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A comparison of behavioral and auditory brainstem response measures of hearing in the laboratory rat (Rattus norvegicus)

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A Comparison of Behavioral and Auditory Brainstem Response Measures of Hearing in the Laboratory Rat (Rattus norvegicus)

By

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Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Doctor of Philosophy Degree in Psychology

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The basic measure of an animal’s hearing is the behavioral, pure-tone audiogram, which shows an animal’s sensitivity to pure tones throughout its hearing range. Because obtaining a behavioral audiogram on an animal can take months, the tone-evoked auditory brainstem response (ABR) is often used instead to obtain thresholds. Although the tone-evoked ABR is obtained quickly and with relative ease, it does not accurately reflect an animal’s behavioral sensitivity to pure tones. Because several lines of evidence suggested that using narrow-band noise to evoke the ABR might give a more accurate measure, ABR thresholds evoked by one-octave noise bands and short-duration tones (tone pips) were compared in rats to determine which most closely estimated the animals’ behavioral, pure-tone thresholds. The results indicated that although the ABR thresholds evoked by octave-band noise (noise-evoked ABR) were a closer match to behavioral thresholds than those evoked by tone pips (tone-evoked ABR), absolute thresholds still did not provide a sufficiently close estimate of the behavioral audiogram. However, when corrected for the mean difference between the noise-evoked ABR and behavioral
thresholds, the noise-evoked ABR did show the potential for estimating high-frequency hearing sensitivity. It should be noted, this finding was a post hoc observation, and requires replication. Three additional findings of this study were: (1) an improved behavioral measure of low-frequency hearing in the laboratory rat, (2) the unexpected finding that damage to the middle ear portion of one ear resulted in transient increased behavioral thresholds for high frequencies in the other ear, and (3) signs of age-related, high-frequency hearing loss that occurred between 14 and 19 months of age.
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Contents

Abstract ........................................................................................................................................... iii
Acknowledgements ...................................................................................................................... v
Contents .......................................................................................................................................... vi
List of Tables ................................................................................................................................... viii
List of Figures ............................................................................................................................... ix

1 Introduction ................................................................................................................................... 1
  1.1 Defining the ABR Threshold ................................................................................................. 4
  1.2 Modified ABR Procedures .................................................................................................... 7

2 Methods ......................................................................................................................................... 16
  2.1 Subjects ................................................................................................................................... 16
  2.2 Instrumentation ..................................................................................................................... 16
  2.3 Behavioral Test Stimuli ......................................................................................................... 19
  2.4 Behavioral Procedure ........................................................................................................... 20
  2.5 ABR Test Stimuli ................................................................................................................ 22
  2.6 Recording the ABR .............................................................................................................. 23
  2.7 Surgical Procedure ............................................................................................................... 25
  2.8 Order of Testing ................................................................................................................... 25
3 Results........................................................................................................................................27

3.1 Behavioral Thresholds – Speaker in front (center) .......................................................27

3.2 Behavioral Thresholds – Speaker 90 degrees to the right...........................................28

3.3 ABR Thresholds – Tones and noise bursts .................................................................30

3.4 Behavioral Thresholds – Speaker to the right: pre- versus post-operation..................35

4 Discussion....................................................................................................................................40

4.1 ABR Thresholds – Tones and noise bursts.................................................................40

4.2 Normal Behavioral Thresholds ..................................................................................43

4.3 Behavioral Thresholds – Differences observed due to speaker location...............44

4.4 Behavioral Thresholds – Effect of unilateral deafening on the intact ear......................45

4.5 Age-related Hearing Loss .........................................................................................47

4.6 Conclusion ..................................................................................................................48

References ...........................................................................................................................50

A ABR Signal Measurements ...........................................................................................57

B Behavioral Threshold Data ..........................................................................................58

C ABR Threshold Data ........................................................................................................61
List of Tables

2.1 Order of ABR testing ..............................................................................................................26

3.1 Correlations between tone- and noise-evoked ABR and behavioral 
thresholds ..................................................................................................................................31

3.2 Difference between corrected individual ABR and behavioral thresholds .............34

3.3 ABR thresholds at 16 kHz: pre- and post-operation ....................................................39

A.1 ABR stimuli – Initial sound pressure level prior to attenuation .................................57

B.1 Behavioral thresholds – Individual and group means ..................................................58

B.2 Individual behavioral thresholds – Front (center) versus right speaker 
orientation ..................................................................................................................................59

B.3 Individual behavioral thresholds – Pre- and post-operation ......................................59

B.4 Individual behavioral thresholds – 1, 4, and 24 weeks post-operation .......................60

C.1 ABR thresholds – Continuous RMS and pulsed peak measurement .........................61

C.2 ABR thresholds – Pulsed RMS measurement ...................................................................62
List of Figures

1-1 Behavioral audiogram for the laboratory rat .................................................................1
1-2 Electrode placement for laboratory rat ABR recording.................................................3
1-3 ABR traces recorded from the laboratory rat .................................................................3
1-4 ABR and behavioral thresholds for the lemur ...............................................................5
1-5 ABR and behavioral thresholds for the laboratory rat ..................................................6
1-6 Behavioral audiograms for the laboratory rat and big brown bat .........................7
1-7 Spectra of stimuli used in behavioral and ABR testing .................................................8
1-8 Behavioral and derived-band ABR thresholds for humans ........................................10
1-9 Behavioral and notched-noise ABR thresholds for humans .......................................11
1-10 Behavioral and sine-wave derived ABR thresholds for the chinchilla ...................13
2-1 Apparatus for behavioral testing ..............................................................................17
3-1 Behavioral thresholds with the standard speaker location – Comparison of individuals and group mean to previously published standards ..................28
3-2 Behavioral thresholds with the speaker located 90 degrees to the right ......................29
3-3 Comparison of noise- and tone-evoked ABR and behavioral thresholds ...................32
3-4 Mean adjusted ABR and behavioral threshold comparison .......................................34
3-5 Behavioral thresholds – Speaker located 90 degrees to the right:
pre- and post-operation comparison..................................................................36

3-6 Signal attenuated by the head when the speaker is located 90 degrees
to the right ..............................................................................................................37

3-7 Post-operative threshold shift over a 24 week period....................................38

4-1 New behavioral standard for low-frequency hearing in the laboratory rat...........43
Chapter 1

Introduction

The basic measure of an animal’s hearing ability is the behavioral pure-tone audiogram, which shows the animal’s auditory sensitivity throughout its hearing range (Heffner & Heffner, 2003). It is obtained by training an animal to make an observable response to pure tones and then determining the lowest intensity to which the animal responds to tones, at octave intervals, across its hearing range. The resulting audiogram can then be used to illustrate the animal’s basic auditory sensitivity, as shown in Figure 1-1.

Because obtaining a behavioral audiogram on an animal can take weeks, if not months, of training and testing, a number of researchers have begun using the auditory brainstem response (ABR) to estimate behavioral thresholds (e.g., Brittan-Powell,
 Unlike the pure-tone audiogram, the ABR is an electrophysiological measure, typically recorded while the animal is sedated, that is dependent on synchronous firing of neurons in the cochlea, VIII\textsuperscript{th} cranial nerve, and brainstem, as well as auditory sensitivity. Sounds that are turned on abruptly evoke synchronous firings of auditory neurons in the brainstem that can be recorded through subdermal electrodes on the head (Figure 1-2). The stimuli presented in ABR testing typically consist of short duration (e.g. 1 ms) tone pips, noise bursts, or clicks. A stimulus is repeated several hundred or more times at a high presentation rate (e.g., 27.7/sec) and the resulting electrical activity is averaged to separate out the neural activity related to the presentation of the auditory stimulus from unrelated, background activity in the brain (Ferguson, Cook, Hall, Grose, & Pillsbury, 1998; Jewett & Williston, 1971). The resulting waveform is characterized by three to seven distinct waves, depending on species, which occur within the first 10-15 ms of stimulus onset. Each peak is labeled P\textsubscript{1} through P\textsubscript{n}, where n is equal to the maximum number of peaks observed (Ozaki, Kurata, Horinouchi, \\& Ando, 1996). Differences in absolute and inter-wave latencies are used to identify abnormalities in hearing sensitivity, and the amplitude of each wave is indicative of neural integrity within the auditory system (Figure 1-3) (Ferguson et al., 1998).
Figure 1-2: Shown is a laboratory rat with subdermal electrodes in place for ABR recording. The active electrode is placed just below the external ear, or pinna, reference electrode placed at the vertex of the skull, and ground electrode (not shown) placed in the muscle tissue in the hind leg.

Figure 1-3: ABR recordings from a laboratory rat at threshold level for a one-octave bandwidth of noise centered at 8 kHz. Triangles indicate waveforms that have been traced from 50 dB SPL until no longer present. Notice how the latency of each wave increases slightly as the signal intensity decreases. Threshold is considered 12.5 dB sound pressure level (SPL) because below this level no latency appropriate response measuring larger than 0.05 μV remains.
1.1 Defining the ABR Threshold

One area of difficulty in using the ABR, regardless of which stimuli is used to evoke the response, has been the identification of threshold because there are generally no objectively agreed upon criteria for threshold detection (Vidler & Parker, 2004). The most common method for identifying an ABR threshold is the visual identification method. This method consists of defining threshold as the lowest intensity at which the ABR is visible above background noise and is replicable in a second trace of an equal number of signal presentations (Jacobson, 1985). However, it has been criticized for its highly subjective nature and lack of agreement between professionals evaluating identical ABR traces (Vidler & Parker, 2004).

An alternative to the visual detection method that has been used to increase the objectivity of threshold detection is the criterion method. In this method, the minimum amplitude that will be considered a response, usually twice the maximum residual background noise (Brittan-Powell, Dooling, & Gleich, 2002; Lasky, Soto, Luck, Laughlin, 1999), is determined a priori and the lowest intensity at which any peak’s amplitude is above this level is considered threshold. This method tends to be slightly less sensitive in the estimation of threshold, but eliminates the subjectivity of the visual detection method.

Recently, many researchers have begun developing methods of threshold detection which are statistically driven in order to further increase the measure’s level of objectivity. One of these is the $F^*$ method, which identifies threshold based on the ratio of variance in the averaged signal at the electrodes to the variance in the averaged residual background noise (Finneran, 2008). The lowest intensity at which the difference
between the two measures of variance is statistically significant is considered threshold. This method tends to give higher (less sensitive) estimates of threshold than other methods.

Another method of threshold detection that is statistically driven is the *linear regression method*. This method specifies the response amplitude used to identify threshold a priori, similar to the criterion method. However, with this method, a regression plot is generated based on the intensity of the signal and amplitude of responses. Threshold is identified by interpolating the intensity at which the signal amplitude will fall below the predetermined value (Brittan-Powell, Dooling, & Gleich, 2002). This method tends to give thresholds that closely match those obtained using the criterion method.

While the detection methods that are statistically driven are becoming more prevalent, their use is limited by the fact that they have not yet been sufficiently refined to provide thresholds that are superior to those obtained using the visual identification method. Ramsier and Dominy (2010) conducted a study using the ABR and all four of the previously mentioned threshold detection techniques to construct an audiogram for the ring-tailed lemur (*Lemur catta*), which is shown in Figure 1-4. As can be seen, the

![Figure 1-4: ABR thresholds from four common detection methods are compared to the behavioral audiogram for *Lemur catta* (Ramsier & Dominy, 2010). Although many of the ABR thresholds are relatively similar, thresholds obtained using the visual identification method are lower than the other three methods in all but one instance (0.71 kHz).]
visual identification method produces thresholds that are a closer match to the behavioral audiogram than the other three methods in all of the frequencies tested, with the exception of 0.71 kHz. Although the visual identification method thresholds diverge from the behavioral audiogram for the lower three octaves (0.5-4 kHz), thresholds for the upper three octaves (8-64 kHz) are relatively similar to the behavioral audiogram, with the exception of 25 and 32 kHz where they differ by approximately 20 dB.

Given that the visual identification method gives the lowest thresholds, the next question is how closely ABR thresholds obtained from the rat compare to the rat’s behavioral audiogram. Although the ABR has been widely accepted as indicative of neural integrity in the auditory system (Ferguson et al., 1998; Jacobson, 1985), there is still considerable debate about its effectiveness as a tool to assess hearing sensitivity. As can be seen in Figure 1-5, the tone-evoked ABR audiograms do not closely match the behavioral audiogram. Not only does the ABR usually tend to underestimate sensitivity, it also does not parallel the audiogram closely enough to be used to estimate behavioral thresholds and areas of best sensitivity with any precision. Moreover, the ABR thresholds obtained by different laboratories often differ significantly—this is in contrast to

![Figure 1-5: Behavioral and ABR audiograms for the laboratory rat are compared. Note that the ABR and behavioral thresholds do not match absolutely and there is a large amount of variation between the four different electrophysiological audiograms (Koay, 2008; Lu, Xu, & Shepherd, 2005; Popelar, Groh, Mazelova, & Syka, 2003; Powers, Widholm, Lasky, & Schantz, 2003).](image)
behavioral audiograms, which consistently do not differ greatly (Figure 1-6). In short, the tone-evoked ABR is not a valid measure of an animal’s hearing sensitivity because of its inaccurate prediction of behavioral thresholds and high level of variation between laboratories.

![Behavioral audiograms for the laboratory rat (Rattus norvegicus) and the big brown bat (Eptesicus fuscus).](image)

Figure 1-6: A comparison of the behavioral thresholds for the laboratory rat and the big brown bat (Dalland, 1965; Heffner et al., 1994; Kelly & Masterton, 1977; Koay, Heffner, & Heffner, 1997). Both of the audiograms for the rats and the bat audiogram by Koay et al. were generated using a conditioned suppression/avoidance procedure. The audiogram by Dalland was generated using a go/no-go behavioral procedure. Note the close agreement between behavioral audiograms, as opposed to the ABR audiograms in Figure 1-5.

1.2 Modified ABR Procedures

Given that the standard tone-evoked ABR procedure does not give valid thresholds, the question is whether modifying the way in which the stimuli are delivered can improve the results. Research aimed at improving the correspondence between behavioral and ABR thresholds has focused on the fact that the stimuli used by the two procedures are not identical. Behavioral thresholds are typically obtained using pure-tone signals that have a relatively long duration (e.g. 400 ms or longer). These stimuli also have a long rise/fall time (e.g. 10-20 ms) in order to prevent signal distortion by the speaker. It is not possible to evoke a synchronous response for the ABR using these
same long-duration signals. This is because the ABR is an onset response, with larger amplitude responses resulting from stimuli with more rapid onsets (Burkard & Don, 2007). Thus, in order to evoke a response, the ABR requires stimuli that are short duration and rapidly repeated. Due to the rapid onset time needed to evoke a response, the spectrums of tone-pip ABR stimuli are much broader than those of long-duration pure tones. This spectral distortion is commonly referred to as ‘spectral splatter,’ which is the generation of additional energy at frequencies surrounding the primary frequency (Gorga & Neely, 2002), as can be seen in top two panels of Figure 1-7. One possibility for the disparity between ABR and behavioral thresholds may be the differences in the stimuli used in the two procedures. In an attempt to rectify this issue, researchers have employed a variety of stimulus modifications in order to obtain an ABR that more closely replicates the behavioral audiogram. The three most common of these are the derived-band technique, the notched-noise technique, and sine-wave derived technique.

Figure 1-7: Spectra of relatively long-duration (400 ms, 20 ms rise/decay time) stimuli used in behavioral tests of auditory sensitivity and the three most common ABR stimuli. Note that the short-duration, 8-kHz tone pip has a much broader spectrum than the 400-ms tone used in behavioral tests. The spectrum of both the broadband click (recorded pulsed at a rate of 27.7 times per second) and octave-band noise (recorded as a long duration signal) also contain energy across a broader range of frequencies than the long-duration tone.
One of the earliest attempts to improve ABR thresholds was the *derived-band technique*, initially described by Teas, Eldredge, and Davis (1962). The derived-band technique is a procedure that uses masking, a phenomenon in which the perception of one sound is blocked or obscured by the presence of another sound (Bess & Humes, 1995), to systematically test different frequency portions of a broadband click (which contains energy across a broad range of frequencies). Specifically, the response to a click stimulus is recorded in the presence of high-frequency masking noise. The masking noise is filtered so that all the frequencies below a specific frequency are filtered out (which is known as a low-pass filter). The low-pass filter is decreased in frequency from one set of recordings to the next, usually in one-half or one-octave steps. The responses from cutoff points above and below the frequency of interest are subtracted from one another resulting in a response that is attributed to the frequency band between the two cutoff points. For example, if the response at 8 kHz is of interest, two sets of responses would be obtained, one with the filter set to remove all frequencies below 5.6 kHz (half an octave below 8 kHz), the other with the filter set to remove all frequencies below 11.2 kHz (half an octave above 8 kHz). The two sets of recordings would be subtracted from each other leaving only the portion of the response associated with the one-octave band between 5.6 and 11.2 kHz. Don, Eggermont, and Brackman (1979) used this method to reconstruct the behavioral audiograms for 10 normal-hearing individuals (Figure 1-8). As can be seen, the average ABR thresholds were found to be 10 dB higher than those published by the International Organization for Standardization (ISO, 1961) at 1, 2, and 4 kHz, but at 0.5 and 8 kHz thresholds were 30 dB higher. It should be noted that in addition to being inaccurate, this method is not able to replicate the entire audiogram
because in order to effectively mask a click, the masking-noise intensity needs to be about 25 dB SPL louder than the click itself. This is an issue at the low-frequency end of the subject’s hearing range, where thresholds increase rapidly. The masking noise needed to prevent a response from the high-frequency end of the hearing range would be loud enough to cause permanent hearing damage (Burkard & Don, 2007).

Another method that has been used in an attempt to improve the correspondence between the ABR and behavioral thresholds is the notched-noise technique, which was first described by Picton, Ouellette, Hamel, and Smith (1979). Similar to the derived-band method, this technique uses noise masking to limit responses to a specific range of frequencies. The key differences lie in the signal used to elicit a response and the bandwidth of the noise masker. First, the signals used in the notched-noise technique are tone pips rather than broadband clicks. These tone pips have a much narrower spectrum than a broadband click, but still contain energy across a broader range of frequencies than a pure tone (see Fig. 7). Second, the masking noise is filtered below a high-frequency cutoff and above a low frequency cutoff, leaving a one-octave ‘notch’ centered at the same frequency as the tone pip (Gorga, 1999). The purpose of the notched noise is to prevent the energy in the tone pips,
that lies more than one-half octave from the fundamental frequency, from evoking a response. In normal-hearing adults, this method has been shown to produce thresholds that differ on average by 10 dB from behavioral thresholds (Figure 1-9) (Stapells, Picton, Durieux-Smith, Edwards, & Moran, 1990). However, out of 20 subjects tested over four frequencies (0.5, 1, 2, and 4 kHz); only 29 percent of the ABR and behavioral thresholds were within 5 dB of one another. On an individual basis, the differences between ABR and behavioral thresholds were sometimes as large as 50 dB. The level of variation in normal-hearing individuals makes it unlikely to accurately replicate the behavioral audiogram using this technique.

There are at least three reasons thresholds obtained using the notched-noise technique cannot be substituted for behavioral testing for experimental purposes in normal-hearing subjects. First, similar to the derived-band technique, the masking noise needed at the extreme ends of the auditory range would be loud enough to cause damage to the most sensitive areas of the auditory system. Second, it has been found that the energy from the masking noise exerts a suppressive effect on responses coming from fibers within the ‘notch’ region, where no noise is present, in individuals with normal hearing (Gorga & Neely, 2002). Finally, the low-frequency energy in the masking noise
has a tendency to spread to the high-frequency end of the cochlea, resulting in a degraded response (Durrant & Boston, 2007).

A more recent procedure for obtaining evoked responses in animals, called the **sine-wave derived technique**, was introduced by Berlin, Hood, Barlow, Morehouse, and Smith (1991). This method consists of recording an ABR response with the stimuli alternating between a tone pip presented in silence and a tone pip presented in the presence of a steady tone of the same frequency. The steady tone is of intensity sufficient to cause a decrease in the amplitude of the recorded response, which is because the neural fibers responding to the steady tone are not firing synchronously with the onset of the tone pip used to generate the ABR. This effect of the steady tone can be observed by subtracting the response elicited by the combination of tone pip and steady tone from the response generated by the tone pip alone. Thresholds are obtained by maintaining a constant intensity level for the tone pip, and reducing the sound pressure level (SPL) of the steady tone until it no longer has an effect on the amplitude of the recorded response—threshold is defined as the lowest sound pressure level where a response with an amplitude greater than or equal to 0.05 μV is observed. This method has been found to give thresholds that were approximately 10 dB closer to the independently-obtained, behavioral thresholds than the standard tone-evoked ABR in guinea pigs, chinchillas, and pocket gophers (Hood et al., 1991). Although the sine-wave derived ABR gives better thresholds than the standard ABR procedure in animals, the method has not been explored as extensively as the derived-band and notched-noise techniques.

The sine-wave derived technique is not optimal for the stand alone assessment of auditory sensitivity because it was not found to reliably predict individual, pure-tone,
behavioral thresholds. Although Hood et al. (1991) found that on average the sine-wave derived thresholds were 10 dB lower than thresholds obtained using the standard tone-evoked ABR, the mean difference between the sine-wave derived and behavioral thresholds was 14 dB (Figure 1-10). As can be seen in Figure 1-10, the majority of the ABR thresholds for the two animals tested do not fall within 5 dB of the behavioral thresholds, which is a more acceptable level of accuracy. Furthermore, none of the frequencies that were within 5 dB of the behavioral thresholds were the same for either animal. Differences between behavioral and ABR thresholds were as large as 40 dB, but not in a consistent or predictable fashion. This degree of variability is not conducive to reliably predicting the auditory sensitivity of an animal using this method alone.

The main difficulty with accepting any of the three previously mentioned methods for predicting behavioral thresholds is that none of these measures estimates the behavioral audiogram with an acceptable degree of accuracy. Although they sometimes
estimate behavioral thresholds to within 5 dB, these measures do not maintain this degree of accuracy across frequencies within an individual or across individuals. All three of these methods also show a tendency to underestimate, and to both over- and underestimate in the case of the sine-wave derived responses, behavioral sensitivity. Moreover, none of them does so in a predictable manner which would allow a simple correction factor to be applied. This makes estimating the behavioral audiogram based solely on ABR thresholds difficult, if not impossible, for any individual subject. Because of these issues, the ABR cannot be considered a reliable estimate of behavioral thresholds using the stimuli discussed previously.

Although the tone-evoked ABR does not accurately estimate pure-tone, behavioral thresholds, there are several reasons to believe that using noise to evoke the response may give more accurate estimates. First, behavioral studies of auditory sensitivity in humans, which used both pure tones and narrow-band noise centered at the same frequencies as the pure tones, ranging from one-tenth to one-octave in bandwidth, found identical thresholds for both stimuli (Berger, 1981; Cox & McDaniel, 1986; Myers, 1957; Sanders & Josey, 1970; Simon & Northern, 1966). Furthermore, a study investigating the auditory sensitivity of harbor seals found that one-third-octave noise bands gave behavioral thresholds that were no different from narrowband, frequency-modulated tones (Kastelein, Wensveen, Hoek, & Terhune, 2009). Second, it has been found that the tone-evoked ABR did not reliably estimate the pure-tone, behavioral threshold shift caused by exposure to loud sound. However, the ABR evoked by octave-band noise did reliably indicate the behavioral threshold shift for that octave noise (Heffner, Koay, & Heffner, 2008). Finally, a study using both tones and clicks found that
although the tone-evoked ABR did not accurately estimate absolute thresholds for tones, the click-evoked ABR accurately estimated the behavioral threshold for a click pulsed at the same rate used to evoke ABR responses (Hill, 2009).

The purpose of the present research was to determine whether the ABR evoked by octave-band noise more accurately estimated pure-tone, behavioral thresholds than the tone-evoked ABR, and whether the entire hearing range of the rat could also be estimated. This was accomplished by making comparisons between the pure-tone, behavioral thresholds of laboratory rats with the ABR thresholds evoked with tone pips and octave-band noise. To reduce between animal variability, ABR and behavioral thresholds were obtained and compared individually for all animals.
Chapter 2

Methods

2.1 Subjects

This study used 5 male, Long Evans, laboratory rats (*Rattus norvegicus*) ranging in age from 149 to 151 days at the beginning of the experiments. Four animals were included in the study. The remaining animal was initially behaviorally trained as a backup animal, but had to be used because one of the primary animals did not survive anesthesia. The animals were bred in the University of Toledo’s Department of Psychology and were thus known to have no previous history of exposure to loud sound or ototoxic medications. Animals had free access to food. Water was only available during the daily training and test sessions. In addition, 10-15 g apple slices were provided as water supplements in the animal’s home cage. The use of animals in this study was approved by the University of Toledo Animal Care and Use Committee.

2.2 Instrumentation

Testing was conducted in a carpeted, double-walled sound chamber (Industrial Acoustics Co., model 1204; 2.55 x 2.75 x 2.05 m), the walls and ceiling of which were
lined with egg-crate foam. The equipment for stimulus generation was located outside the chamber and the subjects were observed over closed-circuit television.

Behavioral testing was conducted in a cage (28 x 13 x 16 cm) constructed with 2.54 cm (1 in.) wire mesh (Figure 2-1). The cage was mounted on a camera tripod and raised 92 cm above the floor. A water spout (15-gauge stainless steel tubing) was mounted vertically up through the floor of the front of the cage so that it projected 5 cm above the cage floor. An oval brass disk (1.2 x 2.0 cm) with a circular hole in the center was mounted directly on top of the spout at a 30 degree angle. This arrangement created a surface where water could collect and permitted the animal to drink off the spout while holding its head in a normal position facing the front of the cage.

The water spout was connected via plastic tubing to a syringe pump (New Era, model NE-1000). A contact switch, connected between the cage and the water spout, operated the syringe pump whenever the animal was in contact with the spout. The syringe pump was set to dispense at a rate of 45 ml/h, and a rat received 8-14 ml of water per daily session. Mild electric shock (60 Hz, 1.25 mA or less) was provided by a shock generator (Grason-Stadler Co., model GSC 700) connected between a foot-plate, the
A 25 W light bulb, located in front of the cage, was turned on and off with the shock.

A variety of different signal generators, filters, amplifiers, and speakers were used in order to ensure that behavioral results were not due to equipment related artifacts. Tones were generated using either Tucker-Davis Technologies (TDT) SigGen software, a Stanford Research Systems (SRS) spectrum analyzer (model SR770), or an arbitrary waveform generator (Agilent Technologies, model 3320A). All noise stimuli were generated using the TDT SigGen software. The TDT SigGen signal was run through a digital to analog converter (TDT, model DA3) prior to filtering. Each signal was then filtered using either a brick-wall filter (Wavetek, model 753A) or a two-channel, band-pass filter (Krohn-Hite, model 3202). The signal was then passed through a programmable attenuator (TDT, model PA4). Each signal was amplified using either a headphone driver (TDT, model HB7), an audio mixer/amplifier (Coulbourn Instruments, model S82-24), or two-channel amplifier (Crown Audio, model D-75). The stimuli with frequencies between 0.125 and 0.5 kHz were delivered via a 12” woofer, between 1 and 4 kHz via a Tang Band W3-319SF 4” small woofer, and between 8 and 80 kHz via either a Technics EAS-10TH100 or Technics EAS-10TH1000 ribbon tweeter.

The sound pressure level (SPL re 20 μN/m²) for the behavioral stimuli were measured using a Brüel & Kjaer (B&K) ¼ inch (6.35 mm) microphone (B&K, model 4939), measuring amplifier (B&K, model 2610), and spectrum analyzer (SRS, model SR770). The spectrum analyzer was used to verify the signal frequency and bandwidth. The measuring amplifier was used to measure the intensity of the signal. The SPL of the behavioral stimuli were measured using the root mean square (RMS) detection setting
and the ‘fast’ averaging time (250 ms) when the sound was turned on as a continuous, non-pulsing signal. The measuring equipment was calibrated with a pistophone (B&K, model 4230) each day, prior to all signal analysis.

The SPL of the ABR stimuli were measured three different ways: a RMS measure of the sound turned on as a continuous, non-pulsing signal (continuous RMS); a RMS measure with the sound pulsing at the rate used to evoke ABR responses (pulsed RMS); and a peak measure with the sound pulsing at the rate used to evoke ABR responses (pulsed peak). In both instances where a RMS measure was conducted, the measuring amplifier (B&K, model 2610) was set to the fast averaging time (250 ms). When the signal was pulsed, the needle would oscillate between one and three decibels for both peak and pulsed-RMS measurements. In this instance a ‘hold’ setting was used which kept the needle at its maximum point of deflection, allowing for an accurate reading of the intensity of the signal. The continuous, non-pulsing signal was measured the same as the signal used in behavioral testing, which is the typical method for ABR signal measurement (Durrant & Boston, 2007). The difference in the sound pressure level of the three different ABR stimuli measurements are shown in Appendix Table A.1.

2.3 Behavioral Test Stimuli

Behavioral thresholds were obtained for pure tones with frequencies of 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 70, and 80 kHz, which span the entire audible range of the laboratory rat. The tones were 400 ms in duration, gated on and off with a rise-fall time of 20 ms and presented with the same speakers mentioned previously. The rise-fall times for 0.125 and 0.25 kHz tones were increased to 80 and 40 ms, respectively, to allow for
10 cycles of each signal to occur during signal onset and offset. This was done to ensure there were no unwanted speaker artifacts, which could serve as a cue for animals to respond to a signal that they otherwise could not hear. Stimuli were presented for 2 s with the tones pulsed 400 ms on, 100 ms off. Animals were tested with the speaker located at a distance of 1 m from the location of the spout. Testing was conducted with the speaker directly in front of the animal (center) and 90 degrees to the right of center facing the animal’s right ear. The condition in which the animals were tested with the speaker to the right was necessary because the ABR is typically evoked with the speaker in this location. The animals were tested with the speaker in front to replicate the testing conditions from previously published audiograms (Heffner et al., 1994; Kelly & Masterton, 1977).

2.4 Behavioral Procedure

It may be noted that young, normal animals show little variation in their auditory thresholds, and studies that establish the audiogram of a species typically use two to three animals (Heffner et al., 1994; Kelly & Masterton, 1977). Thus, the decision to use 4 rats (with 1 animal as a backup) was based on the time commitment involved in testing and the risk of death due to repeated anesthetization. An animal was considered trained when it gave a threshold that was within 3 dB of the expected hearing sensitivity of the rat, as indicated by the audiogram published by Heffner, et al. (1994), for the pure-tone frequency used during training (8 kHz). It took approximately 34 days of training for each animal to give a behavioral threshold that met the established criterion. After each animal was trained it took approximately 237 days to complete all behavioral testing.
Behavioral thresholds were obtained using a standard condition suppression/avoidance procedure (Heffner, Koay, & Heffner, 2006). A water deprived animal was placed in the test cage and allowed to drink from the water spout. Test sessions were divided into 2 s trial intervals separated by 1 s intertrial intervals while the animal maintained continuous contact with the spout. When an animal broke contact with the spout, the program paused at the end of the most recent trial and did not continue until the animal resumed contact with the spout. Each trial was either a sound trial (22%) or a silent trial (78%) with the acoustic signal being presented at an interval between 1 and 6 silent trials. Trials were presented by a computer program in a pseudorandom sequence in order to maintain the previously mentioned sound trial to silent trial ratio. Immediately following the offset of the fourth pulse of the acoustic signal, a mild shock was delivered through the foot-plate and spout, thus the tonal signal served to warn the animal to avoid the upcoming shock. The shock was 2 s in duration, and was avoidable by breaking contact with the spout and footplate and moving to the back of the cage. A warning light, mounted above the speaker, served as a visual cue that the shock was turned on. The animals quickly learned to avoid the shock by breaking contact with the water spout and moving off of the foot-plate whenever a tone was presented.

Any instance where the animal broke contact with the water spout for more than the final 75 ms of a test trial was recorded as a response by the computer. Responses were classified as a hit if they occurred during a sound trial, and as a false alarm if they occurred during a silent trial. Both the hit and false alarm rates were determined for each block of 6 to 8 sound trials (which include approximately 25 silent trials) for each stimulus condition. The hit rate was corrected for false alarms according to the formula:
Performance = Hit rate – (False alarm rate x Hit rate), with the hit and false alarm rates expressed in proportions of one (Heffner & Heffner, 1995). This measure reduced the hit rate by the false alarm rate observed under each stimulus condition and varied from 0 (no hits) to 1.0 (1.0 hit rate and 0.0 false alarm rate). False alarm rates were typically less than 0.1, and if the rate was at or above 0.2 the data were not used, however this was not a frequent occurrence. The starting intensity of the acoustic signal was approximately 40 dB above the behavioral threshold for each frequency as indicated by Heffner et al (1994). The starting intensity at 0.125, 0.25, and 80 kHz was 100 dB SPL because higher intensities at these frequencies created tactiley detectable vibrations in the chamber, distortion in the signal, and were potentially damaging to the animal’s hearing. Absolute thresholds were determined by reducing the intensity of a tone by 5 dB in successive blocks of 6 to 8 sound trials until the subject no longer responded to the signal above the 0.01 chance level (binomial distribution). Threshold was defined as the stimulus level that yielded a corrected performance score of 0.5, which was typically identified through a linear interpolation.

2.5 ABR Test Stimuli

Two different types of sound were used to obtain ABR thresholds: tone pips and octave-band noise. Tones had the same frequencies as the pure tones used in behavioral testing (i.e. 1, 2, 4, 8, 16, 32, and 64 kHz). The octave-band noise was centered on the same frequencies as the pure tones (e.g. for a noise band centered on 2 kHz there was a high-pass filter set at 1.4 kHz and a low-pass filter set at 2.8 kHz, resulting in one-half-octave band of noise above and below this frequency). The tone pips were generated in
2-1-2 cycles (2 cycle rise-fall, 1 cycle plateau) which is a standard practice (Stapells, 2000), and pulsed at a 27.7/s rate. The noise bands were 1 ms in duration (0.01 ms rise-fall, 0.998 ms plateau), and were also pulsed at a 27.7/s rate. The speaker was located 90 degrees to the right of center facing the animal’s right ear. This speaker configuration is typical of ABR measures taken in animals.

2.6 Recording the ABR

The ABR was obtained by anesthetizing the rat using isoflurane, then inserting subdermal electrodes at the vertex (reference), below the right pinna (active), and in the animal’s hind leg (ground). Once electrodes were in place the animal was visually inspected to ensure that the position of the pinna was consistent with the positioning that was observed behaviorally. Impedance measurements were required to be less than 7 kΩ for each electrode, and interelectrode impedance less than 2 kΩ, before testing could begin. Thresholds were obtained for both octave-band noise and tone-pip stimuli of the same frequency within one session, with each session lasting between 45-75 min. The order in which the ABR stimuli were presented was counterbalanced, alternating between animals. This allowed for any order effects of frequency presentation and interactions between frequencies to be identified.

ABR data were collected using a Nicolet electrodiagnostic system (Nicolet Instrument Corporation, model CA 2000). The biological signal was bandpass filtered (0.15-3.0 kHz) and amplified with the artifact rejection level set at 10 μV. The recording window was 15 ms in duration and triggered by a timing pulse from the TDT system at the stimulus onset. Thresholds were identified using the criterion method. As specified
by Hood (1998), threshold was defined as the lowest intensity at which a latency-appropriate response with amplitude of 0.05 µV was present. The ABR responses typically show an increase in latency as the intensity of the signal is decreased (see Figure 1-3), however increases in latency of more than 0.5 ms were not considered to be latency appropriate and were no longer considered a response. Thus, threshold was determined by reducing the intensity of the stimulus in 5 dB steps until no latency-appropriate response measuring more than 0.05 µV was evident. The number of samples taken at threshold level was 4000. Consistent with past practice, at least two recordings were taken above and below threshold and visually compared to identify whether the peaks matched (Vidler & Parker, 2004). Then the traces were combined and the amplitude of the peaks was determined.

It is not typical to get clear responses for the frequencies in the lower region of an animal’s hearing range, as was the case for this study. Preliminary testing indicated that obvious responses could not be observed at 1 kHz below intensities of 85 dB SPL for noise and 90 dB SPL for tones. Therefore, no attempt was made to record responses at frequencies below 1 kHz (i.e. 0.125, 0.25, and 0.5 kHz), because of the potential damage to hearing caused by prolonged exposure to signals above 90 dB SPL. Although the starting intensities for several of the behavioral stimuli were higher than 90 dB SPL, the exposures to these stimuli were relatively brief. However, this was not the case for ABR stimuli, where the animals are exposed to a continuous pulse train for up to 2.5 min per intensity, which puts the animals at a higher risk for hearing damage.
2.7 Surgical Procedure

After the behavioral audiogram had been obtained with the speaker located directly in front of the animal and with the speaker 90 degrees to the right of center, each animal was deafened in its left ear and retested behaviorally with the speaker to the right. This was done to confirm that the head and pinna reduced the intensity of the tones reaching the left ear so that the resulting thresholds reflected only the sensitivity of the right ear. The surgical procedure consisted of anesthetizing the animal with isoflurane and removing the left eardrum with a double pronged pick (Fine Science Tools, model 18067-11). After the left eardrum was removed, the bulla was packed with permanent, non-absorbable Gelfoam (The Upjohn Company, model 7867). Care was taken to make sure the cochlea was left intact to avoid affecting the vestibular system, which could have affected behavioral auditory thresholds by causing an animal to hold its head in a tilted position.

2.8 Order of testing

Behavioral audiograms and ABR measures were obtained prior to the surgical deafening of the left ear, which was done to determine whether the left ear may have contributed to the behavioral thresholds when the loudspeaker was located to the right. The only exception to this was the ABR threshold at 1 kHz, which was obtained after the surgical procedure because the starting intensity of this signal was potentially damaging to the animals’ hearing. Behavioral training began at 8 kHz, and thus this was the first frequency where a threshold was obtained. Frequencies were then increased sequentially in octave steps up to 64 kHz, and then one-third-octave steps from 64 to 80 kHz, until
reaching the upper limit of the animal's hearing range, which was defined as any point at which an animal's threshold exceeded 70 dB SPL. Then, beginning again at 32 kHz, the frequencies were decreased sequentially in octave steps until reaching the lower limit of the animal's hearing range. Thresholds were obtained with the speaker located in the center and the right before moving on to the next frequency. The ABR for each frequency was recorded within 3 days of obtaining the corresponding behavioral threshold with the speaker located to the right (no ABR responses could be evoked above 64 kHz). ABR thresholds were counterbalanced by obtaining the tone ABR first for half of the animals, and the octave-band noise ABR first for the other half because of the potential for the responses to change during a recording session due to anesthesia. Table 2.1 indicates the order in which the first ABR stimulus was presented for each animal.

<table>
<thead>
<tr>
<th>Frequency (in kHz)</th>
<th>First ABR in Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A, B</td>
</tr>
<tr>
<td>2</td>
<td>C, D</td>
</tr>
<tr>
<td>4</td>
<td>A, B</td>
</tr>
<tr>
<td>8</td>
<td>C, D</td>
</tr>
<tr>
<td>16</td>
<td>A, B</td>
</tr>
<tr>
<td>32</td>
<td>C, D</td>
</tr>
<tr>
<td>64</td>
<td>A, B</td>
</tr>
</tbody>
</table>
Chapter 3

Results

Although the main goal of this study was to determine how accurately the noise-evoked ABR estimated the pure-tone, behavioral audiogram, the data are also relevant to other issues. For this reason, the results are presented in four sections: (1) behavioral thresholds with the loudspeaker in front of the animal, (2) behavioral thresholds obtained with the speaker 90 degrees to the right of center, (3) ABR thresholds for tone and octave-band noise, and (4) effect of removing the left eardrum on the behavioral thresholds of the right ear.

3.1 Behavioral Thresholds – Speaker in front (center)

Pure-tone thresholds were obtained for the rats with the loudspeaker directly in front of the animal, which is the standard speaker position used to test an animal’s hearing sensitivity and allows for comparison between the current results and those in the literature. The individual and mean behavioral thresholds obtained from the four animals are shown on the left panel of Figure 3-1 (and Appendix Table B.1). Note that there was little variability between animals across all frequencies tested. The extreme high and low
frequencies showed the most variability, which is typical (Heffner et al., 1994; Kelly & Masterton, 1977). The mean behavioral thresholds obtained in the current study are compared to the results reported by Heffner et al. and Kelly and Masterton in the right panel of Figure 3-1 (and Appendix Table B.1). As can be seen, the audiogram obtained in the current study does not differ greatly from the two previous publications, especially at the frequencies where comparisons between behavioral and ABR thresholds were made (1-64 kHz). Thus, the behavioral procedure used here gave results consistent with previous studies.

Figure 3-1: Left: Audiograms for the four animals (A, B, C, D) in the current study. All values were obtained with both ears intact and the speaker located directly in front (0 degrees from midline) of the animal. Right: Comparison of average behavioral thresholds from the current study and those published by Heffner et al. (1994) and Kelly & Masterton (1977).

3.2 Behavioral Thresholds – Speaker 90 degrees to the right

Because the auditory brainstem potential is obtained on only one ear at a time, it was necessary to obtain thresholds for one ear in each rat (which was the right ear in all cases). This was done by obtaining pure-tone thresholds with the loudspeaker positioned 90 degrees to the right of the animal, which was the speaker position used to obtain the ABR thresholds—note that the left ear was still intact at this point. The mean behavioral
thresholds obtained from the four animals with the speaker to the right are compared to the standard audiogram speaker orientation (center) in the right panel of Figure 3-2 (and Appendix Table B.2). Similar to the audiogram obtained with the speaker in front of the animal, individual variation was greatest at the extreme ends of the hearing range. This is especially true at the high frequency end of the hearing range, which was found to vary more between animals (left panel of Figure 3-2) with the speaker located to the right than in the front. The first notable difference between the center and right thresholds is the absence of a decrease in sensitivity, or ‘notch’, at 16 kHz, which is due to the external ear, or pinna (Shaw, 1974; Wotton, Haresign, & Simmons, 1995). Second, the animals were found to have a greater sensitivity to tones above 32 kHz when the speaker was located to the right, which is also due to the directionality of the pinna and indicates that the acoustic axis of the rat ear is directed to the side, rather than to the front, of the animal.

Figure 3-2: Left: Audiograms for the four animals (A, B, C, D) with the speaker located to the right (90 degrees from midline) of the animal. All values were obtained with both ears intact. Right: Comparison of average behavioral thresholds obtained with the speaker directly in front (0 degrees from midline) and to the right (90 degrees right of midline) of the animals.
3.3 ABR Thresholds – Tones and noise bursts

Making a comparison between behavioral and ABR thresholds is complicated by the transient nature of the ABR stimuli for which there are several different methods of measuring the sound pressure level, and no agreed upon standard (see the Method section for details). To determine which measure showed the closest relationship to the behavioral audiogram, ABR thresholds calculated using three different measurement techniques were correlated with the behavioral thresholds, the results of which are shown in Table 3.1 (the correlations were calculated with and without the 1 kHz thresholds because, as will be seen, the 1 kHz ABR thresholds appear to be outliers). As the table shows, the thresholds calculated with the pulsed-RMS measurement revealed a better correlation with the behavioral thresholds than the other two ABR signal measurements. Although this method is not typically used for ABR signal measurement, it was the one used here; thus, the following analysis is purposely biased in favor of finding a good correspondence between behavioral and ABR thresholds for both stimuli types (thresholds calculated using the other two calibrations can be found in Appendix Table C.1).
A comparison of the behavioral and ABR thresholds for both tone pips and octave-band noise are shown in Figure 3-3 (and Appendix Table C.2). All behavioral and ABR thresholds shown here (with the exception of the ABR thresholds for 1 kHz) were obtained prior to removal of the left eardrum. The ABR thresholds obtained using both octave-band noise and tone pips were found to underestimate the behavioral thresholds at most frequencies (Figure 3-3). The largest underestimate of sensitivity occurs at 1 kHz for both stimuli, with the mean differences between behavior and ABR thresholds being 62 dB for the tone-evoked and 50 dB for the noise-evoked ABR.
Behavioral and ABR thresholds for noise and tones

Figure 3-3: Behavioral thresholds are compared to the thresholds for noise- and tone-evoked ABR. Responses were obtained from each animal with the speaker located 90 degrees to the right of midline. The signal intensity was the RMS measure of the pulsed ABR signal for both the tone and the narrow-band noise. Notice that the noise ABR thresholds were better at most frequencies, especially below 8 kHz and above 32 kHz.
The noise- and tone-evoked ABR were both found to give thresholds most closely matching the behavioral scores at 8 kHz, while the tone-evoked ABR was found to give thresholds that also closely matched the behavioral thresholds at 32 kHz. The noise-evoked ABR was found to be, on average, 5.5 dB more sensitive than the tone-evoked ABR across all frequencies.

While the ABR was unable to accurately estimate the absolute behavioral threshold at most frequencies, the question arose as to whether the frequency of best sensitivity could be predicted using this measure. Although the frequency of best sensitivity was correctly indicated for rat A with the noise-evoked ABR and rat C with tone-evoked ABR, this result was not observed consistently enough to be considered a valid predictor. When the group mean is considered, the ABR estimated frequency of best sensitivity was 8 kHz, whereas behaviorally this frequency was found to be 16 kHz (Figure 3-3) when the speaker is located to the right.

One final consideration was whether the behavioral thresholds could be accurately estimated by the ABR if an adjustment were made to compensate for the difference between the two measures. The simplest adjustment was to subtract a constant from the ABR at each frequency. This constant was calculated as the mean of the difference between the group ABR and group behavioral thresholds at frequencies from 2-64 kHz; differences at 1 kHz were not included in the calculations because of the large differences between ABR and behavioral thresholds at this frequency. The correction factor for the tone-evoked ABR was 13 dB, and the noise-evoked ABR was 9 dB; the corrected audiogram for the group mean can be seen in Figure 3-4 and corresponds with the bottom, center panel in Figure 3-3.
The range of individual differences after correction can be seen in Table 3.2, the mean corresponds to the difference between the ABR and behavioral threshold shown in Figure 3-4. The adjusted noise-evoked ABR was found to more closely estimate the behavioral thresholds than the tone-evoked ABR. However, it is noted that this is a post hoc correction and thus it is not known whether this correction factor would apply to rats tested in other laboratories, or even additional rats tested in this laboratory.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Tone ABR difference</th>
<th>Noise ABR difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>2 kHz</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>4 kHz</td>
<td>-12</td>
<td>5</td>
</tr>
<tr>
<td>8 kHz</td>
<td>-22</td>
<td>-6</td>
</tr>
<tr>
<td>16 kHz</td>
<td>-9</td>
<td>4</td>
</tr>
<tr>
<td>32 kHz</td>
<td>-14</td>
<td>-9</td>
</tr>
<tr>
<td>64 kHz</td>
<td>11</td>
<td>22</td>
</tr>
</tbody>
</table>
3.4 Behavioral Thresholds – Speaker to the right: Pre- versus post-operation

The behavioral thresholds obtained from the right ear in previous sections were tested with the left ear still intact, which was done on the assumption that the head and pinna would reduce the intensity of the tones reaching the left ear so that the resulting thresholds reflected the sensitivity of the right ear alone. To verify this assumption, behavioral thresholds were obtained after removing the left eardrum and packing the middle ear with Gelfoam; although the inner ear remained intact, the surgical procedure would be expected to reduce the sound reaching the cochlea by approximately 24 dB (Aibara, Welsh, Puria, & Goode, 2001; Bess & Humes, 1995). The animals’ behavioral thresholds with the speaker located to the right were then retested one week after the surgical procedure. Testing was begun at 8 kHz and proceeded to the upper limit (80 kHz), followed by the lower frequencies, starting at 4 kHz and proceeding to the lower limit (.125 kHz). A comparison between the mean pre- and post-operation thresholds, with the speaker located to the right, for the four animals used in the study can be seen in Figure 3-5 (and Appendix Table B.3). An unexpected result of the surgical removal of the left eardrum was a decrease in sensitivity in the right ear thresholds for all animals at frequencies above 8 kHz, with the greatest change occurring at 16 kHz (Rat D’s 1 kHz threshold also increased substantially).
When the sound source is located 90 degrees to the right of midline the right ear is closer to the source of the sound than the left ear, thus the signal would be expected to have a higher sound pressure level at the right ear than the left. However, this only holds true for high-frequency sounds which can be blocked by the head and the pinna. In order to verify that this was not the source of the observed post-operation differences, a series of sound measurements were made at each ear of a euthanized adult male, weighing 361 g and with no obvious physical abnormalities of the head or pinnae. Sound measurements were made with the microphone placed at the entrance to the ear canal of both the left and right ear for all frequencies used in the study. Figure 3-6 shows the intensity differences when the measurement at the right ear is subtracted from that at the left ear. As can be seen in the figure, the signal at the left ear canal was not observed to have a higher sound pressure level than at the right ear canal for any frequency tested. Specifically, above 8 kHz, where the largest post-operation differences were observed,
the signal had an appreciably greater sound pressure level at the right ear canal than the left.

Due to the unexpected threshold shift in the high-frequency hearing range, the behavioral thresholds were then retested 4 and 24 weeks post-operation to determine if the increased high-frequency thresholds might be the result of initial post-operative effects that decreased over time. The resulting thresholds from this additional behavioral testing can be seen in Appendix Table B.4. The post-operative threshold shifts for three animals, rats A, B, and C (rat D had died before this test was conducted), tested at 1, 4, and 24 weeks, are shown in Figure 3-7\(^1\). As can be seen, the 8 kHz threshold, which did not change post-operation, was relatively stable over the 24 week period, showing that the animals remained careful observers. It should be noted that 80 kHz was not retested at 24 weeks post-operation because the intensity of the signal needed to elicit a response would have been greater than 100 dB SPL. The threshold shift at higher frequencies can be described as follows: An average threshold shift of more than 5 dB was observed from 16 kHz to 80 kHz one week post-operation; the threshold shift was still seen 4 weeks later for all but 80 kHz, which showed complete

\[\text{Figure 3-6: Difference in the signal intensity measured at the left and right ears. Negative numbers indicate that the signal was louder at the right ear. Positive numbers indicate that the signal was louder at the left ear. Notice that the signal was never measured to be louder at the left ear.}\]

\[\text{Figure 3-6: Signal attenuation caused by the head (speaker 90° right).}\]

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\(^1\) Since these results were obtained, a recent test of 3 additional rats failed to find an increase in the behavioral 16 kHz threshold following removal of the contralateral middle ear.
recovery; complete recovery was seen at 24 weeks for 16 and 32 kHz; however, an even greater threshold shift was then observed at 64 and 70 kHz.

**Post-operative threshold shift by frequency**

![Threshold shift graphs for 8 kHz, 64 kHz, 16 kHz, 70 kHz, 32 kHz, and 80 kHz](image)

Figure 3-7: Comparison of the threshold shift measured 1, 4, and 24 weeks post-operation for each individual animal. Notice that the thresholds at 16 and 32 kHz return to their original values between 4 and 24 weeks, but thresholds increased at 64 and 70 kHz during this time period.
Measurements of hearing sensitivity were also conducted via ABR on each of the three remaining animals 24 weeks post-operation at 16 kHz to identify whether the thresholds matched their pre-operative levels. This frequency was chosen because it was the frequency that showed the most threshold shift immediately after the operation. On average, the three animals’ ABR thresholds at 16 kHz were not found to differ from their original thresholds any more than the behavioral thresholds in the same time period (Table 3.3). However, the individual ABR thresholds were found to have larger differences pre-operation and 24 weeks post-operation than were observed behaviorally. The amount of difference between the ABR thresholds depended on the method used to measure the initial intensity of the ABR, but overall the noise-evoked ABR showed the largest differences. Unfortunately, ABR data were not collected immediately post-operation, nor at any other frequency 24 weeks post-operation, due to concerns for the animal’s survival after repeated anesthetization.

<table>
<thead>
<tr>
<th>ABR Measurement</th>
<th>Rat A Pre-</th>
<th>Rat B Pre-</th>
<th>Rat C Pre-</th>
<th>Rat A Post-</th>
<th>Rat B Post-</th>
<th>Rat C Post-</th>
<th>Mean Pre-</th>
<th>Mean Post-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavior</td>
<td>1</td>
<td>2</td>
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<td>3</td>
<td>2.5</td>
<td>5</td>
<td>1.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Steady RMS</td>
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<td>33.5</td>
<td>30</td>
<td>31</td>
<td>25</td>
<td>18.5</td>
<td>27.5</td>
<td>27.7</td>
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<td>Pulsed RMS</td>
<td>11</td>
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<td>13.5</td>
<td>16</td>
<td>9.5</td>
<td>3.5</td>
<td>11.3</td>
<td>12.7</td>
</tr>
<tr>
<td>Pulsed Peak</td>
<td>34.5</td>
<td>43</td>
<td>37</td>
<td>40.5</td>
<td>32.5</td>
<td>28</td>
<td>34.7</td>
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<table>
<thead>
<tr>
<th>Tone-evoked ABR</th>
<th>Rat A Pre-</th>
<th>Rat B Pre-</th>
<th>Rat C Pre-</th>
<th>Rat A Post-</th>
<th>Rat B Post-</th>
<th>Rat C Post-</th>
<th>Mean Pre-</th>
<th>Mean Post-</th>
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<tr>
<td>Behavior</td>
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<td>2</td>
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<td>3</td>
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<td>Steady RMS</td>
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<td>33</td>
<td>37.5</td>
<td>39.2</td>
<td>40.8</td>
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</table>

Table 3.3: 16 kHz ABR thresholds (in dB SPL): pre-operation and 24 weeks post-operation. Values are displayed for all three ABR signal measurement techniques. Notice that the pre- and post-operative thresholds are nearly identical for the group mean, whereas individually, there is some variation in agreement.
Chapter 4

Discussion

The purpose of this study was to determine how well the ABR evoked by octave-band noise estimated the pure-tone, behavioral audiogram throughout the hearing range of the rat. The results of this study also yielded several additional outcomes which merit consideration. Thus, the results are discussed in the following five sections: (1) ABR thresholds for tone and noise, (2) normal behavioral thresholds, (3) threshold difference based on speaker location, (4) effect of removing eardrum on behavioral thresholds and (5) age-related hearing loss.

4.1 ABR Thresholds – Tones and noise bursts

The primary focus of this study was to identify whether the noise-evoked ABR thresholds more accurately estimated behavioral thresholds than the tone-evoked ABR. While it would appear that the noise-evoked ABR gave thresholds that were closer estimates of the absolute behavioral thresholds than the tone-evoked ABR, the strength of the relationship between the ABR and behavior depends upon whether 1 kHz is included in the data set (Table 3.1). When 1 kHz is included, the correlations between the tone-evoked ABR and behavior indicate that it is more closely related to the behavioral
audiogram than the noise-evoked ABR. However, when this frequency is not included, the noise-evoked ABR correlates better with the behavior. The ABR has previously been found to underestimate behavioral sensitivity in humans more for low-frequency than for high-frequency stimuli (Gorga, Kaminski, Beauchaine, & Jesteadt, 1988). Thus, the results at 1 kHz are not surprising and their exclusion is probably acceptable. It can be concluded that for a large portion of the rat hearing range, the noise-evoked ABR is more accurate in the estimation of hearing sensitivity.

Regardless of the inclusion of 1 kHz in the data set, the majority of the ABR thresholds were more strongly correlated with the behavioral thresholds when the pulsed-RMS measurement of the ABR signal was used, as opposed to the continuous-RMS or pulsed-peak measurements. This suggests that, although not commonly used, the pulsed-RMS measure of signal sound pressure level may be a better method for the measurement of ABR signals. Further research is necessary to establish whether this method of signal measurement is more appropriate for ABR research than the currently accepted measuring methods.

The overall finding of this study is a strong correlation between noise- and tone-evoked ABR and behavioral measures of hearing, regardless of how the ABR stimuli are measured. However, this does not necessarily indicate that the ABR is sufficiently accurate in estimating behavioral thresholds on an individual basis. For example, the ABR was unable to accurately predict the range of hearing for any animal. This is especially significant with regards to the lower limit of hearing, which was underestimated by three octaves for each animal. The ABR was also unable to accurately estimate the frequency of best sensitivity. This indicates that even comparisons between
frequencies in the same animal may yield results that are misleading and do not precisely represent the relative level of sensitivity. Furthermore, the ability to identify and make comparisons between the hearing ranges and frequencies of best sensitivity in different species is of importance for research involving the evolutionary aspects of hearing. With regards to the needs of this type of research, the ABR does not prove to be a sufficient tool for the evaluation of hearing.

One final consideration involves the potential to compensate for the difference between the two measures. Indeed, when the average noise- and tone-evoked ABR are adjusted, as shown in Figure 3-4, the noise-evoked ABR thresholds appear to more closely match behavioral thresholds than the tone-evoked ABR thresholds. However, it would be up to the discretion of the researcher to decide if the range of individual variation after correction, shown in Table 3.2, was acceptable. Furthermore, the lack of standardized ABR methodology serves as a limiting factor that prevents the application of this adjustment to data obtained in other laboratories. First, differences in methods for measuring ABR signal intensity could result in differing ABR thresholds from identical signals. Second, differing ABR threshold detection criteria could cause the adjustment to be inadequate and inapplicable. Finally, while developing a laboratory specific adjustment based on behavioral and ABR differences would be useful for comparisons made within a specific laboratory, it may not be applicable to results obtained in other laboratories.

Further research is needed to standardize the method of stimulus measurement for ABR research. Once a common standard is in place the most appropriate stimulus type for using the ABR to estimate behavioral thresholds can be identified. This may allow
for correction factors, like the one identified in this study, to be used. Until such time, the differing techniques for ABR stimulus measurement will remain a significantly limiting factor in the comparison of ABR research conducted in separate laboratories.

4.2 Normal Behavioral Thresholds

The behavioral thresholds obtained in the current study closely matched the previously published audiograms, although there were minor differences at the extreme ends of the hearing range. The similarities between the audiograms themselves, and the testing methods used to obtain the data, permit the combination of individual animal’s thresholds to obtain a more complete picture of the hearing sensitivity for the laboratory rat. The average of the 11 individual rats from the three different studies is shown in Figure 4-1. The largest difference between the two previously published rat audiograms was at the low-frequency end, from 0.25 to 0.5 kHz, with Kelly and Masterton (1977) having better low-frequency sensitivity than Heffner et al. (1994). The results of the present study found low-frequency sensitivity similar to that published by Kelly and Masterton. Thus, the combined results of the three

![Figure 4-1: The line represents the mean calculated from the 11 individual animals from the three different studies. This new rat behavioral audiogram agrees closely with the previous studies and may better represent the overall auditory sensitivity of the laboratory rat than any one previous study.](Image)
studies give a new standard for low-frequency hearing in the laboratory rat (see also Appendix Table B.1).

4.3 Behavioral Thresholds – Differences observed due to speaker location

Behavioral audiograms are typically obtained with the speaker located in front of the animal; however this study also investigated hearing with the speaker located to the right of the animal. This resulted in two important differences due to speaker position: (1) A general increase in sensitivity to high-frequency tones, and (2) the absence of an area of decreased sensitivity at 16 kHz.

The most notable difference between the audiograms obtained with the speaker in front (center) and to the right of the animal (Figure 3-2) was an increase in sensitivity to frequencies above 8 kHz. The only exception was at 32 kHz where thresholds were found to be nearly identical. Specifically, thresholds for 16, 64, 70, and 80 kHz were found to have an average increase in sensitivity of 13.5 dB when the speaker was directly facing the ear. These results are similar to those observed in humans. An investigation of human hearing, conducted by Sivian and White (1933), found that thresholds varied systematically as the speaker was rotated around the head in the horizontal plane. Specifically, single-ear thresholds were found to have the greatest sensitivity when the speaker was 90 degrees to the right/left of center, directly facing the test ear. The increase in sensitivity due to the speaker facing the test ear was found to be greater than 5 dB for frequencies ranging from 4 to 15 kHz, which is within one octave of the upper limit of human hearing. These differences in behavioral thresholds as the sound source changes location along the horizontal plane highlight the role that the high frequencies play in sound localization for mammals. Specifically, the changes in the spectrum of a
multi-frequency sound as the location of the sound source changes are used by the auditory system to determine the location of the sound source (Butler, 1999).

The second observed difference between the audiograms obtained with the two speaker locations was the absence of the area of decreased sensitivity at 16 kHz. This decrease in sensitivity is caused by the pinna, which acts to enhance or deflect certain frequencies depending on the location of the sound source (Wotton, Haresign, & Simmons, 1995). As Figure 3-2 shows, when the sound source is directly facing the animal’s ear, the effect of the pinna is eliminated resulting in a curve with no area of decreased sensitivity. The effect of the pinna enhancing the intensity of a signal can also be observed at 32 kHz in Figure 3-6. Although the general trend in this figure is an increasing intensity difference between the two ears as the frequency is increased, there is an anomaly occurring at 32 kHz. This is likely the effect of the pinna enhancing the intensity of the signal, and may explain why thresholds did not improve at this frequency when the speaker was moved to the right.

4.4 Behavioral Thresholds – Effect of unilateral deafening on the intact ear

An unexpected result of this study was the increase in behavioral thresholds above 8 kHz in the right ear that was observed after deafening of the left ear. The resulting threshold shift appeared to be temporary, lasting between 4 and 24 weeks, and only affected the upper three octaves of the animals’ hearing ranges. The idea that this was because the left ear was contributing to the threshold even though the speaker was on the right side of the animal was ruled out by showing that the head and pinnae greatly attenuated the high frequencies reaching the left ear (Figure 3-6) and that the right ear
threshold shift was temporary (Figure 3-7). At this time, only two other possible explanations seem plausible: (1) The damage to the eardrum and middle ear bones of the left ear caused a semi-permanent contraction of the muscles attached to the middle ear bones in the right ear, and/or (2) the surgical removal of the left ear caused transient tinnitus, which served to mask the detection of high frequency sounds presented to the right ear.

The first possibility considers a semi-permanent contraction of the middle ear muscles, called the acoustic reflex, which occurs in response to loud sounds and when an animal vocalizes. During the acoustic reflex, the stapedius and tympani tensor muscles contract, causing the stapes to be pulled away from the oval window of the cochlea and the malleus to be pulled away from the eardrum (Bess & Humes, 1995). This results in a decrease in the ability of the middle ear bones to transmit sound from the eardrum to the cochlea. It is known that a loud sound presented unilaterally will evoke a bilateral acoustic reflex. Therefore, the potential exists that the damage to the middle ear caused by the surgical procedure may have resulted in a semi-permanent contraction of the middle ear muscles in the right ear which would affect the post-operative thresholds. However, research investigating the effect of the acoustic reflex on thresholds indicates that the acoustic reflex attenuates the low frequencies to a much greater extent than the high frequencies (Borg, 1968; Murata, Ito, Horikawa, & Minami, 1986). Because the opposite effect was observed in the current study, it is unlikely that a semi-permanent contraction of the middle ear muscles was the source of the post-operative hearing loss.

The other potential explanation for the temporary increase in thresholds is that the operation may have caused tinnitus, which served as a masking noise for the thresholds at
the high frequency end of the animals’ hearing ranges. Although tinnitus resulting from damage to the middle ear would not be surprising, there does not appear to be any note of it in the human literature, perhaps because tinnitus has, until recently, been considered a minor disorder. In order to investigate this explanation, the finding of a contralateral, high-frequency hearing loss would first have to be replicated. If the result proved to be reliable, then a second study could determine whether the animals developed tinnitus. In addition, this provides the opportunity to identify a new area where the ABR may be used as a diagnostic tool. Research in humans has indicated that there is no appreciable difference when comparisons are made between ABRs from normal humans and from those who experience tinnitus (Jacobson, 2000). If damage to the middle ear results in tinnitus that masks high-frequency tones, then it would be expected that behavioral testing would show a threshold shift, while the ABR thresholds would remain unchanged. Thus, the combination of ABR and behavioral testing may serve to identify the presence of tinnitus. Unfortunately, no immediate post-operative ABRs were conducted in the current study to make such a comparison.

4.5 Age-related Hearing Loss

It is not unusual for an animal’s hearing sensitivity to decrease as it ages. This age-related hearing loss, or presbycusis, is commonly observed in humans older than 50 years (Bess & Hume, 1995). Presbycusis typically consists of a bilateral, high-frequency hearing loss due to damage to outer hair cells in the cochlea. One of the results of the extensive behavioral testing conducted on the rats in the current study was the

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2 A recent test of 3 additional rats failed to find an increase in the 16 kHz behavioral or tone-evoked ABR threshold following the removal of the contralateral middle ear.
observation of an increase in the behavioral thresholds above 32 kHz when the rats were between the ages of 14 and 19 months. This high-frequency hearing loss appears to correspond to the typical pattern of presbycusis that is observed in humans. Using a conditioned-suppression technique, Borg (1982) investigated the effects of age on the hearing of Winstar strain rats and found a high-frequency hearing loss of approximately 20 dB at 48 kHz in rats between the ages of 15 and 22 months. Frequencies above 48 kHz were not tested in the Borg study, so a frequency-by-frequency comparison to the current study is not possible. However, comparison of the current results and those reported by Borg appear to show a hearing loss that is of a similar magnitude, occurring in the same frequency range, and at approximately the same age. This finding indicates that the results of this study are not unusual, and the high-frequency hearing loss above 32 kHz is likely related to the animal’s age. Furthermore, these results provide evidence of presbycusis in the Long-Evans strain of laboratory rat, which has not been previously demonstrated behaviorally.

4.6 Conclusion

The results of this study indicate that although the noise-evoked ABR was found to be more accurate in the estimation of the behavioral audiogram than the tone-evoked ABR, it was not accurate enough to serve as a stand alone replacement for behavioral testing. However, it was found that the noise-evoked ABR may be able to accurately estimate the high-frequency hearing of an animal if a correction factor is applied to compensate for the difference between the ABR and behavioral measures. Further research is required to establish whether the correction factor can be applied to species
other than the laboratory rat and/or across laboratories. In addition to these findings, this study also establishes a new standard for the low-frequency hearing of the laboratory rat. This study resulted in three additional noteworthy findings: (1) the location of a sound source in space influences high-frequency hearing sensitivity, (2) unilateral trauma to the ear drum and middle ear may result in hearing loss in the unaffected ear (although a recent test of 3 rats was unable to replicate this finding), and (3) the Long-Evans strain of rat appears to develop presbycusis-like hearing loss between 14 and 19 months of age.
References


Appendix A

ABR Signal Measurements

<table>
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<th>Frequency</th>
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<th>Pulsed Peak</th>
<th>Continuous RMS</th>
<th>Pulsed RMS</th>
<th>Pulsed Peak</th>
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<td>78</td>
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<td>70</td>
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<td>81.5</td>
</tr>
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Appendix B

Behavioral Threshold Data

Table B.1: Behavioral thresholds (in dB SPL) for individual rats and the group mean. All values were obtained with the speaker in front (center) of the animal at a distance of 1 m. The values on the left are the three means that can be seen plotted in Figures 3-1 and 4-1.

<table>
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<th>Rat C</th>
<th>Rat D</th>
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<th>Heffner et al. (1994)</th>
<th>Kelly &amp; Masterton (1977)</th>
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Table B.2: Individual, behavioral thresholds (in dB SPL) with the speaker in front (center) and to the right of the animal. All values were obtained in their respective locations with the speaker at a distance of 1 m while both of the animal’s ears were intact.

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<th>Frequency</th>
<th>Rat A</th>
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<th></th>
<th>Rat C</th>
<th></th>
<th>Rat D</th>
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Table B.3: Pre- and post-operative, behavioral thresholds (in dB SPL). All values were obtained with the speaker located 90° to the right of midline at a distance of 1 m.

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<th>Rat C</th>
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Table B.4: High frequency thresholds (in dB SPL): pre-operation, 1, 4, and 24 weeks post-operation. All high-frequency, behavioral thresholds below 64 kHz were found to be within 3 dB of their original scores within 24 weeks.

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<th>Rat C</th>
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### Appendix C

**ABR Threshold Data**

Table C.1: ABR thresholds (in dB SPL) for the continuous RMS and pulsed peak signal measurements. All values were obtained with the speaker 90° to the right of midline.

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<th>A (Peak)</th>
<th>B (Continuous)</th>
<th>B (Peak)</th>
<th>C (Continuous)</th>
<th>C (Peak)</th>
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Table C.2: ABR thresholds (in dB SPL) for the pulsed RMS signal measurement. All values were obtained with the speaker 90° to the right of midline.

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