and for derived responses at 4 kHz using a 55 dB peak SP toneburst and 0, 10 and 20 dB peak SP tones (right).

speakers were placed 30 cm from the animal's ears at ear level and 0 degree incidence. The intensity was measured in the region of the animal's ears using a 1/4" B & K microphone and B & K Model 2610 Measuring Amplifier.

Surface recordings were obtained using subdermal placement of needle electrodes at the vertex, dorsal midline between the bullae, and a ground in the hind leg. Physiologic filters were set at 0.03 to 3 kHz for lower frequency stimuli and from 0.1 to 3 kHz for stimuli above 1 kHz. Stimuli were presented at 27.7 per second using a time window of 15 ms that incorporated a 5 ms pre-stimulus baseline. A total of five-thousand sweeps was accumulated for each condition in an interleaving manner as in Experiment 1.

Results and Discussion

Chinchillas

Examples of toneburst and derived response intensity series are shown in Fig. 6 for a chinchilla. Comparison of the behavioral and evoked potential thresholds for each of the chinchillas tested indicated best agreement between methods for the frequencies between 0.05 and 8 kHz (Fig. 7). Behavioral thresholds were generally about 15 dB more sensitive than toneburst thresholds and less than 5 dB more sensitive than the derived response thresholds. Thus, toneburst and derived response thresholds differed from each other by an average of about 10 dB with derived responses being more similar to behavioral thresholds. Differences between the two animals occurred at 16 and 32 kHz, where behavioral and derived response thresholds were similar for Chinchilla 1. However, behavioral thresholds for Chinchilla 2 were better at 16 and 32 kHz than evoked potential thresholds. Below 500 Hz, behavioral thresholds were more sensitive than either of the evoked potential threshold techniques.

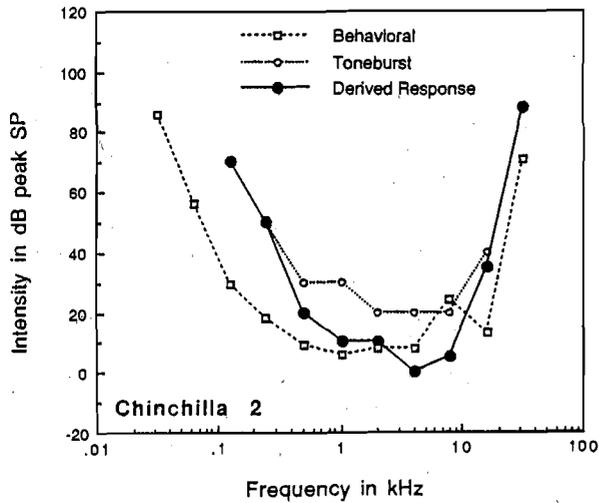
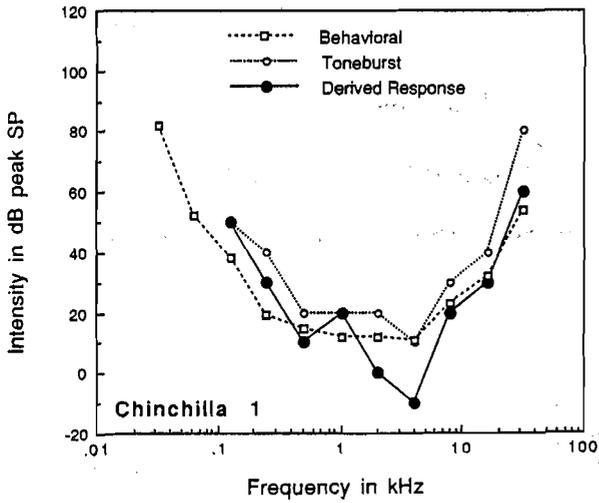


Fig. 7. Comparison of behavioral, toneburst and derived response thresholds obtained from two individual chinchillas.

Pocket gophers

Examples of toneburst and tone-toneburst derived responses are shown in Fig. 8 for a pocket gopher.

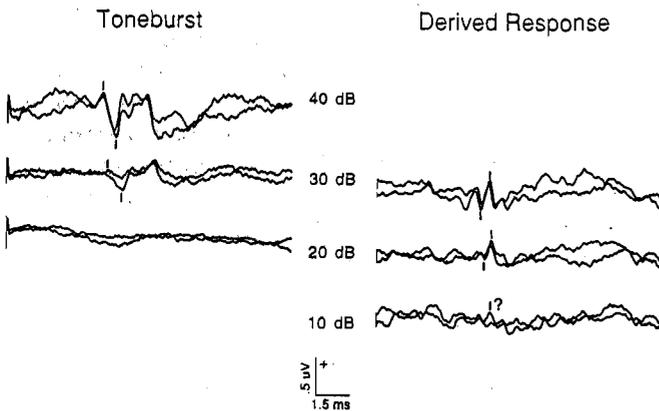


Fig. 8. Intensity series obtained from a pocket gopher for tonebursts centered at 2 kHz at 20, 30 and 40 dB peak SP (left) and for derived responses at 2 kHz using a 55 dB peak SP toneburst and 10, 20 and 30 dB peak SP tones (right).

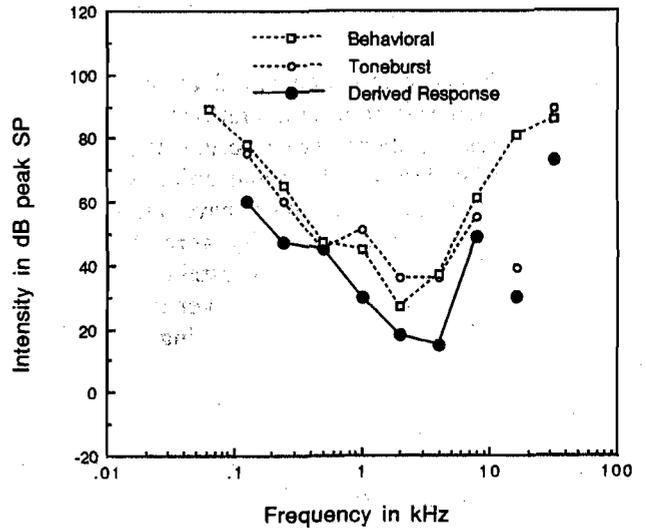


Fig. 9. Comparison of mean behavioral, toneburst and derived response thresholds for two pocket gophers.

Derived response thresholds for the pocket gophers were generally 10 to 15 dB more sensitive than either behavioral or toneburst thresholds across the frequency range of 0.125 to 32 kHz with the exception of a large and consistent difference at 16 kHz (Fig. 9).

Evoked potential thresholds at 16 kHz indicated 40 to 50 dB greater sensitivity than suggested by the behavioral thresholds. Since the behavioral thresholds showed a rapid decrease in sensitivity in the higher frequencies, we reasoned that if the data at 16 kHz represented a response from a frequency area below 16 kHz, then an increase in latency of the potential would be expected. To address this possibility, we plotted a latency-intensity function for the derived response data. As shown in Fig. 10, latency decreased predictably as frequency increased. We currently have no explanation

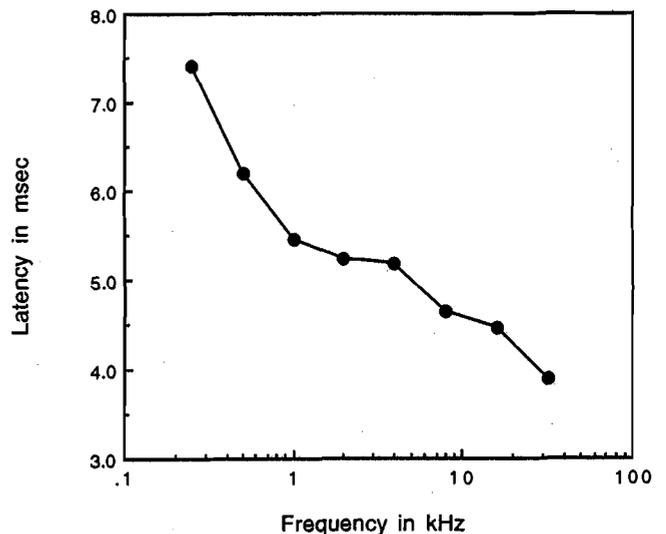


Fig. 10. Latency-frequency function for the pocket gophers based on derived responses.

for the discrepancy at 16 kHz although some possibilities include possible spectral spread associated with an abrupt high-frequency slope of hearing as demonstrated on the behavioral measures, or the possibility of a gating transient in the evoked potential system which might generate a spurious response. We also cannot exclude the possibility of a gender difference in hearing sensitivity at 16 kHz between the female gophers tested behaviorally and the male gophers tested with evoked potentials, but, if such a difference were to occur, it would be unique among mammals.

General Discussion

Data from these experiments indicate that derived responses yield thresholds that are approximately 10 dB more sensitive than those obtained using tonebursts alone. Derived responses appear most useful at 1 kHz and above. Lower-frequency responses, even in animals with good low-frequency hearing, are more difficult to detect which is a consistent feature of frequency-specific evoked potentials.

In a recent study, Salt and Vora (1990) compared action potentials recorded at the round window in guinea pigs using both toneburst and derived response methods. They reported that derived response thresholds averaged about 10 dB better than toneburst thresholds in the frequency range of 1 to 30 kHz with the greatest differences in the lower frequencies. Derived responses were approximately 10 dB poorer than the guinea pig behavioral thresholds reported by Prosen et al. (1978). Salt and Vora's results, obtained at the round window, are very similar to the current comparisons of toneburst and derived response thresholds. This is particularly encouraging since the current study utilized surface recordings in which the recording electrodes are even more remote from receptors. As mentioned in the previous discussion, the agreement of results with behavioral studies is dependent on the stimuli, presentation mode, and calibration among other factors. In fact, even comparison among behavioral studies shows differences on the order of 10 dB or more (cf Heffner and Heffner, 1991).

When the toneburst alone and derived response methods are compared for latency changes as a function of intensity, differences between methods are observed. When stimulating the cochlea with progressively more intense tonebursts, we expect the latency of the response to decrease as intensity increases. This presumably is due to a basalward spread of excitation. When the latencies of the derived responses are compared at various intensities, such latency changes with intensity are not observed (refer to Fig. 2). We consider this a very important feature because, in contrast to increasing the intensity (and therefore the side-

bands) of the tonebursts which brings in more basal elements quite rapidly, increasing the intensity of a single frequency sine wave may not activate many more basal units and may provide greater frequency specificity of measurement along the cochlear partition. However, it is also true that the latency is dominated by the donor stimulus and therefore latency/frequency specificity may be narrowed but not highly specific with the continuous sine wave.

When comparing the ability to identify responses near threshold using the standard toneburst and the derived potential techniques, two useful advantages are seen in the latter method. First, comparison of peak-to-peak amplitude for the toneburst and derived responses as a function of intensity showed that amplitudes of the derived responses (and presumably detectability) were greater than amplitudes of the toneburst responses. Second, since the latency of the derived response does not change as intensity is varied, the latency region in which a response should appear at progressively lower intensities is highly predictable (refer to Figs. 2, 6 and 8). This increases confidence in the isolation of responses visually and may accommodate automated identification techniques. However, it is also possible that a continuous tone may exert a mechanical effect on the basilar membrane sufficient to interfere with the response to a transient tone even when the continuous tone does not itself activate afferent fibers (and therefore is not heard). Therefore, signs of audibility may be confounded with small, near threshold mechanical disturbances of the cochlear partition.

Finally, the amount of time necessary to obtain threshold data using the derived technique is considerably less than that involved in behavioral training and threshold measurement. Complete threshold data for a single frequency were obtained in well under one hour in a quiet preparation. The assessment of frequencies across the audibility range obtained in the above experiments for each of the species was completed in two or three sessions, depending on the duration of sedation that the animal could safely tolerate. Because we are able closely to approximate behavioral audiograms both reported in the literature and obtained on the same individuals, the derived response technique may provide a means of assessing hearing sensitivity in laboratory animals in studies requiring rapid assessment of thresholds. This technique would be especially useful in situations where changes over short time periods are of interest.

Acknowledgements

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