Comparison of behavioral and auditory brainstem response measures of threshold shift in rats exposed to loud sound

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The purpose of this study was to determine how closely the auditory brainstem response (ABR) can estimate sensorineural threshold shifts in rats exposed to loud sound. Behavioral and ABR thresholds were obtained for tones or noise before and after exposure to loud sound. The results showed that the ABR threshold shift obtained with tone pips estimated the initial pure-tone threshold shifts to within ±5 dB 11% of the time and the permanent pure-tone threshold shifts 55% of the time, both with large errors. Determining behavioral thresholds for the same tone pips used for the ABR did not improve the agreement between the measures. In contrast, the ABR obtained with octave noise estimated the initial threshold shifts for that noise to within ±5 dB 25% of the time and the permanent threshold shifts 89% of the time, with much smaller errors. Thus, it appears that the noise-evoked ABR is more accurate in estimating threshold shift than the tone-evoked ABR.
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I. INTRODUCTION

The first step in studying hearing loss is to determine the degree of the loss and the frequencies at which it occurs. Although it is usually not difficult to obtain a behavioral audiogram for adult human subjects, this can be a time-consuming process with animals. For this reason, hearing loss in animals is often assessed with a physiological measure, such as the compound action potential or auditory brainstem response (ABR). The question, then, is how accurately do such physiological measures reflect changes in behavioral thresholds?

To determine the accuracy of physiological measures of hearing loss, it is necessary to compare behavioral and physiological measures in the same animals, which four previous studies have done. Three of these studies recorded the neural responses evoked by sound from electrodes either in the cochlea (Dallos *et al.*, 1978) or in the inferior colliculus (Davis and Ferraro, 1984; Henderson *et al.*, 1983). The results of these studies indicated that although the physiological measures agreed closely with some of the behavioral threshold shifts that resulted from ototoxic drugs or exposure to loud sound, there were significant differences with no way to determine which estimates were accurate and which were not. The fourth study compared the ABR with behavioral thresholds before and after exposure to an ototoxic drug or loud sound and found better agreement between the behavioral and physiological measures than the previous three studies, although their results were based on only two animals (Borg and Engström, 1983). Nevertheless, these results suggested that the ABR is a promising technique for assessing hearing loss in animals.

Indeed, the ABR has been used for many years to assess hearing loss in humans, especially in infants and individuals with developmental disabilities who cannot be tested behaviorally. As a result, a number of studies have been conducted to determine the accuracy of the ABR for estimating behavioral thresholds at different frequencies [for reviews, see Gorga (1999), Gorga and Neely (2002), and Stapells (2000a and 2000b)]. In general, the ABR appears to be 10–20 dB less sensitive than pure-tone behavioral thresholds in adults with normal hearing (e.g., Stapells, 2000a, 2000b). Interestingly, for individuals with sensorineural hearing loss, the ABR usually falls within 5–15 dB of the behavioral thresholds (Gorga and Neely, 2002; Stapells, 2000a, 2000b). In other words, the relation between the ABR and behavioral thresholds changes following sensorineural hearing loss. However, it should be noted that such comparisons have only been done for frequencies in the human midrange (500 Hz to 4 kHz), and it has been shown in mice that the ABR diverges significantly from the behavioral thresholds at the high and low-frequency ends of the audiogram (Heffner and Heffner, 2003), an effect also seen in other animals (Finneran and Houser, 2006; Szymanski *et al.*, 1999).

Recently, we have been studying tinnitus in animals caused by exposure to loud sound (Heffner and Harrington, 2002; Heffner and Koay, 2005). In doing so, we have used the ABR to estimate the accompanying hearing loss to determine whether the hearing loss, rather than the tinnitus, is associated with the increased activity in the dorsal cochlear nucleus that occurs following such exposure (Zhang *et al.*, 2004). The purpose of this study, then, was to determine how well the ABR estimates behavioral hearing loss in rats exposed to loud sound. As will be seen, our results indicate that the ABR evoked by octave noise provides a much more accurate estimate of hearing loss than the tone-evoked ABR.

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II. METHODS

Behavioral and ABR thresholds for tones and noise were obtained on monaural rats. For optimal accuracy, behavioral and ABR thresholds were obtained for only one sound at a time. The animals were then exposed to a loud tone for 10 min, followed 1 h later by behavioral and ABR testing to determine the resulting threshold shift. Both thresholds were then tracked over subsequent days until they had stabilized, and the ABR threshold shifts were compared with the behavioral threshold shifts. To avoid potential bias during testing, the behavioral and ABR results for an animal were not compared until testing was complete.

A. Subjects

The subjects were 18 male Long Evans laboratory rats (*Rattus norvegicus*) ranging in age from 70 to 115 days at the beginning of the experiments. The animals had been bred in the Department of Psychology of the University of Toledo and were thus known to have no previous history of exposure to loud sound, such as transportation noise. They were given free access to rodent blocks. Water was available only during the daily training and test sessions. The use of animals in this study was approved by the University of Toledo Animal Care and Use Committee.

B. Surgical procedure

Prior to training and testing, each animal was deafened in its left ear so that all testing was conducted on its right ear. This involved anesthetizing an animal with halothane, removing the left eardrum and middle ear bones, and packing the bulla with a piece of foam rubber earplug (E-A-R Classic earplug, Aearo Corp.) to prevent sound from entering the bulla. The cochlea was purposely left intact to avoid affecting the vestibular system, which could affect behavioral auditory thresholds by causing an animal to hold its head in a tilted position. Subsequent ABR testing failed to reveal any response in the deafened ear to the sounds used in this study.

C. Behavioral apparatus

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

The animals were tested in a cage (28 cm long $\times$ 13 cm wide $\times$ 16 cm high) constructed with 1 in. (2.54 cm) wire mesh [for a drawing of the test cage, see Heffner *et al.* (1994)]. 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 (1.2×2.0 cm) was mounted on top of the spout at a 30 deg angle. This arrangement permitted an animal to lick water 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 (NE 1000, New Era, Wantagh, NY). 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 42 ml/h, and a rat received 8–14 ml of water per daily session. Mild electric shock was provided by a Coulbourn ac-resistive small animal shocker connected between the water spout and the cage floor. A 25 W light bulb located beneath the cage was turned on and off with the shock.

D. Acoustic apparatus

Behavioral thresholds were obtained for pure tones ranging from 2 to 45 kHz as well as for octave-band noise (approximately 20–40 kHz); these signals had a duration of 400 ms and a rise-fall time of 10 ms. In addition, thresholds were obtained for two of the same sounds used to obtain the ABR: a 16 kHz tone and the 20–40 kHz noise, both of which were 1 ms total duration with a rise-fall time of 0.5 ms (no plateau).

The 400 ms pure tones were digitally produced, gated with a 10 ms rise-fall time, amplified, and sent to either a Motorola piezoelectric speaker (2 kHz) or a Foster ribbon tweeter (4, 8, 16, and 45 kHz). The speaker was located 90E to the right of an animal's head at a distance of 1 m when it was drinking from the water spout. The noise was generated using Tucker-Davis Technologies (TDT) SIGGEN software. The output of the digital to analog converter (TDT, model DA3) was passed to a programable attenuator (TDT, model PA4), filtered, amplified, and sent to the ribbon tweeter. Sound pressure levels were measured using a Bruel & Kjaer (B&K) 1/4 in. (0.64 cm) microphone (Model 4135, B&K, Naerum, Denmark), a measuring amplifier (B&K model 2608), and a spectrum analyzer (Zonic 3525). The measuring equipment was calibrated with a pistonphone (B&K model 4230). The spectra of the 20–40 kHz noise and the 16 kHz tone stimuli are shown in Fig. 1. (The ABR stimuli are described below.)

E. Behavioral procedure

A standard conditioned suppression procedure was used to obtain the behavioral thresholds (Heffner *et al.*, 2006). A thirsty animal was placed in the test cage and allowed to drink from the water spout. Sounds were presented at random intervals and followed at their offset by a mild electric shock delivered through the spout. The animal quickly learned to avoid the shock by breaking contact with the spout whenever it heard a tone.

Test sessions were divided into 2.0 s intervals separated by 1.0 s intertrial intervals. Each trial contained either a sound ("warning" signal) or silence ("safe" signal), with 22% of the trials containing a sound. A response was recorded if an animal broke contact for more than half of the last 150 ms of a trial. The response was classified as a hit if the trial contained a sound and as a false alarm if no sound

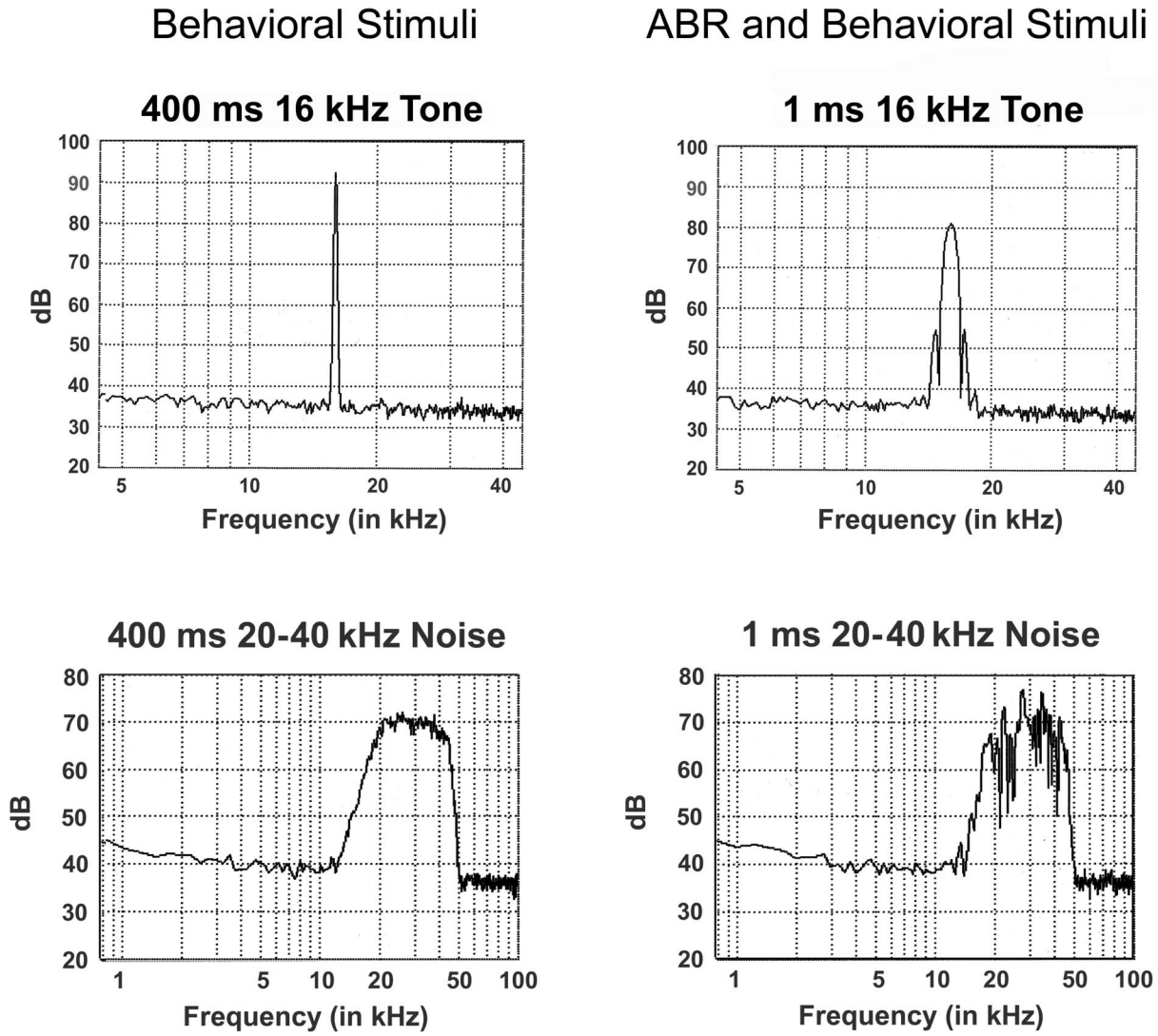

FIG. 1. Spectra of the noise and the 16 kHz tone stimuli used in the tests. Note that the 1 ms tone and noise stimuli are the same as those used for the ABR. The side lobes of the 1 ms, 16 kHz tone burst, which are caused by the rapid onset and offset, peak at 14.75 and 17.25 kHz.

was presented. Both the hit and false alarm rates were determined for each block of six to eight warning trials (which also included approximately 25 safe trials) for each stimulus condition. The hit rate was corrected for false alarms according to the formula: performance=hitrate-(false alarm rate $\times$hit rate), with the hit and false alarm rates expressed in proportions of 1. Absolute thresholds were determined by reducing the intensity of a tone in successive blocks of six to eight warning trials until the animal no longer responded to the signal above the 0.01 chance level (binomial distribution). Thresholds were obtained for only one stimulus at a time so that an entire session could be devoted to obtaining a reliable threshold.

F. Recording the auditory brainstem response

ABR testing was conducted in a double-walled sound chamber identical to that used for behavioral testing. To obtain the ABR, a rat was anesthetized with isoflurane, and subdermal electrodes were inserted at the vertex and behind the right ear, with the ground electrode in the animal's hind leg. The speaker was positioned directly above the animal's ear at a height of 12 cm. Body temperature was maintained by electrically heating the chamber. Because thresholds were obtained for only one sound, the procedure was usually completed in 15–25 min.

The sound noise were generated using the same equipment and loudspeaker used to obtain the behavioral thresholds. The main difference was that the stimuli were 1 ms in duration, 0.5 ms rise-fall time (no plateau), and pulsed 27.7 times/s. The spectra of the 16 kHz tone and the noise are shown in Fig. 1.

Data were collected using a Nicolet model CA 2000 electrodiagnostic system (Nicolet Instrument Corporation, Madison WI). The biological signal was bandpass filtered (0.15–3.0 kHz) and amplified (sensitivity setting of 25 μV) with the artifact rejection level set at 10 μV. The recording window was 10 ms in duration and was triggered by a timing pulse from the TDT system at the stimulus onset. Thresholds were determined by reducing the intensity of the stimulus in 10 dB steps until no latency-appropriate responses were evident. The intensity of the stimulus was then increased in 2.5 or 5 dB steps until a response could once again be discerned. Threshold was then defined as the lowest intensity at which a

latency-appropriate response with an amplitude greater than 0.05 μV could be detected. The number of samples per average varied with the clarity of the response, ranging from a minimum of 1000 at higher stimulus intensities to 6000–8000 around the threshold. At least two recordings were taken above and below the threshold and were compared to see if the peaks matched. The traces were then combined and the amplitude determined.

G. Exposure to loud sound

For exposure, an animal was anesthetized with isoflurane and its right ear exposed to a loud tone for 10 min. The exposure tones used were 1.4, 2.8, 5.6, 11.2, 16, and 31.5 kHz at intensities of 110, 115, or 120 dB sound pressure level (SPL). The 16 kHz tone was chosen because we had previously used it to induce tinnitus in rats (Imig *et al.*, 2007). The other frequencies were chosen because Davis *et al.* (1950) had found that the maximum hearing loss caused by exposure to loud tones generally, although by no means always, occurred half an octave above the frequency of the exposing tone. Thus, in measuring threshold shifts at frequencies half an octave above the frequency of the exposing tone, we expected to see differing degrees of hearing loss, which we did.

Some rats were exposed more than once as part of a study of the cumulative effects of exposure to loud sound. Specifically, rat 06-07 was exposed again 32 days after the first exposure, rat 07-08 was exposed 20 days after the first exposure, rat 06-01 was exposed 20 days after the first exposure and again 33 days after the second exposure, and rat 06-02 was exposed 16 days after the first exposure and again 34 days after the second exposure.

The tone was produced by a digital signal generator (Model 3525, Zonic, Tokyo, Japan), amplified (Model MPA, 100-w/channel, Radio Shack, Fort Worth, TX), and sent either to an Electro-Voice Model 1823M driver (for frequencies of 1.4 and 2.8 kHz) or to a Motorola KSN 1005A piezoelectric loudspeaker (for frequencies of 5.6 kHz and higher). The sound was directed to an animal's ear through a plastic funnel with a 4 mm inner diameter tip that was attached to the loudspeaker with thermoplastic adhesive. The sound was measured with the $\frac{1}{4}$ in. microphone placed at the tip of the plastic tube.

A behavioral threshold was obtained 1 h after the exposure, following which the animal was reanesthetized and its ABR threshold obtained. Behavioral and ABR thresholds were then obtained daily until they had stabilized, with the ABR threshold taken immediately following the behavioral threshold.

III. RESULTS

The results consist of a comparison between behavioral and ABR measures of threshold shift following a 10 min exposure to a loud tone.

To obtain maximum reliability, each animal was tested daily on the same stimulus until thresholds had stabilized. Because initial threshold shifts were obtained beginning 1 h after exposure, a control test was conducted to determine whether any lingering effects of the anesthesia might have affected the thresholds.

The results of the exposures are described in terms of (1) the size of the initial hearing loss determined behavioraly 1 h after the exposure (followed immediately by the ABR recording, (2) the time to recover from the temporary portion of the hearing loss (defined as the number of days it took for a threshold to fall to within 3 dB of its final value), and (3) the magnitude of the permanent hearing loss

A. Behavioral and ABR threshold shifts for tones

The behavioral threshold shifts for 400 ms pure tones are compared with the ABR threshold shifts (1 ms tone pips) in Figs. 2 and 3, where they are arranged by the frequency of the test tone. Because preliminary tests showed that the ABR threshold did not vary from day to day, only one or two pre-exposure ABR thresholds were obtained to minimize the number of times that a rat had to be anesthetized. Pre-exposure behavioral thresholds were also quite stable, generally varying by less than 3 dB.

As can be seen in Figs. 2 and 3, the ABR does not provide a reliable estimate of the initial behavioral threshold shift. Differences between the ABR and behavioral thresholds ranged from an underestimate of more than 28 dB [Fig. 2(a)] to an overestimate of 28.8 dB [Fig. 2(e)] with the ABR as likely to overestimate as to underestimate the behavioral threshold shift.

With regard to the time to recover from the temporary threshold shift (defined as the number of days it took for a threshold to fall to within 3 dB of its final value), in only 3 of the 11 cases did the ABR agree with the behavioral recovery time [Figs. 2(a), 3(c), and 3(e)].

An analysis of the permanent hearing loss, on the other hand, shows some agreement between the ABR and the behavioral measure, with the final ABR falling within ± 5 dB of the final behavioral measure of hearing loss in 6 of the 11 cases [Figs. 2(a), 2(b), 2(d), 3(a), 3(b), and 3(f)]. However, it should be noted that the two cases showing the best agreement were two exposures on the same animal that did not have a permanent hearing loss [Figs. 3(a) and 3(b)]. In other cases, the ABR underestimated the permanent hearing loss by 7.8 dB [Fig. 3(c)] and overestimated it by up to 37.3 dB [Fig. 2(e)]. In short, it would appear that the tone ABR does not provide a reliable estimate of pure-tone sensorineural hearing loss.

B. Anesthesia controls

The consistent disagreement between the behavioral and ABR measures of the initial hearing loss raised the possibility that the behavioral measure might have been affected by lingering effects of the anesthesia, even though the animals were given an hour to recover before testing. To investigate this possibility, we determined the behavioral thresholds of four rats for the 16 kHz, 1 ms tone pips used in the ABR test. The animals were then given a "sham" exposure in which they were anesthetized for 10 min, but not exposed to sound, and then tested 1 h later. As shown in Fig. 4, the anesthesia

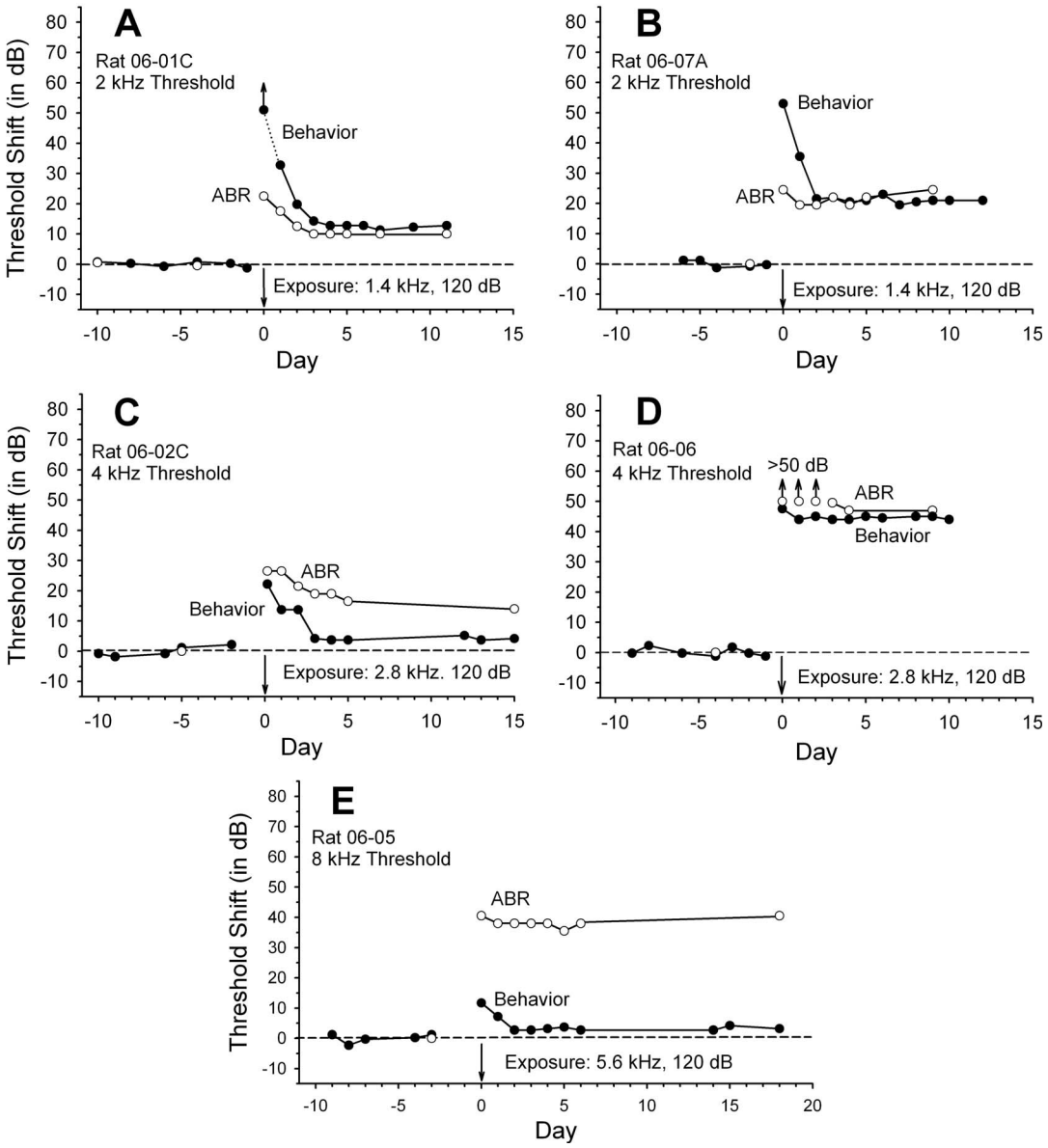

FIG. 2. Behavioral (closed circles) and ABR (open circles) threshold shifts for 2, 4, and 8 kHz tones. Upward pointing arrows and dotted lines indicate that the threshold that day was greater than the maximum stimulus intensity that could be produced. The intensity and frequency of the exposure tone is listed in each graph; all exposure durations were 10 min. Note that here and in Fig. 3 the letters A, B, and C following a rat's designation indicate that the results are from the animal's first, second, and third exposures; most rats were exposed only once.

alone had no effect on their behavioral thresholds, nor, for that matter, did it affect the subsequent ABR threshold. Thus, the large discrepancies observed between behavioral and ABR measures of the initial hearing loss was not caused by any lingering effects of the anesthesia.

C. Behavioral and ABR threshold shifts using the same 1 ms 16 kHz stimulus

Because the tone ABR uses a different stimulus than the pure-tone behavioral audiogram (Fig. 1), it is possible that there might be better agreement between the two measures if the same auditory stimulus were used for both. To test this possibility, behavioral thresholds were obtained for four rats using the same 1 ms, 16 kHz tone pips used for the ABR. The rats were tested before and after exposure to 11.2 kHz at 120 dB for 10 min.

Despite using the same stimuli, the ABR still did not provide a reliable estimate of the behavioral threshold shift for tones (Fig. 5). Differences between the initial behavioral and ABR threshold shifts ranged from 5.1 to 18.6 dB (Fig. 5). With regard to the time to recover from the temporary portion of the hearing loss, the ABR indicated that recover occurred by day 1 in each case, but the behavioral thresholds of rats 08-01 and 08-02 took 2 and 3 days, respectively, to recover to within 3 dB of their final value (Fig. 5).

Finally, the ABR and behavioral measures of the permanent threshold shift fell to within ±5 dB of each other for

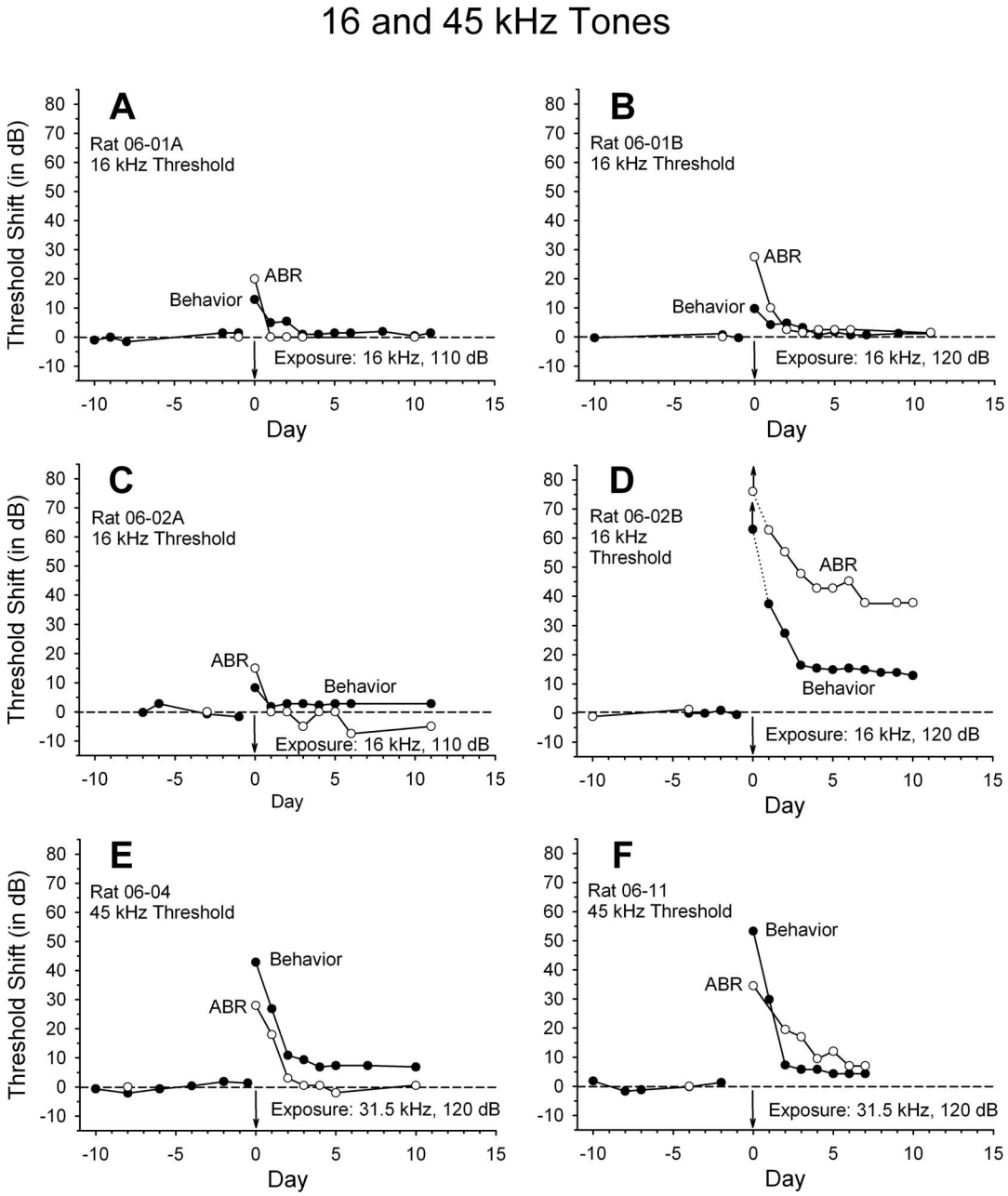

FIG. 3. Behavioral (closed circles) and ABR (open circles) threshold shifts for 16 and 45 kHz tones. Upward pointing arrows indicate that the threshold was greater than the maximum stimulus intensity that could be produced. Exposure frequency and intensity are listed in each graph; all exposure durations were 10 min.

two of the animals [Figs. 5(b) and 5(c)]. In the other two cases, the ABR overestimated the behavioral threshold shift by 15.1 dB in one case [Fig. 5(a)] and underestimated it by 15.6 dB in another [Fig. 5(d)]. Thus, the ABR did not estimate the behavioral threshold shift for the 1 ms tone pip used in the ABR any better than it estimated the 400 ms pure-tone threshold shift.

D. Behavioral and ABR threshold shifts for 20–40 kHz noise

In recent studies of tinnitus induced by exposure to loud sound, we estimated the accompanying hearing loss by determining the shift in the ABR threshold using band filtered noise (Heffner and Harrington, 2002; Heffner and Koay, 2005; Imig *et al.*, 2007). Therefore, we were interested in determining whether the ABR reliably estimated the behavioral hearing loss for the 20–40 kHz noise we have used elsewhere (Imig *et al.*, 2007). The behavioral thresholds in this experiment were obtained using the 400 ms duration noise.

In contrast to the tests using tonal stimuli, the results of this test indicated relatively good, although not perfect, agreement between the ABR and behavioral measures of threshold shift (Fig. 6). As with the tone ABR, the noise-evoked ABR was least accurate in estimating the initial hearing loss, with none of the four estimates within ±5 dB of the behavioral threshold shifts. However, it was fairly accurate in estimating the time course, being off by one day in one case in which the behavioral thresholds took 3 days to stabilize whereas the ABR threshold stabilized in 2 days [Fig.

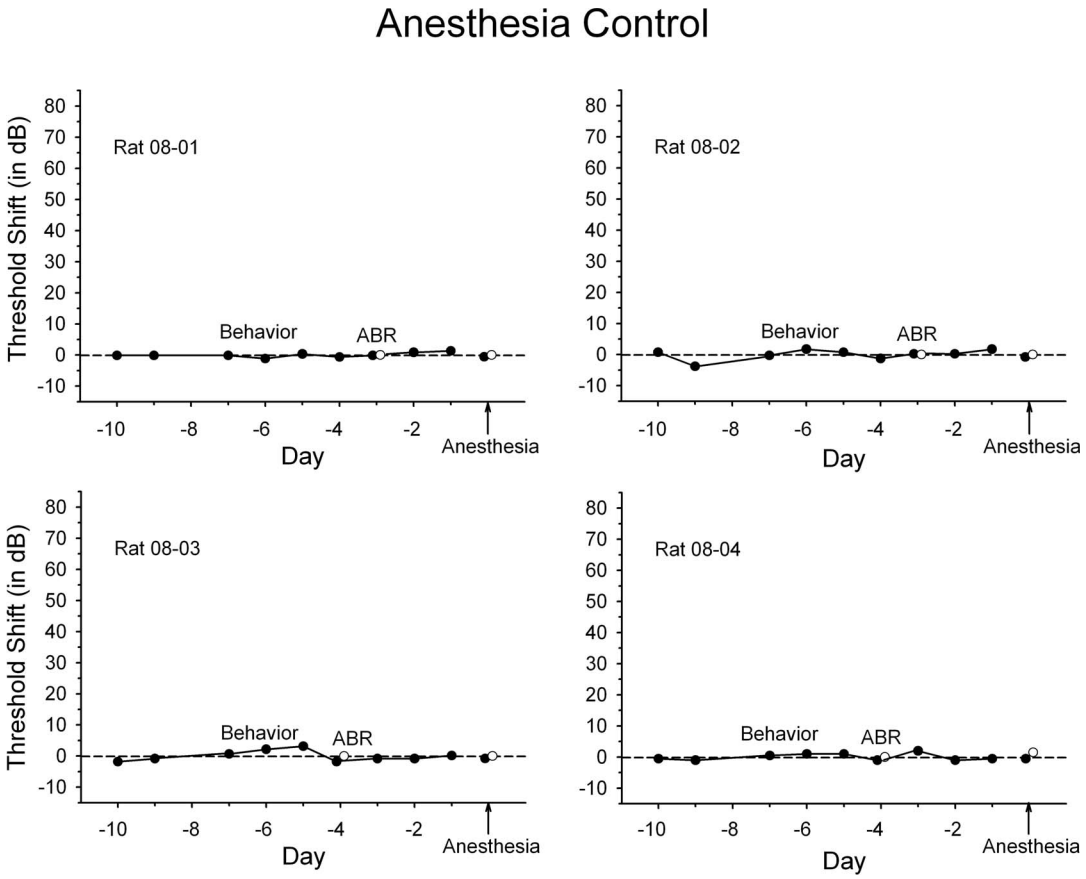

FIG. 4. Behavioral thresholds are not affected by 10 min of gas anesthesia (isoflurane) delivered 1 h before behavioral testing. The behavioral auditory stimulus was the same 1 ms, 16 kHz stimulus used in the 16 kHz ABR. Closed circles indicate behavioral thresholds; open circles indicate ABR thresholds.

6(c)]. Similarly, it was fairly accurate in estimating the permanent threshold shift, with the final scores for all four animals differing by no more than ±3.7 dB.

E. Behavioral and ABR threshold shifts using the same 1 ms noise stimulus

Given the relatively good agreement between the ABR and behavioral measures of threshold shift using the noise stimulus, we investigated whether the agreement would be greater if the same 1 ms noise burst were used for both the behavioral and the ABR thresholds.

With regard to the initial hearing loss, two of the four ABR estimates of threshold shift fell within ±5 dB of the behavioral threshold shifts (Fig. 7)—which is a slightly better agreement than was found for the 400 ms noise thresholds. With regard to the time course of the recovery, the ABR disagreed with the behavioral recovery in two of the five cases where in one it indicated a five versus a four day recovery [Fig. 7(a)], and in the other it indicated a one versus a three day recovery [Fig. 7(e)]. Finally, with regard to the permanent threshold shift, the ABR estimate showed slightly less agreement than was found for the 400 ms noise thresholds with four of the five threshold estimates falling within ±5 dB.

To determine if using the same stimulus for the behavioral and ABR thresholds resulted in a better agreement, the results of this test were compared with those of the previous one using the Mann–Whitney *U* test in which the difference between the behavioral and ABR threshold shifts were rank ordered. The results of the analysis indicated that using the same stimuli for both tests did not significantly improve the agreement ($p > 0.2$). However, given the small sample sizes, we cannot rule out the possibility that increasing the number of animals tested might yield a statistically reliable difference.

F. Hearing loss

It can be seen from these results that the magnitude of a threshold shift often varied between animals exposed to the same loud tone, a phenomenon that has been seen in both humans and animals (e.g., Davis *et al.*, 1950; Heffner and Harrington, 2002). One possibility is that the magnitude of the threshold shift is related in some way to pre-exposure absolute sensitivity. For example, perhaps those animals with better sensitivity are more susceptible to the traumatic effects of loud sound, or the reverse. Because of the variety of exposing tones and test stimuli that were used, we do not have many instances for comparison. Thus, Table I shows the two sets of data for which three or more animals were exposed and tested in the same way. In the first group, in which four animals were exposed to 11.2 kHz at 120 dB and tested on the 1 ms 16 kHz tone pip (Fig. 5), the rank ordering of the rats by pre-exposure sensitivity indicates that the better the pre-exposure sensitivity, the greater the hearing loss. However, the second group, in which three animals were exposed to 16 kHz at 120 dB and tested on 1 ms noise (Fig. 7), shows

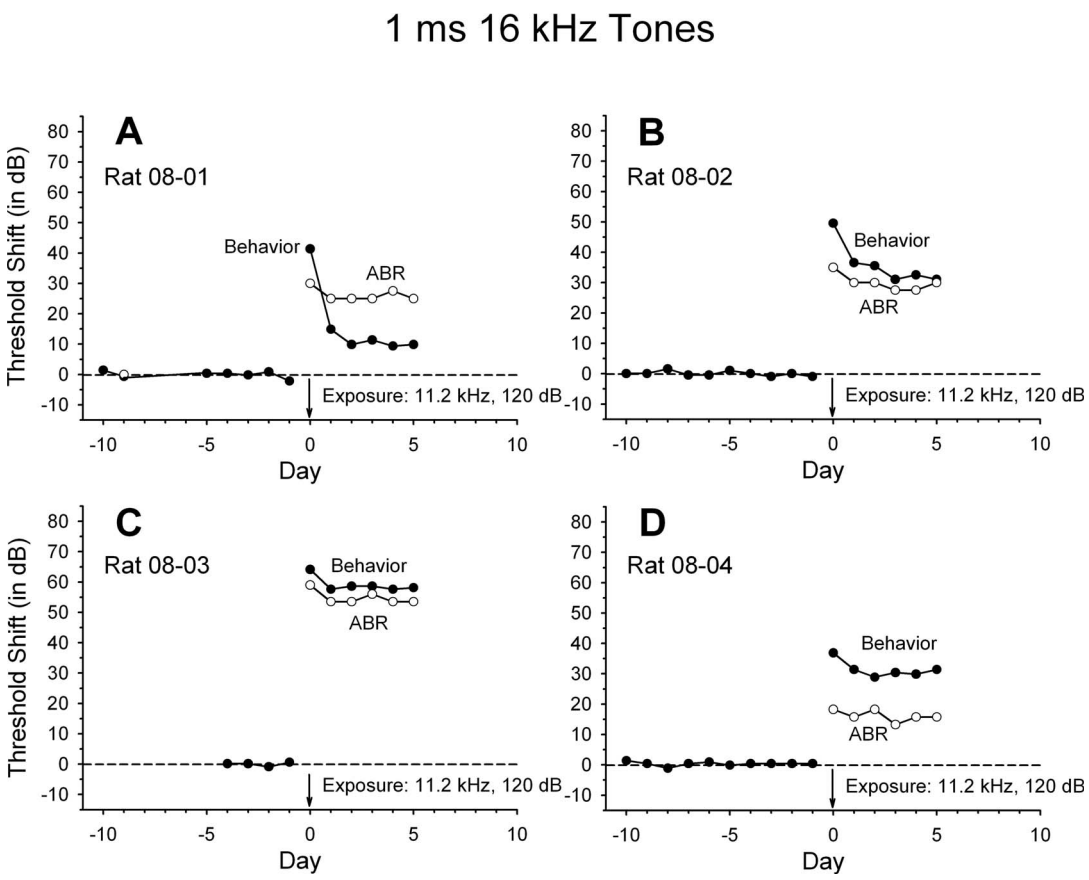

FIG. 5. Behavioral (closed circles) and ABR (open circles) threshold shifts for the 1 ms, 16 kHz stimulus. Using the same tonal stimuli for the behavioral and ABR thresholds does not noticeably improve the agreement between the two procedures. (The last of two pre-exposure ABRs for rats 08-02 and 08-03 were done 14 days before exposure; the last one for rat 08-04 was done 26 days before exposure.)

the opposite, that the less sensitive animals had the greater hearing loss. However, it should be noted that the animals' absolute thresholds do not vary that much and that whereas the pre-exposure thresholds of rats 08-01 and 08-02 differed by only 0.1 dB, their threshold shifts differed by over 20 dB (Table I). Although it is possible that there is some relationship between absolute sensitivity and threshold shift that varies with the frequency of the exposing and test stimuli, we do not have sufficient data to address this question. Similarly, we do not yet have sufficient data to determine if previous exposure to loud sound affects the results of subsequent exposures.

IV. DISCUSSION

A. The behavioral thresholds

The interpretation of these results rests on the degree of confidence in the behavioral thresholds. The method of conditioned suppression used here is a relatively simple procedure that has long been used to determine the auditory thresholds of mammals (Masterton *et al.*, 1969). Indeed, thresholds obtained for rats with this method in different laboratories and many years apart show remarkably good agreement (cf. Heffner *et al.*, 1994 and Kelly and Masterton, 1977). One factor contributing to this reliability is that the act of drinking from a spout fixes an animal's head in the sound field, thus making accurate measurement of the sound reaching its ears possible. Another is that an animal need only make the simple and natural response of freezing when it detects a sound. Finally, by devoting an entire test session to a single sound, we ensured that a sufficient number of trials could be obtained to precisely determine an animal's threshold. The stability over time also supports our confidence that the behavioral thresholds are both reliable and valid.

It should be noted, however, that exposing animals to a sound loud enough to cause a hearing loss may also cause tinnitus; moreover, given the levels of exposure used here, the severity of the tinnitus would be expected to be greatest immediately after the exposure and then to gradually decline (Heffner and Koay, 2005; Imig *et al.*, 2007). Thus, the question arises as to whether the greater initial difference between the behavioral and ABR measures of hearing loss for noise could be attributed to tinnitus. Although plausible, there are at least two reasons why this is probably not the case. First, the behavioral stimulus was pulsed to prevent it from being confused with tinnitus. Although some patients do describe their tinnitus as pulsing, it would seem unlikely that the animals would develop tinnitus that was close in pitch and pulsing at the same rate as the sounds on which they were tested. Thus, the characteristics of the physical sounds should have prevented them from being confused with tinnitus. Second, unlike human patients who typically have little experience in auditory testing, the rats in this study were trained observers, having received 30 or more

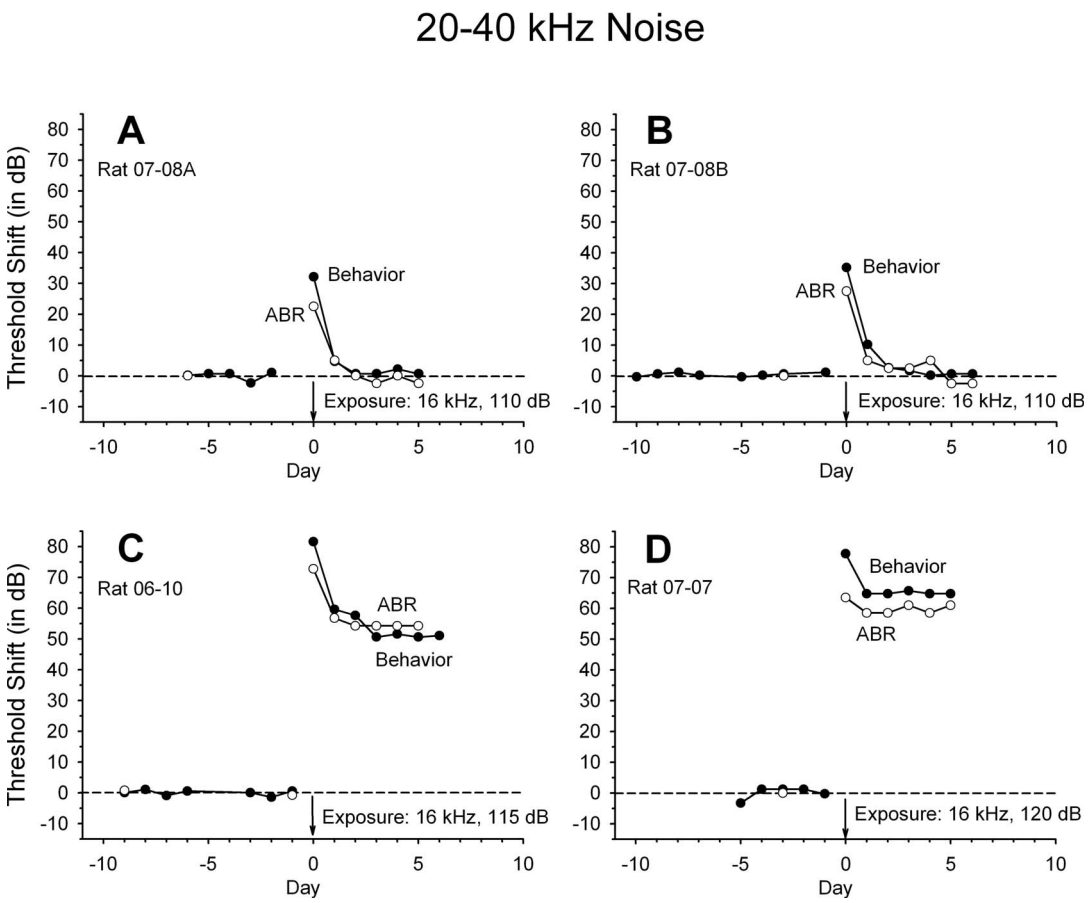

FIG. 6. Behavioral (closed circles) and ABR (open circles) threshold shifts for 20–40 kHz band noise in which behavioral thresholds were determined with 400 ms noise bursts and ABR thresholds with 1 ms noise bursts. Note the relatively good agreement between the two measures.

days of training to detect one specific sound prior to testing. Thus, we think it unlikely that the animals' thresholds were affected by tinnitus.

B. Estimating sensorineural hearing loss from the ABR

One of the main conclusions of this study is that tonal ABR thresholds do not provide a reliable estimate of sensorineural hearing loss for tones, regardless of whether the behavioral tests use pure tones or the ABR tone pips. Interestingly, the problem is not that the ABR errs by a consistent amount and direction (in which case a correction factor could be applied), but that its correspondence with the behavioral threshold shift is erratic. For example, the tone ABR estimated the permanent threshold shift to within ±5 dB in 8 of the 15 cases (see Figs. 2, 3, and 5), but over- or underestimated the other 7 cases by as much as 37 dB [Fig. 2(e)]. Because the tone ABR provides an accurate measure in about half of the cases, we re-examined the records of each of the animals to see if there was some factor, such as background noise level in the ABR, that might indicate whether or not the ABR was accurate, but could find none. Nevertheless, it is conceivable that there might be some other measure that, used in conjunction with the ABR, would indicate those animals for which the ABR provides an accurate estimate of threshold shift (e.g., evoked otoacoustic emissions, middle latency, and other responses).

In contrast, the noise-evoked ABR gave a more accurate picture of the behavioral threshold shift for that noise stimulus, regardless of whether the behavioral stimulus was 400 ms or 1 ms pulses (Figs. 6 and 7). As shown in Table II, rank ordering the animals on the ABR estimate of threshold shift for noise results in only one reversal for the initial hearing loss for noise and only a minor reversal for the permanent hearing loss. However, it should be noted that octave-band noise is a relatively broad frequency stimulus, and the question is whether the ABR evoked by narrow band noise would provide a reliable indication of the frequencies at which a hearing loss occurs.

Finally, we evaluated the noise-evoked ABR as an estimate of behavioral hearing loss because we have previously used it to estimate hearing loss in studies of tinnitus (Heffner and Harrington, 2002; Heffner and Koay, 2005; Zhang *et al.*, 2004). These studies suggested that the increase in spontaneous activity in the dorsal cochlear nucleus that follows exposure to loud sound is due not to tinnitus, but to the hearing loss resulting from the exposure (for a discussion of this issue, see Heffner and Koay, 2005). One outcome of the present study is that the noise-evoked ABR is a reliable measure of behavioral hearing loss. This finding supports the view that increase in spontaneous activity in the DCN, which begins to occur about a week after exposure to loud sound, is related not to tinnitus, but to the accompanying hearing loss.

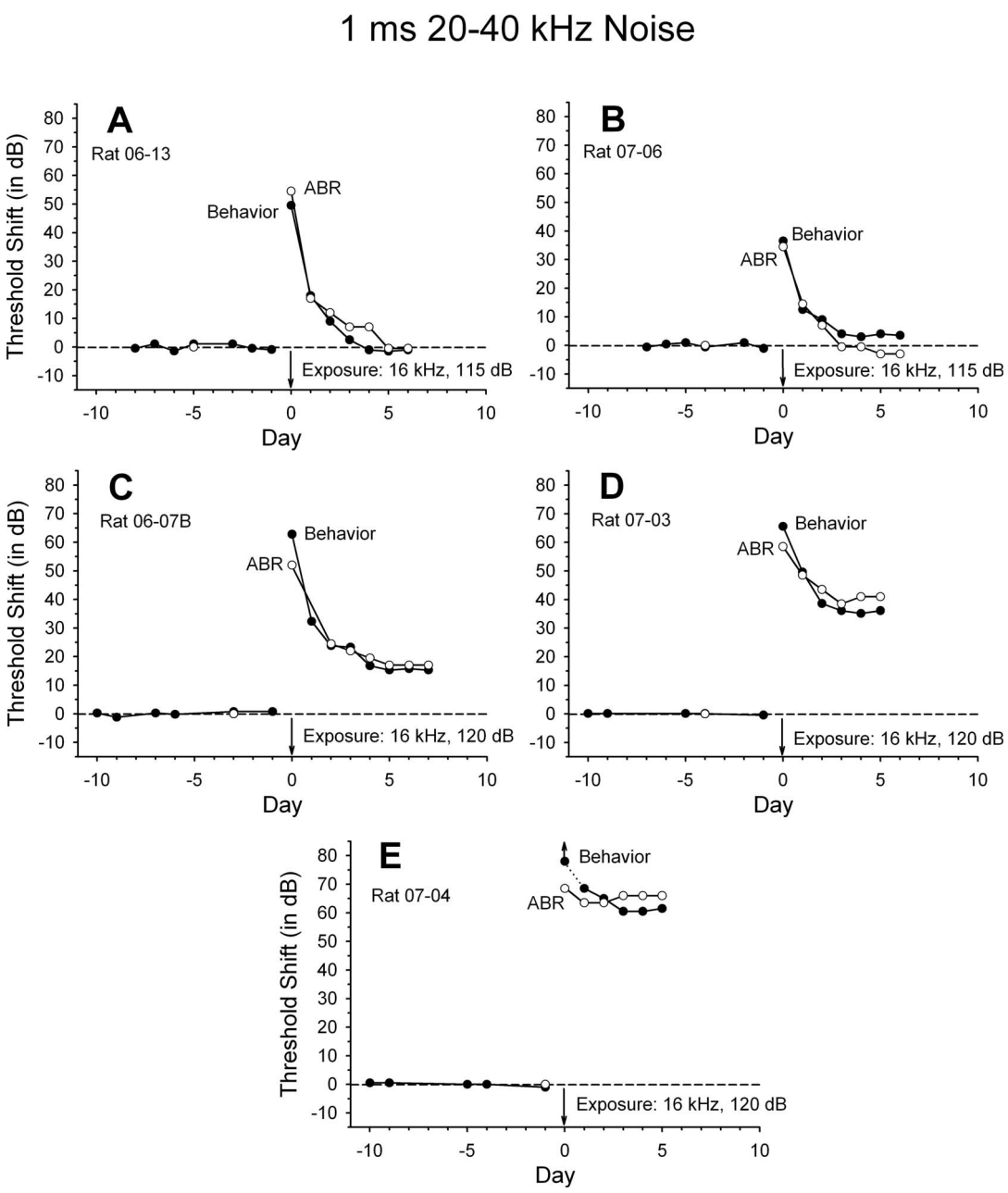

FIG. 7. Behavioral (closed circles) and ABR (open circles) threshold shifts for 1 ms high-frequency noise bursts in which both the behavioral and the ABR thresholds were determined for 1 ms noise bursts. Using the same noise stimulus in the behavioral and ABR tests did not improve the correspondence between the two measures beyond that seen in Fig. 6.

C. Previous comparisons of behavioral and physiological measures of hearing loss in animals

As noted in the Introduction, there have been four previous studies that compared behavioral and physiological measures of hearing loss in the same animals. These differed from the present study in several important ways. First, although the previous studies tested fewer animals, they obtained thresholds from each animal for a number of different frequencies. Second, with one exception, they measured the permanent but not the initial hearing loss. Third, again with one exception, the previous studies did not calculate threshold shifts but rather compared the posttreatment behavioral and physiological absolute thresholds with each other. Thus, where possible, we reanalyzed their data to determine threshold shifts. Finally, only one of the studies recorded the ABR; the others recorded either the compound action potential from inside the bulla or the evoked response recorded with bipolar electrodes implanted in the inferior colliculus.

The first study, conducted by Dallos *et al.* (1978), compared tone-evoked compound action potentials with behavioral thresholds in gerbils and chinchillas whose cochleas had been damaged with kanamycin. The physiological stimuli were short-duration tones with a 1 ms rise time, whereas the behavioral thresholds were obtained using 3.8 s tones with a 10 ms rise time. Because their physiological measures were obtained in terminal experiments, the comparisons were limited to posttreatment measures of the permanent effects of the kanamycin. Their results for four ger-

TABLE I. Pre-exposure sensitivity and the magnitude of postexposure threshold shift.

Rat	Pre-exposure threshold (in dB SPL)	Threshold shift (in dB)
Exposed to 11.2 kHz at 120 dB[a] Tested on 1 ms 16 kHz:		
08-03	19.9	58.1
08-04	22.6	31.4
08-02	23.5	31.1
08-01	23.6	9.9
Exposed to 16 kHz at 120 dB[a] Tested on 1 ms noise:		
06-07B	13.7	15
07-03	17.9	36.1
07-04	18.5	61.5

[a]All exposures were 10 min in duration.

bils and four chinchillas, tested on multiple frequencies, showed that the thresholds for the compound action potential paralleled the behavioral thresholds fairly well and could thus indicate the frequencies at which kanamycin caused a behavioral threshold shift. However, the compound action potential was less successful in indicating the magnitude of the behavioral threshold shift. In the case of the gerbils, the compound action potential sometimes indicated the actual behavioral threshold although in most cases it overestimated the hearing loss, sometimes by as much as 40 dB. In the case of the chinchillas, the compound action potential threshold was almost always higher than the behavioral threshold with an average difference of 18 dB. Thus, the compound action potential can indicate the relative pattern of a behavioral hearing loss but is less successful in indicating the magnitude of the loss.

Five years later, Henderson *et al.* (1983) compared auditory evoked potentials recorded from a bipolar electrode implanted in the inferior colliculus with behavioral thresholds in three monaural chinchillas. The animals were tested before and after a 1 h exposure to loud noise (a mixture of continuous and impulse 2–4 kHz band noise). The physiological and behavioral stimuli in this study were both 20 ms duration tones (5 ms rise-fall times). They obtained a threshold for seven different frequencies (ranging from 0.5 to 8 kHz) 24 h after exposure and again 30 days after exposure. Although they did not calculate threshold shifts, it is possible to derive threshold shifts from Table 2 of their paper. Their data, like ours, show that the evoked response does not reliably correspond to the initial behavioral threshold shift. Specifically only 12 of their 21 physiological estimates (57%) fell within ±5 dB of the behavioral threshold shifts with some being off by as much as 25 or 30 dB. With regard to the permanent behavioral hearing loss, the evoked response estimated 13 out of 21 threshold shifts to within ±5 dB. However, the chinchillas had little or no permanent hearing loss, with only a third of the thresholds elevated by 5 dB or more, and thus these results do not indicate how well this method estimates a permanent loss; for this it is necessary to turn to the next study.

The third study, by Davis and Ferraro (1984), also compared auditory evoked potentials recorded from a bipolar electrode implanted in the inferior colliculus with behavioral thresholds in monaural chinchillas before and after exposure to loud sound, in this case a 2 kHz tone (120 dB, 4 h). Although they did not measure the initial hearing loss, they determined the permanent behavioral hearing loss (5 weeks after exposure) for two sets of tones, the same tones used in the physiological measures (20 ms duration, 5 ms rise-fall) as well as longer duration tones (500 ms duration, 5 ms rise-fall). They obtained pre- and postexposure evoked potentials and behavioral thresholds for six chinchillas at seven frequencies from 500 Hz to 4 kHz. Their results, reanalyzed to reveal the amount of threshold shift for each measure, can be summarized as follows: First, the evoked response estimated the behavioral threshold shifts for 500 ms tones to within ±5 dB, 10 out of 35 times (29%) with misestimates as high as 25 dB or more. However, it was more accurate in estimating the threshold shifts for the 20 ms tones in which 26 of the 42 threshold estimates (62%) were within ±5 dB and the largest misestimates appeared to fall within 15–19 dB of the behavioral thresholds. Second, the evoked response accurately estimated the 20 ms behavioral threshold shifts for some animals, but not for others; for example, the tone be-

TABLE II. Rank ordering by ABR estimate of hearing loss for 20–40 kHz noise.

	Initial hearing loss (in dB)			Permanent hearing loss (in dB)			
Rat	ABR	Behavior	Difference	Rat	ABR	Behavior	Difference
400 ms noise:							
07-08A	22.5	32.2	−9.7	07-08A	−2.5	0.7	−3.2
07-08B	27.5	35.2	−7.7	07-08B	−2.5	0.7	−3.2
07-07	63.5	77.7	−14.2	06-10	54.3	50.6	3.7
06-10	72.8	81.6	−8.8	07-07	61.0	64.7	−3.7
1 ms noise:							
07-06	34.5	36.5	−2.0	07-06[a]	−3.0	3.5	−6.5
06-07B	52.0	62.8	−10.8	06-13	−0.5	−1.0	−1.7
06-13[a]	54.5	49.5	5.0	06-07B	17.0	15.3	0.5
07-03	58.5	65.6	−7.1	07-03	41.0	36.1	4.9
07-04	68.5	>78.0	−9.5+	07-04	66.0	61.5	4.9

[a]Indicates an incorrect ranking (in both cases, the ABR underestimated the behavioral hearing loss).

havioral threshold shifts of one chinchilla (animal 5) fell within ±5 dB of the evoked response shifts for all seven frequencies whereas only two of the seven thresholds of another (number 3) fell within ±5 dB. Finally, the evoked response was more accurate in estimating the behavioral threshold shift for lower frequencies (0.5, 0.75, and 1 kHz) than for higher frequencies (1.5, 2, 3, and 4 kHz).

The final study, by Borg and Engström (1983), recorded the ABR in rabbits using subcutaneous needle electrodes. The physiological stimuli were brief tone bursts, whereas the behavioral stimuli were 10 s tones. The authors reported threshold shifts for two rabbits, one that had been exposed to loud sound and the other that had been treated with kanamycin. The results of the animal exposed to loud sound indicated a fairly good agreement with the two measures differing by 7 dB or less at 0.5, 1, 2, and 4 kHz; behavioral thresholds at higher frequencies could not be obtained, so no further comparisons were available. The results of the animal given kanamycin also showed good agreement; with the exception of 0.5 kHz where the threshold shifts differed by 25 dB, the threshold shifts at 1, 2, 4, 8, and 16 kHz were within 4 dB of each other. The authors mentioned that they made two other noise exposure but did not report the results. Thus, their results are based on complete data from one animal and on partial data from another.

In conclusion, the most comprehensive study for which measures of threshold shift are available is that by Davis and Ferraro (1984). Their results indicate that the evoked response recorded from the inferior colliculus can estimate the permanent behavioral threshold shifts for short-duration (20 ms) pure tones to within ±5 dB about 60% of the time, with misestimates ranging near 20 dB. In comparison, our results indicate that the ABR evoked by octave noise estimated the permanent behavioral threshold shift to an accuracy of ±5 dB eight out of nine times (89%), with the largest misestimate being 6.5 dB (Table II). This suggests that the accuracy of physiological measures of hearing loss is improved by using noise rather than tone stimuli. However, to obtain frequency-specific information, it would be necessary to narrow the width of the noise band, and we do not know at this time if this would reduce its accuracy.

D. The hearing loss

One of the outcomes of this study is the observation that animals exposed to the same loud sound may develop different hearing losses. As can be seen in Table I, this variation cannot be easily accounted for by variation in pre-exposure sensitivity. This is not a new observation as we have seen such variation in hamsters exposed to loud tones (Heffner and Harrington, 2002). Indeed, in their classic study of temporary hearing loss caused by exposure to loud sound, Davis *et al.* (1950) noted that the same exposures to loud sound result in different patterns and degrees of hearing loss in different individuals; as they stated, "…some individuals are systematically more susceptible than others." This leads to the question of why some ears are more resistant to overstimulation by sound. Is there individual variation in some biochemical or physiological mechanism that protects the ear from loud sounds? Is there a way to identify those individuals with susceptible ears so that they might take precautions to protect them? Are there treatments that might make ears less susceptible to damage?

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