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Tinnitus and Hearing Loss in Hamsters (*Mesocricetus auratus*) Exposed to Loud Sound

Henry E. Heffner and Gimseong Koay
University of Toledo

Hamsters were trained to go left and right to sounds on their left and right sides, respectively. Silent trials were occasionally given in which no sound was presented. Hamsters exposed to a loud 2- or 10-kHz tone in 1 ear often shifted their responding on the silent trials to the side of the exposed ear, suggesting that they perceived a sound in that ear (i.e., tinnitus). The degree of tinnitus was related to the degree of the accompanying hearing loss (estimated by the auditory brainstem response). However, a conductive hearing loss (plugging 1 ear) did not cause a hamster to test positive for tinnitus. Tinnitus could be demonstrated within minutes following tone exposure, indicating an immediate onset, as occurs in humans.

Keywords: auditory brainstem response, hamster, hearing loss, operant behavior, tinnitus

Recent studies have indicated that exposing animals to loud sound can cause them to respond as though they hear sound in the absence of any physical stimulus, that is, that they develop tinnitus (Bauer & Brozoski, 2001; Brozoski, Bauer, & Caspary, 2002; H. E. Heffner & Harrington, 2002). This suggests that it may be possible to use animals to determine the physiological basis and treatment of sound-induced tinnitus in humans. To do so, however, it is necessary to determine how closely the sound-induced tinnitus observed in animals resembles that found in humans.

One of the first studies of sound-induced tinnitus in humans is the classic study of temporary deafness following exposure to loud sounds by Hallowell Davis and his colleagues (Davis, Morgan, Hawkins, Galambos, & Smith, 1950). Using themselves and college students as subjects, they exposed one ear to a loud sound and then observed the resulting changes in sensitivity, loudness, and pitch perception. Subjects were tested once or twice a week, with time allowed for recovery between tests, thus providing multiple observations with replication on the same subject. In commenting on the tinnitus that accompanied the hearing loss, they noted that the tinnitus resulting from exposure to a loud tone was more likely to have a "definite and constant pitch" than was that resulting from exposure to noise. Moreover, the pitch of the tinnitus typically occurred at the high-frequency edge of a sharply localized hearing loss, an observation suggesting that tinnitus occurs when a section of the basilar membrane is rendered partly or completely unresponsive to sound, with the pitch of the tinnitus corresponding to the less affected portion of the basilar membrane at the high-frequency end of the damaged section. Judging from the illustrations in their report, it appears that the pitch of the tinnitus was generally matched to a tone 1–1.5 octaves above the frequency of the exposing tone, well above the frequency of maximum hearing loss, which usually occurred about 0.5 octaves above the frequency of the exposing tone.

Two other studies of sound-induced tinnitus in humans have appeared since 1950 (Atherley, Hempstock, & Noble, 1968; Loeb & Smith, 1967). The study by Loeb and Smith (1967), which exposed subjects to both loud tones and octave-band noise, found the same relationships between the frequency of an exposing tone, the frequency of maximum hearing loss, and the pitch match of the resulting tinnitus as did Davis et al. (1950). The pitch of the tinnitus induced by exposure to octave-band noise, which was not investigated by Davis et al., was found to range from 0.04 to 0.61 octaves above the center frequency of the noise. The study by Atherley et al. (1968), which exposed subjects to 0/3-octave filtered noise, found the resulting tinnitus to match the pitch of tones from about 0.1 to 0.6 octaves above the center frequency of the exposing noise band, values similar to those found by Loeb and Smith for octave-band noise. Although both of these studies found considerable between-subjects variation in matching the pitch of tinnitus induced by the same stimulus, this may have been due in part to variation in the ability of subjects to make pitch matches, a fact that caused Davis and his colleagues to confine their observations to their most experienced subjects (who were three of the authors themselves).

Given the results of these human studies, we expected that acute tinnitus in animals resulting from exposure to loud sound would have the following characteristics. First, such tinnitus would be associated with a hearing loss. Second, the onset of the tinnitus would be immediate. Third, exposure to loud tones would be more likely to produce tinnitus that had a definite and constant pitch than that caused by exposure to broadband noise. Fourth, the pitch of the tinnitus resulting from exposure to loud tones would generally match a tone 1–1.5 octaves above the frequency of the exposing tone; the pitch of tinnitus induced by a noise band, on the other hand, would match tones from about the center frequency of the

Henry E. Heffner and Gimseong Koay, Department of Psychology, University of Toledo.

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Correspondence concerning this article should be addressed to Henry E. Heffner, Department of Psychology, University of Toledo, Toledo, OH 43606. E-mail: hheffne@pop3.utoledo.edu

noise to 0.5 octaves higher. Finally, the fact that the frequency of maximum hearing loss induced by exposure to a loud tone occurs about 0.5 octaves above the frequency of the exposure tone, whereas the pitch of the tinnitus is typically matched to a frequency 1–1.5 octaves above the exposing tone, could provide a way of distinguishing between the physiological effects of tinnitus and those of hearing loss in animals exposed to loud tones.

Recently, we have begun to investigate the characteristics of sound-induced tinnitus in hamsters with the goal of comparing it with that found in humans. To do this, we needed a procedure that gave results that could be more readily compared with those of human studies. Specifically, we wanted a method in which a subject served as its own control, the determination of tinnitus could be made quickly before it had time to change, and the effect of the tinnitus could be clearly separated from the effects of the accompanying hearing loss. The procedure we chose is a two-choice task in which an animal is trained to go left or right to sounds coming from its left or right side (i.e., to lateralize sound). The animal is also given silent trials in which no sound is presented. One ear of the animal is then exposed to an intense sound, and the animal is retested. The results, which can be obtained in a single session of less than 30 min, show that many of the exposed animals shift their responding on the silent trials to the side of their exposed ear, suggesting that they hear a sound in that ear—that is, tinnitus. Moreover, the accompanying hearing loss has the opposite effect on sound trials in that animals often make more errors by responding to the side of their unexposed (normal) ear when a sound is presented on the side of their exposed ear.

Our purpose in the present study was to use the two-choice procedure to (a) determine the relationship among the intensity of a 10-kHz exposing tone, the degree of the resulting tinnitus, and the degree of hearing loss in hamsters; (b) determine whether tinnitus could be detected immediately in animals exposed to loud tones for short durations (3–60 min); and (c) determine the effect of a unilateral hearing loss unaccompanied by tinnitus by putting an earplug in one ear.

Method

The experiments involved four procedures: (a) the two-choice tinnitus test, (b) exposure to loud sounds to induce tinnitus, (c) the auditory brainstem response (ABR) to measure hearing loss, and (c) construction and placement of earplugs.

Subjects

The subjects were male Syrian golden hamsters (*Mesocricetus auratus*), which were obtained from Charles River Laboratory and ranged in age from 50 to 70 days at the beginning of the experiments. They were housed on corn cob bedding in standard solid-bottom cages with grid covers and given free access to rodent blocks supplemented occasionally with pieces of apple. Water was available only during the daily training and test sessions.

Care was taken to avoid exposing the hamsters to significant noise, including that made when placing the lid on the metal pan in which they were weighed each day (this noise was reduced by fitting the lid with a rubber gasket). The ambient noise level of the hamster colony room was 60 dB sound pressure level (SPL) re 20 $\mu N/m^2$ (for details, see H. E. Heffner & Harrington, 2002).

Behavioral Apparatus

Testing was conducted in a carpeted, double-walled sound chamber (IAC Model 1204, Industrial Acoustics, Bronx, NY; 2.55 m × 2.75 m × 2.05 m), the walls and ceiling of which were lined with eggcrate foam. The equipment for behavioral control and stimulus generation was located outside the chamber, and the hamsters were observed over closed-circuit TV. The chamber was carefully checked for the presence of extraneous sounds, including frequencies above the range of human hearing, by use of a sound level meter (described below). Because dimming the incandescent ceiling lights in the chamber caused them to emit an audible tone, the light bulbs were replaced with lower wattage bulbs that were never dimmed. In addition, a careful check of the video camera for the presence of sonic and ultrasonic sounds within the range of the hamster's hearing revealed no detectable sound.

The hamsters were tested in a cage (18 cm long × 10 cm wide × 12 cm high) constructed of 0.5-in (1.27-cm) wire mesh. The cage was mounted on a table 1 m above the chamber floor. Three water bottle sipper tubes served as the response manipulanda. The tubes were mounted in a horizontal row at the front of the cage, 2.5 cm apart and 5.5 cm above the cage floor. Each tube, which had an LED mounted on it 2.5 cm back from the tip, was connected to a separate lick circuit. A tiny water bowl (1.2 cm diameter) was mounted below the center sipper tube 1.5 cm above the cage floor and was connected to a syringe pump (NE 1000, New Era, Wantagh, NY), with the water feeding up through a hole in the bottom of the bowl.

Acoustical Apparatus

Sine waves were generated by a tone generator (Model 2400, Krohn-Hite, Avon, MA) and broadband noise by a noise generator (Model S81-02, Coulbourn, Lehigh Valley, PA). The signals were combined, amplified (Model S82-24, Coulbourn), and sent to one of two piezoelectric tweeters (Model KSN 1005A, Motorola, Chicago) that were located just outside the cage at 90° to the left and right of a hamster's head when it was licking the center sipper tube.

The SPL was measured with a Bruel & Kjaer (B&K) 0.25-in (0.64-cm) microphone (Model 4135, B&K, Naerum, Denmark) and measuring amplifier (Model 2608, B&K). The measuring equipment was calibrated with a pistonphone (Model 4230, B&K).

The intensity of the broadband noise was 35 dB SPL. The tones were set 20–30 dB above the published hamster threshold for a frontally placed sound source (R. S. Heffner, Koay, & Heffner, 2001): 10 kHz at 30 dB, 12.5 kHz at 35 dB, 16 kHz at 45 dB, 20 kHz at 40 dB, 25 kHz at 40 dB, and 32 kHz at 40 dB. Tones were presented with the noise during training because it was expected that tinnitus induced by exposure to a loud tone would be tonal. Noise alone was presented during tinnitus testing.

Behavioral Procedure

A hamster was first trained to lick the dry center sipper tube in order to get a water reward delivered to the water bowl located below it. It was then trained to lick the center sipper tube and then a side sipper tube to get the water reward. At first, the correct side response was indicated both by turning on the loudspeaker on that side and by illuminating the LED over the correct sipper tube. Once the hamster had learned to perform the task at 80% or better, both side LEDs came on to signal that a side response should now be made without indicating the correct side.

In the next stage of training, a hamster was placed in the test cage with the LED above the center sipper tube turned on. Licking the center tube turned off the center LED, illuminated both side LEDs, and turned on the noise/tone signal from either the left or right loudspeaker. Licking a side sipper tube turned off the side LEDs, with correct responses rewarded by the dispensing of 0.04 mL of water into the water bowl and incorrect responses punished by the delivery of a mild electric shock between the tube and the cage floor. The center LED was then turned back on, and the hamster was permitted to initiate another trial. The frequency of the tone embedded in the noise was changed from session to session to accustom the hamsters to responding to different tones.

In the final stage of training, silent trials were randomly inserted in which licking the center tube initiated a trial (signaled by the LEDs), but no sound was presented. The hamster was required to make a side response, but was neither rewarded with water nor punished with shock—in other words, it received no feedback on the silent trials. Approximately 24% of the trials in a session were silent trials. At this time, the hamster was also placed on a 50% feedback schedule in which, randomly, half of the sound trials were also not followed by water reward or shock, regardless of the hamster's side response. Instead, the side LEDs were turned off and the center LED turned back on so that the hamster could initiate another trial. The purpose of the 50% feedback schedule was to accustom the hamster to a partial reward schedule so that it would be less likely to learn that silent trials were never rewarded or punished. The use of the partial feedback did not decrease the hamsters' performances on the sound trials, and their responses on the silent trials were generally stable—partial feedback has been previously used in generalization tests on animals (e.g., Blough, 1975; H. Heffner, 1975; Page, Hulse, & Cynx, 1989).

The left–right trial sequence was determined by a quasi-random schedule (Gellermann, 1933). A correction procedure was used in which the correct side did not change following an error. The results of a correction trial were not counted in a hamster's score.

The hamsters were trained until they consistently performed at a level of 90% correct or better on the sound trials, a criterion that all of the hamsters easily met (scores of 100% correct were not unusual). The entire training period took 42–47 days, by the end of which the hamsters were typically receiving 100–120 sound and 30–40 silent trials in a session.

The tinnitus test consisted of exposing one ear of a hamster to a loud tone and then testing the hamster to determine whether its responding on silent trials shifted to the side of the exposed ear. Overall, the hamsters' preexposure silent scores were evenly distributed, with about half the hamsters favoring the left and the other half favoring the right side.

Calculating a Score on the Tinnitus Test

A hamster's score on the tinnitus test was defined as the difference between its average score on silent trials for the five sessions preceding exposure (or, in the case of the controls, prior to being anesthetized; see below) and its postexposure score. For example, a hamster that responded 20% left on the silent trials before exposure and then shifted to 50% left following exposure of the left ear would be given a score of +0.30 for that session. Although we experimented with using other measures, such as determining the percentage of change in a hamster's performance, a simple subtraction seemed more straightforward, and as the hamsters never responded entirely to one side or the other (i.e., there was no floor or ceiling effect), there seemed to be no reason not to use it. The .01 probability that a postexposure score was reliably different from the preexposure scores was calculated by use of the mean of a hamster's five preceding preexposure sessions plus the standard deviation multiplied by the *z*-score for the one-tailed .01 level, that is, $M + (SD \times 2.33)$.

Inducing Tinnitus

10-kHz, 4-hr exposure. Twenty-one hamsters were exposed in the left ear to a 10-kHz tone for 4 hr at 80, 110, or 125 dB SPL (7 hamsters per exposure group); the decision of left-ear exposure was for the convenience of subsequent electrophysiological recordings (Zhang, Heffner, Koay, & Kaltenbach, 2004). Seven additional hamsters were anesthetized, but not exposed, and were used as controls. 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), attenuated (L-pad, Model AT-100, Speco technologies, Amityville, NY), and sent to a piezoelectric loudspeaker (KSN 1005A, Motorola). A funnel (8-cm diameter) was attached to the front of the tweeter with thermoplastic adhesive, allowing the sound to be directed into a 7-mm inner diameter plastic tube (30-cm long) with a 4-mm inner diameter plastic tip. The sound was measured with a microphone placed at the tip of the plastic tube. A spectrum analysis of the acoustic signal showed that the 10-kHz, 125-dB tone was accompanied by a single harmonic at 20 kHz, which was 45 dB below the level of the 10-kHz tone. For reference, the average hamster thresholds at 10 and 20 kHz are 1.5 and 19 dB SPL, respectively (R. S. Heffner et al., 2001).

For exposure, a hamster was anesthetized (ketamine 90 mg/kg and xylazine 9 mg/kg), and a foam earplug was placed in its right ear with the pinna taped over the meatus. The hamster was then placed on its side, with its right ear against the table top, and the plastic tip of the sound tube placed 1–2 mm from the concha. We closely observed the hamster for the duration of the exposure to ensure that the tube remained in place. Control hamsters were anesthetized, but not exposed to the tone.

10-kHz, 3–10-min exposure. Three hamsters were exposed in one ear to the 10-kHz tone at 120 dB under halothane anesthesia and then tested for tinnitus as soon as they recovered from the anesthesia. For exposure, a hamster was placed in an anesthesia box containing 2% halothane and a 50:50 mixture of oxygen and nitrous oxide. Once unconscious, the hamster was removed from the box, the hose from the anesthesia machine was placed over its snout, the nonexposed ear was plugged as described above, and the tube from the loudspeaker inserted into the other ear. The hamster was exposed to the tone for 3–10 min and testing began 3–17 min after exposure.

2-kHz, 6–60-min exposure. Four hamsters were exposed in one ear to a 2-kHz tone at 120 dB by means of the same audio equipment described above, except that the exposing tone was produced by a midrange driver (Model 1823M, Electrovoice, Burnsville, MN). The hamsters were anesthetized with the halothane and oxygen–nitrous oxide mixture described above. They were exposed for 6–60 min and tested 3–15 min after exposure. Three of the hamsters that did not test positive for tinnitus were reexposed to a longer duration a week later.

Recording the ABR

To estimate the degree of hearing loss caused by the tone exposures, we determined thresholds using the ABR for the left and right ears of tone-exposed and unexposed (control) hamsters. To obtain a single number that reflected the overall hearing loss in an exposed ear, we determined thresholds for a 10–50 kHz band-pass noise burst 7–10 days after tone exposure.

ABR testing was conducted in a double-walled sound chamber identical to that used for behavioral testing. To obtain the ABR, a hamster was anesthetized (ketamine 90 mg/kg and xylazine 9 mg/kg) and one ear temporarily blocked by inserting a small foam plug into the auditory canal, taping the pinna over the meatus, and attaching a piece of 3-mm thick foam tape (approximately 3 cm × 3 cm) to the side of the hamster's head. This served to attenuate the signal in the plugged ear and to stabilize the hamster's head when it was placed on its side for testing. Subdermal electrodes were inserted at the vertex and behind the ear to be tested, with the ground electrode in the hamster's hind leg. The speaker was positioned directly above the hamster's ear at a height of 12 cm.

The noise stimulus was generated by use of Tucker-Davis Technologies (TDT; Alachua, FL) SigGen software at a sampling rate of 111.1 kHz (9-μs sampling period). The stimulus was 1 ms in duration and pulsed 27.7 times per second. The output of the DA converter (Model DA3, TDT) was passed to a programmable attenuator (Model PA4, TDT), through two filters (Model 3550, Krohn-Hite; 10–50 kHz bandpass settings providing 48-dB/octave roll-off), to a headphone driver (Model HB7, TDT), and then to a ribbon tweeter (Model 110T02, Foster Culver, Gardena, CA). The maximum intensity of the stimulus, determined with the previously described sound measuring system, was 77 dB SPL.

Data were collected with 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 with the artifact rejection level set at 25 μV. The recording window was 10 ms in duration and was triggered by a timing pulse from the TDT system at 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 2,000 at higher stimulus intensities to 4,000 around threshold.

Earplugs

Earplugs were used to determine the effect of a hearing loss, in the absence of tinnitus, on the performance of hamsters in the tinnitus test. Because occluding the ear canal causes internal sounds, such as licking and swallowing, to be physically louder in the plugged ear, that is, the *occlusion* effect (e.g., Tonndorf, 1976), we used vented earplugs. The importance of using vented plugs was indicated by tests showing that unvented or partially vented plugs caused a hamster in the tinnitus test to respond to the side of the plugged ear—that these plugs were in fact producing the occlusion effect was verified by demonstrating that the cochlear microphonic to bone-conducted sounds was increased when the plug was in place. Therefore, we used earplugs that were sufficiently vented so as not to cause the occlusion effect.

The earplugs used in this test were constructed from 4.5-mm outer diameter heat shrink tubing that was shrunk to 3.5-mm outer diameter (2.8-mm inner diameter) to fit the hamster auditory canal and cut into 4-mm long pieces. One end of the tubing was then either shrunk to a 1.0-mm opening or left unshrunk (2.8-mm opening).

To inserted an earplug, we anesthetized a hamster with the ketamine/xylazine anesthesia and placed the plug into the entrance of the auditory canal of one ear, with the fully open end toward the eardrum. The earplug was held in place with surgical glue (cyanoacrylate). The hamsters were allowed to recover from the anesthesia and given the tinnitus test the following day. The effect of three open and four partly closed earplugs was determined on four hamsters. After being given the tinnitus test, a hamster was again anesthetized and the degree of attenuation determined by the ABR with the same 10–50 kHz noise burst used to measure hearing loss following exposure to the 10-kHz tone.

Results

Analysis of Individual Tinnitus Test Performance

Examples of the performances of hamsters exposed in the left ear to the 10-kHz tone are shown in Figure 1. The hamster exposed to 10 kHz at 125 dB for 4 hr shifted its responding on silent trials to the left side on all 5 postexposure test days, suggesting that it had tinnitus (see Figure 1A). The exposure also resulted in a large, transient increase in errors on sound trials when the sound was presented on the exposed (left) side, a sign of a sudden hearing loss in that ear (the ABR, taken 9 days after exposure, indicated a 30-dB threshold shift in the left ear). Note that these two changes in responding are in the opposite direction—a shift to the left on silent trials and a shift to the right on sound trials—and therefore cannot be attributed to a general shift in the hamster's side preference.

The hamster exposed to a 10-kHz tone at the lower intensity of 110 dB for 4 hr shifted its responding on silent trials to the left side on postexposure Days 1 and 3 (see Figure 1B). This hamster showed a small increase in errors on sound trials that was slightly greater for sounds on the left side, again, probably because of the sudden hearing loss in the exposed ear (the ABR, taken 8 days after exposure, indicated a 15-dB threshold shift in the left ear).

The hamster exposed to 10 kHz at only 80 dB for 4 hr failed to test positive for tinnitus (see Figure 1C). In addition, this hamster showed no increase in errors on the sound trials, indicating that its hearing loss at that time, if any, was not sufficient to noticeably affect sound localization (the ABR, taken 8 days after exposure, also showed no threshold shift in the left ear).

As can be seen from these examples, whether a hamster developed tinnitus was indicated by whether it shifted its response on silence trials to the side of the exposed ear, implying that it was perceiving a sound in that ear. Moreover, it appears possible to obtain a measure of the degree and duration of a hamster's tinnitus. Specifically, the size of the shift following exposure may indicate the degree or salience of the tinnitus, although factors such as how well a hamster generalized from external tones to its tinnitus may also play a role; the persistence of tinnitus may be indicated by the number of days that a hamster tested positive, although factors such as habituation and spontaneous shifts in side preference could affect this measure.

Effect of 10-kHz, 4-hr Tone Exposures on Tinnitus Test Performance

The effect of exposing hamsters to 10 kHz for 4 hr at 80, 110, and 125 dB is shown in Figure 2A. In case of the 110- and 125-dB groups, the highest scores were almost always on the first postexposure day, and a hamster that did not test positive on the first day did not test positive on subsequent days. The performance of the 80-dB exposure group, however, was variable with one hamster testing positive on Day 1, two on Days 2–4, and three on Day 7 (which accounts for the slightly higher average score on the last day). In contrast, none of the control hamsters ever tested positive. One explanation is that the hamsters in the 80-dB exposure group developed a low-level tinnitus to which they occasionally responded.

Because tinnitus is related to hearing loss, and individuals exposed to the same loud sound can vary in their hearing loss (Davis et al., 1950), the tinnitus scores were examined as a function of hearing loss. The ABR estimate of hearing loss was taken 7–10 days after exposure, when behavioral testing was complete, and therefore represents the permanent portion of a hamster's hearing loss, as it would have recovered from the temporary portion by that time. The degree of each hamster's hearing loss was estimated by comparing its ABR to that of the two control hamsters for which ABR thresholds were available. The exposed hamsters were then grouped as follows: 0-dB threshold shift (thresholds within 5 dB of the control hamsters), 10-dB threshold shift (7.5–12.5 dB above control average), 20-dB threshold shift (17.5–22.5 dB above control average), and 30-dB threshold shift (27.5–32.5 dB above control average). Arranging the hamsters by hearing loss slightly reduces the number of crossovers between groups and shows that the greater the hearing loss the higher the average tinnitus score (see Figure 2B).

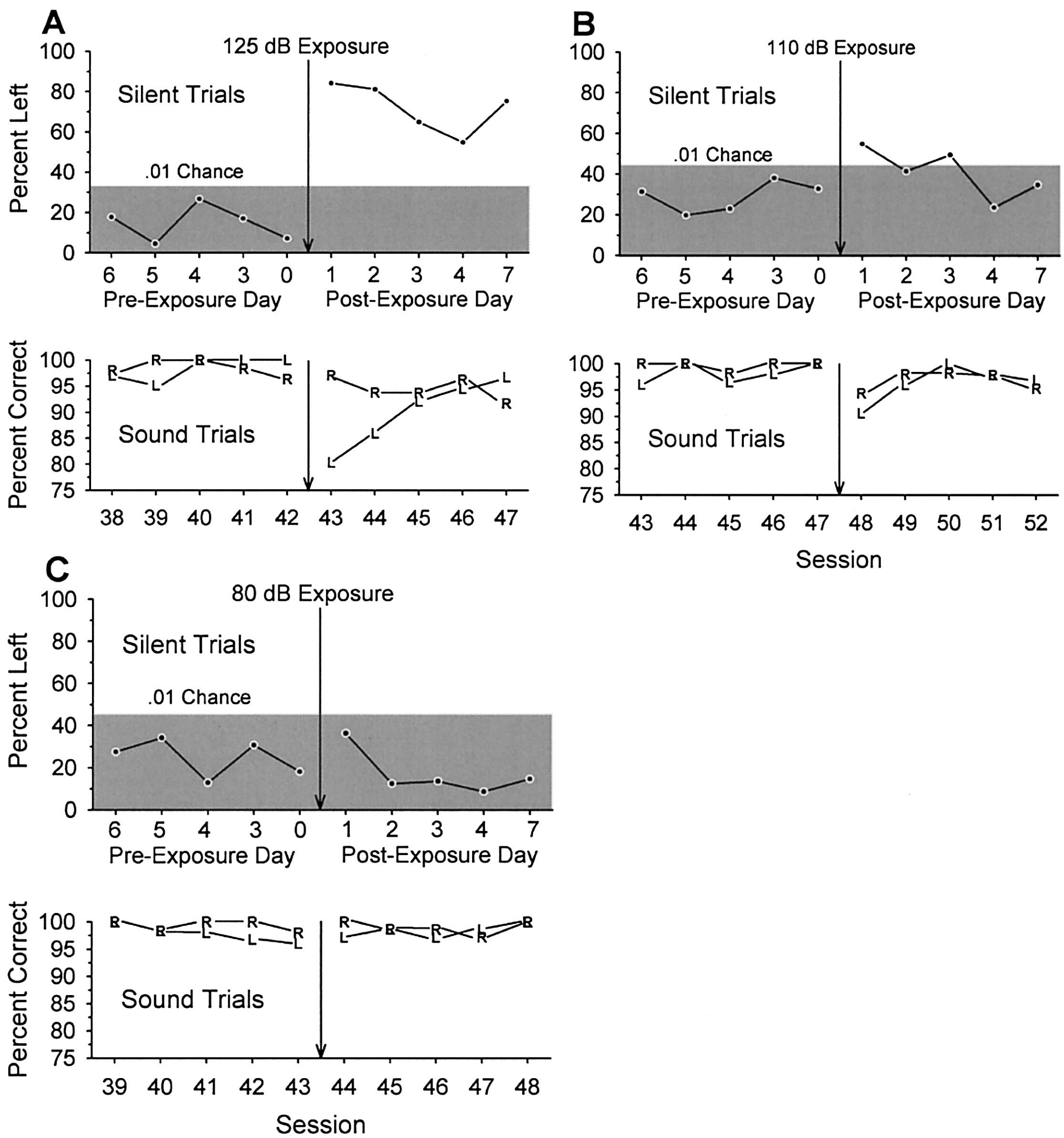

Figure 1. Effect on silent and sound trials of exposing the left ear to a 10-kHz tone for 4 hr. L and R indicate left and right sound trials, respectively. Performances are based on an average of 34–40 silent and 106–134 sound trials per session. A: Hamster exposed at 125 dB shifted its response on silent trials to the exposed (left) side, indicating that it had developed tinnitus in its left ear. It also showed a transient increase in errors on sound trials for sounds presented on the left side, due to the estimated 30-dB hearing loss in its left ear. B: Hamster exposed at 110 dB shifted its response on silent trials to the exposed (left) side on postexposure Days 1 and 3, indicating that it had developed tinnitus in its left ear. It also showed a temporary increase in errors on sound trials that was slightly greater for sounds presented to the left side, which had an estimated hearing loss of 15 dB. C: Hamster exposed at 80 dB did not shift its response on silent trials and showed no decrement in its performance on sound trials, and its auditory brainstem response indicated no hearing loss in its left ear.

Hearing Loss and Tinnitus

The relationship between hearing loss and tinnitus is apparent in Figure 3, which shows the correlation between hearing loss and the tinnitus score on the first postexposure day ($r = .791$, $p = 3 \times 10^{-5}$). As can be seen, a threshold shift of 12.5 dB or more always resulted in a positive score on the tinnitus test, although one hamster with no detectable hearing loss also scored positive. Note that the magnitude of the tinnitus score increased with threshold shift suggesting that the greater the hearing loss, the more noticeable the tinnitus.

The finding that hearing loss and tinnitus are related is consistent with the observation that, in humans, tinnitus induced by loud sounds is always accompanied by, and is probably the result of, a hearing loss. However, an alternative interpretation of the present results is that the tinnitus test is measuring the degree of hearing loss, an

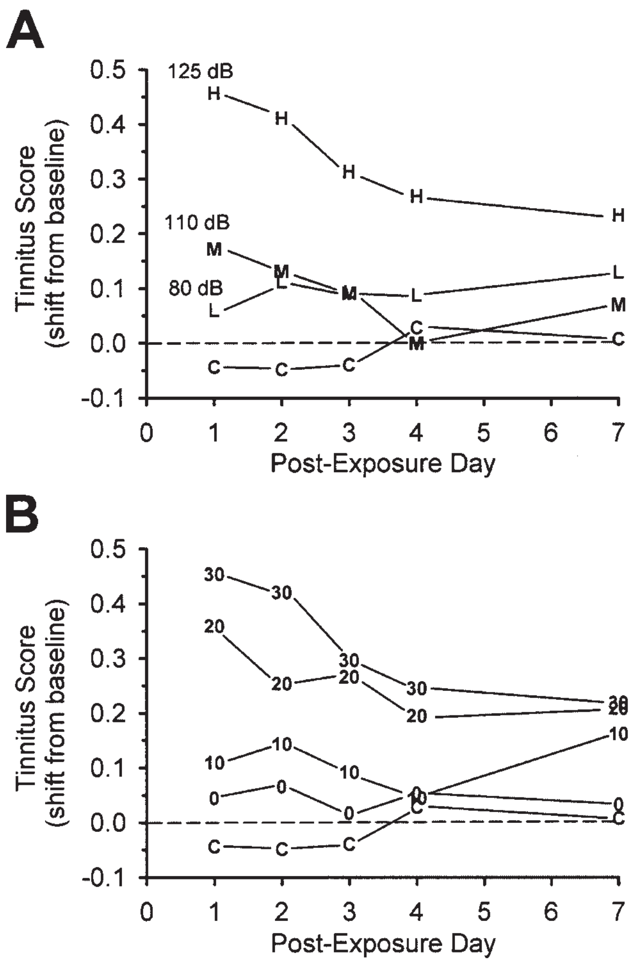

Figure 2. Average performances of animals exposed to 10 kHz for 4 hr. Scores indicate the amount of shift on silent trials toward the exposed (left) ear. A: Animals grouped by exposure, 80 dB (L), 110 dB (M), 125 dB (H), and control (C). B: Animals grouped by the hearing loss resulting from the exposure (estimated by the auditory brainstem response), no hearing loss (0), 10 dB (10), 20 dB (20), 30 dB (30), and control animals (C).

interpretation that we investigated by testing hamsters with unilateral hearing loss, but not with tinnitus, by placing an earplug in one ear.

Effect of Earplugs on Tinnitus Test Performance

The effect of seven different vented earplugs on a hamster's performance in the tinnitus test was determined by inserting an earplug in one ear and testing the hamster the following day. The degree of attenuation, determined by ABR immediately after testing, ranged from 10 to 25 dB. Although the plugs often caused an increase in errors on sound trials when the sound was presented to the side of the plugged ear, none of the hamsters tested positive for tinnitus. This contrasts with the results of the tone-exposed hamsters (see Figure 3), in which all hamsters with a hearing loss of 12.5 dB or greater tested positive for tinnitus. Thus, it appears that a hearing loss, in the absence of tinnitus, does not result in a positive score on the tinnitus test.

Testing for Tinnitus Immediately After Exposure

Seven hamsters were exposed to 2 or 10 kHz at 120 dB for 3–60 min under halothane anesthesia and then immediately tested for tinnitus. We found that the hamsters regained motor coordination and were able to begin performing the two-choice task within 3–17 min after exposure. The hamsters performed at high levels, indicating that there were no lingering effects of the anesthesia that interfered with their performance.

2-kHz tone, 120 dB. Of the four hamsters exposed for 6–30 min, only the hamster receiving the 30-min exposure tested positive for tinnitus (see Table 1). Three of the hamsters were reexposed a week later for 45–60 min, and all tested positive for tinnitus. Although there may have been a cumulative effect of the exposures, it appears that an exposure of at least 30 min is necessary to produce a positive score. Note that hamster 03–04, which had tested positive on the day of exposure to the 30-min duration but not afterward, tested positive again after being exposed for 60 min. This suggests that the hamster may have stopped testing positive following the first exposure because its tinnitus had subsided, not because it was ignoring it—the hamster tested positive again when the second exposure reinstated the tinnitus.

10-kHz tone, 120 dB. Of the three hamsters exposed for 3, 6, or 10 min, only the 3-min exposure failed to produce a positive score on the tinnitus test. Not only was the 10-kHz tone more effective in producing tinnitus at shorter durations than the 2-kHz tone, but the hamsters tested positive for longer.

Three conclusions may be drawn from this test. First, the onset of tinnitus in hamsters exposed to loud tones is immediate. Second, the reason that, at the same SPL, it takes a longer exposure at 2 kHz than at 10 kHz to produce tinnitus may be because the hamster is 30 dB less sensitive to 2 kHz than it is to 10 kHz (31 dB SPL vs. 1.5 dB SPL, respectively; R. S. Heffner et al., 2001). Finally, the results demonstrate that it is possible to test for temporary tinnitus in hamsters with the same exposure durations as those used in human studies (Davis et al., 1950).

Discussion

The Two-Choice Tinnitus Test and Hearing Loss

Because tinnitus induced by exposure to loud sound is always accompanied by a hearing loss, it is possible that a positive score on a tinnitus test might be due to the hearing loss rather than to the tinnitus. However, there are several reasons for believing that the two-choice procedure is detecting tinnitus. First, the animals are trained to respond to sounds on their left and right sides by going left and right, respectively. Therefore, it is logical that, on silent trials, they would respond to unilateral tinnitus by going to the side of the affected ear. Second, a unilateral hearing loss, if anything, would be expected to cause the animal to go to the side of its unexposed ear as external sounds would then be relatively louder in that ear—indeed, this occasionally happens as animals with large unilateral hearing losses often make more errors on sound trials by incorrectly going to the side of their unexposed ear (see Figure 1A). Finally, the results of the earplug test demonstrate that a unilateral hearing loss of up to 25 dB, unaccompanied by tinnitus, does not cause a hamster to test positive for tinnitus, whereas tone exposures that result in a hearing loss of as little as 12.5 dB (and presumably also cause tinnitus) consistently result in

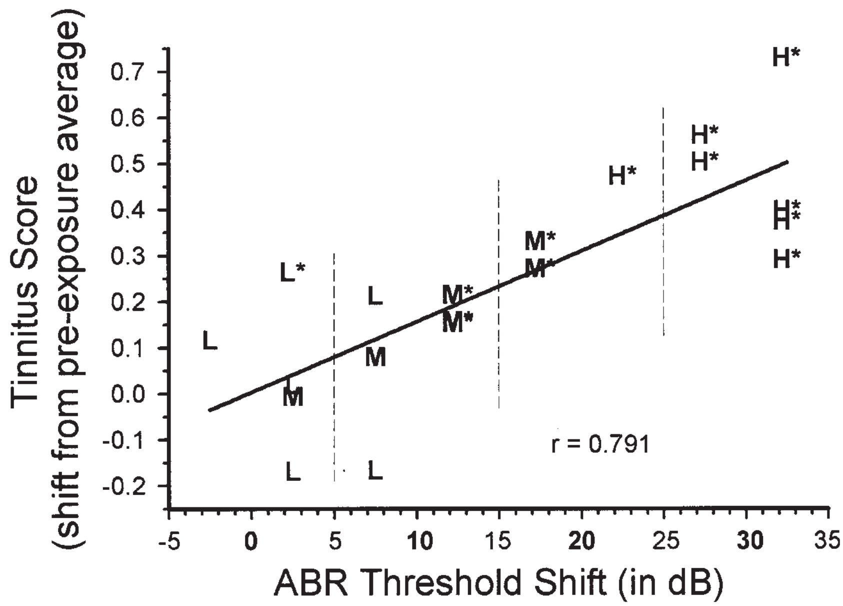

Figure 3. Relationship between auditory brainstem response (ABR) threshold in the exposed ear and first-day tinnitus score. The correlation coefficient, $r = .791$, $p = 3 \times 10^{-5}$, indicates that the greater the hearing loss, the greater the magnitude of the tinnitus score. L, M, and H indicate animals exposed to 10 kHz at low (80 dB), medium (110 dB), and high (125 dB) intensities for 4 hr. The vertical dashed lines divide the animals by hearing loss (0, 10, 20, and 30 dB). Asterisk indicates significant tinnitus score ($p < .01$, one-tailed).

a positive score (see Figure 3). Thus, a positive score on the two-choice test appears to indicate tinnitus, not hearing loss.

Unilateral Tinnitus

An underlying assumption of the two-choice test is that exposing an ear to a loud sound will induce tinnitus that will be lateralized to that ear, that is, that the tinnitus will neither be lateralized to the unexposed ear nor be bilateral. This assumption appears to be supported by the three previously mentioned studies of sound-induced tinnitus in humans (Atherley et al., 1968; Davis et al., 1950; Loeb & Smith, 1967). Specifically, each of these studies exposed one ear to loud sound, determined absolute thresholds in both ears, and had the subjects compare the resulting tinnitus to tones presented to the unexposed ear. In spite of the fact that the subjects were carefully attending to both ears, none of these studies reported that the tinnitus was bilateral or that it was lateralized to the unexposed ear. Moreover, we have since confirmed that the tinnitus observed by Davis et al. (1950) was always lateralized to the exposed ear (J. E. Hawkins, Jr., personal communication, February 25, 2003). Thus, the results of careful human studies support the conclusion that as long as the exposing sound is presented to one ear and does not reach the other ear either by air or bone conduction, the resulting tinnitus will be lateralized to the exposed ear.

Our results with hamsters support the conclusion that unilateral exposure results in tinnitus that is lateralized to the exposed ear. Specifically, once an exposure caused an ABR threshold shift of 12.5 dB or more, the animals invariably tested positive for tinnitus lateralized to the exposed ear (see Figure 3). If unilateral exposure were to occasionally cause bilateral tinnitus (or tinnitus in the opposite ear) we would have expected some of these animals to fail to test positive, but none did. With regard to those hamsters that did not test positive for tinnitus, it should be noted that none showed any sign of having tinnitus lateralized to the unexposed ear. Although we cannot rule out the possibility that these hamsters might have developed bilateral tinnitus, the fact that their ABR threshold shift was 7.5 dB or less suggests that the tone exposure was probably insufficient to induce tinnitus. It should also be noted

Table 1
Testing for Tinnitus Immediately After Tone Exposure

Animal	Exposure duration (min)	Response on silent trials to exposed ear		Duration of effect (days)
		Pre (%)	Post (%)	
2 kHz, 120 dB				
03–10	6	24.5	46.4	—
03–13	10	25.2	31.6	—
03–02	15	20.4	19.0	—
03–04	30	19.6	62.5*	1
03–10	45	24.8	82.1*	3
03–13	45	33.6	88.9*	3
03–04	60	24.8	62.5*	3
10 kHz, 120 dB				
03–12	3	16.1	4.3	—
03–03	6	35.7	88.5*	7
03–11	10	27.1	84.8*	>12

Note. Testing began 3–17 min after exposure under halothane anesthesia. The preexposure score (Pre) is the average of the last five preexposure sessions, whereas the postexposure score (Post) is the score on the first postexposure session. Dashes indicate no effect of exposure.
$^*p < .01$ (one-tailed).

that this conclusion applies only to the initial effects of unilateral tone exposure as we cannot rule out the possibility that the animals might have eventually developed bilateral tinnitus. However, in the absence of any behavioral evidence, either in humans or animals, this possibility remains unsupported.

Features of the Two-Choice Tinnitus Test

There are several features of the two-choice tinnitus test that may be noted. First, each animal serves as its own control, thus permitting individual diagnoses and eliminating reliance on group averages. As a result, the number of animals that are needed is greatly reduced, as even one animal can give an indication of whether a particular exposure can cause tinnitus. Second, an animal's performance is not disrupted by the aftereffects of halothane anesthesia, thus allowing animals to be tested within minutes of being exposed to a loud sound. Thus, animals may be tested by use of the same exposure durations that have been used to study temporary tinnitus in humans, instead of the long durations that have previously been used (e.g., H. E. Heffner & Harrington, 2002). Finally, the magnitude and duration of the tinnitus appears to be reflected in the degree of an animal's shift to the side of the exposed ear and the length of time that it continues to test positive; whether these measures may be affected by other factors, such as habituation, can be determined by simulating tinnitus with tones presented via earphones.

As has been noted, the two-choice test depends on the tinnitus being lateralized to one ear and thus would probably not detect bilateral tinnitus, such as that resulting from salicylate and other tinnitus-inducing drugs. As a remedy for this, there are a number of other procedures that do not depend on the tinnitus being unilateral (e.g., Brozoski et al., 2002; Guitton et al., 2003; H. E. Heffner & Harrington, 2002; Jastreboff, Brennan, Coleman, & Sasaki, 1988; Rüttiger, Ciuffani, Zenner, & Knipper, 2003). However, it is not difficult to train animals in a two-choice task to go to one side in the presence of sound and to the other side in the absence of sound (e.g., H. E. Heffner, 1983). Thus, the two-choice procedure could be adapted to testing for bilateral tinnitus by training animals to go to one side when no sound is presented and to go to the other side in the presence of sound, regardless of its perceived locus.

Tinnitus in Hamsters

The results presented here indicate that the tinnitus resulting from exposure to loud sound is related to the hearing loss that accompanies it, a relationship that shows up in several ways. First, once a particular level of hearing loss was reached, the hamsters invariably tested positive for tinnitus (see Figure 3). Second, the greater the hearing loss, the higher the tinnitus score (see Figure 3), suggesting that large hearing losses may cause more noticeable or louder tinnitus. Finally, the greater the hearing loss, the longer the hamsters continued to test positive for tinnitus (see Figure 2).

We did, however, find some signs of tinnitus in hamsters that did not have a noticeable hearing loss as estimated by the ABR, although it is possible that a detailed audiogram, or one taken immediately after tone exposure, might have revealed a loss. Specifically, of the 7 hamsters exposed to the 10-kHz 80-dB tone for 4 hr, 1 tested positive on the first 4 postexposure days, whereas 5 hamsters that had not initially tested positive did so on a subsequent day. In contrast, none of the 7 control hamsters ever tested positive. One possible explanation is that the exposure generated a low-level tinnitus to which the hamsters only occasionally responded.

Finally, we note that tones of the same SPL can differ in their effectiveness in producing tinnitus. Specifically, a 2-kHz tone at 120 dB required an exposure of at least 30 min before producing tinnitus, whereas a 10-kHz tone at the same SPL produced tinnitus with a 6-min exposure (see Table 1). Because hamsters are about 30 dB more sensitive at 10 kHz than at 2 kHz (1.5 dB SPL vs. 31 dB SPL, respectively; R. S. Heffner et al., 2001), this suggests that it is the intensity of the tone relative to absolute threshold that determines whether it will induce tinnitus.

Implications of Behavioral Studies for the Physiology of Tinnitus

The observations that Hallowell Davis and his colleagues (1950) made regarding the tinnitus they observed have relevance for the physiological as well as the behavioral study of tinnitus in animals. In particular, they noted that the pitch of the tinnitus typically occurred at the high-frequency edge of a sharply localized hearing loss. This observation suggests that tinnitus occurs when a section of the basilar membrane is rendered partly or completely unresponsive to sound, with the pitch of the tinnitus corresponding to the less affected portion of the basilar membrane at the high-frequency end of the damaged section. It also suggests that this form of tinnitus occurs because auditory units have lost inhibitory input (lateral inhibition) from adjacent lower-frequency units, causing them to respond as if a tone were present (though it should be noted that no inhibitory circuits that could account for this have been described). Although it had previously been speculated that such tinnitus results from increased activity in the auditory nerve, this does not appear to be the case, as damage to hair cells in the cochleas of cats treated with ototoxic drugs results in the elimination of activity in auditory nerve units with no sign of hyperactivity (Kiang, Moxon, & Levine, 1970). This suggests that the tinnitus caused by damage to the basilar membrane is actually generated in the central nervous system, one possible site being the cochlear nucleus where the afferent fibers from the cochlea terminate.

That tinnitus might be generated in the cochlear nucleus was suggested by electrophysiological studies that demonstrated an increase in spontaneous activity in the dorsal cochlear nucleus (DCN) of hamsters following a 4-hr exposure to a 125–130-dB, 10-kHz tone (e.g., Kaltenbach & McCaslin, 1996). Although this hypothesis was supported by the discovery that the level of spontaneous DCN activity is positively correlated with a behavioral measure of tinnitus (Kaltenbach, Heffner, & Afman, 1999), the possibility remained that the increased activity was the result not of tinnitus but of the accompanying hearing loss. Recently, this possibility was investigated in a study that measured spontaneous activity in the DCN of hamsters that had been tested for both tinnitus (by use of the two-choice tinnitus test) and hearing loss (by use of the ABR) (Zhang et al., 2004). The results showed that although the level of spontaneous DCN activity was significantly correlated with the behavioral measure of tinnitus, it was more highly correlated to hearing loss. Partial correlational analysis subsequently revealed that when the effect of hearing loss was held constant, the correlation between spontaneous activity in the DCN and tinnitus was no longer significant. On the other hand, the correlation between DCN activity and hearing loss remained significant when the effect of tinnitus was held constant. Thus, statistical analysis indicates that increased spontaneous activity in the DCN is not the cause of tinnitus—the fact that DCN activity and tinnitus are correlated is because both are caused by hearing loss.

The observation that increased spontaneous activity in the DCN is directly related to hearing loss rather than to tinnitus is supported by two other observations. First, the maximum increase in DCN activity caused by exposure to the 10-kHz tone occurs at the 12.5-kHz isofrequency contour. As previously noted, the pitch of tinnitus is typically matched to frequencies 1–1.5 octaves above the frequency of the exposing tone, whereas the frequency of maximal hearing loss occurs at about 0.5 octaves above the exposing tone (Davis et al., 1950; Loeb & Smith, 1967). Because 12.5 kHz is about 1/3 octave above the 10-kHz exposing tone, it is much closer to the typical frequency of maximum hearing loss than it is to the pitch of the expected tinnitus. Second, the onset of tinnitus induced by a loud sound is immediate, whereas it takes more than 2 days for the increased spontaneous activity to appear in the DCN (Kaltenbach & Afman, 2000), a time course more consistent with changes in central neural activity as the result of damage to the basilar membrane. Although it is conceivable that the increased DCN activity represents a change in the generation of tinnitus from peripheral to central neural structures, there is no behavioral evidence to support this notion.

Finally, a recent study has suggested that tinnitus in tone-exposed chinchillas is related to elevated spontaneous activity of the fusiform cells in the DCN (Brozoski et al., 2002). In this study, the chinchillas were exposed to a 4-kHz tone at 80 dB SPL for 30–60 min and then tested 1 week later. The results showed that the exposed animals were better able to detect a 1-kHz tone than were control animals and was interpreted as indicating that they had tonal tinnitus that was similar in pitch to a 1-kHz tone. Although these results are intriguing, the question arises as to how closely they match what is known about tinnitus in humans. First, tinnitus in humans resulting from tone exposure is generally matched to a tone 1–1.5 octaves higher than the exposing tone, whereas the chinchilla results suggest a tinnitus 2 octaves lower than the exposing tone (Davis et al., 1950; Loeb & Smith, 1967). Second, whether tinnitus in humans interacts with external tones in the way it is claimed it does in chinchillas remains to be determined.

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