

# Tinnitus in hamsters following exposure to intense sound

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## Abstract

Hamsters were trained with a conditioned suppression/avoidance procedure to drink in the presence of a broadband noise and/or a tone and to stop drinking in the absence of sound. A variety of tones and loudspeaker locations were used during training so that the animals would respond to a sound regardless of its frequency or location. Four groups of animals then had their left ears exposed to a 10-kHz tone at 124 or 127 dB for 0.5, 1, 2 or 4 h. They were then tested for tinnitus by comparing their performance with that of unexposed animals to determine if they behaved as if they perceived a sound when no external sound was present. The groups exposed for 2 and 4 h tested positive for tinnitus whereas those exposed for 0.5 and 1 h did not. The degree of hearing loss produced by the tone exposure was assessed using behavioral and auditory brainstem response (ABR) procedures. A partial dissociation was found between the hearing loss, as estimated by the ABR, and the results of the tinnitus test in that animals exposed for 1 h had the same hearing loss as the 2- and 4-h exposed animals, but did not test positive for tinnitus. This suggests that the positive scores on the tinnitus test were not due to hearing loss. These results are discussed along with those of previous behavioral studies of tinnitus in animals. © 2002 Published by Elsevier Science B.V.

*Key words:* Audiogram; Auditory brainstem response; Hamster; Hearing loss; Tinnitus

## 1. Introduction

Determining the occurrence and characteristics of tinnitus in humans presents little difficulty. With the exception of mental patients and cases involving financial compensation, one has little reason to doubt the statements of those who report the perception of a sound inside their head that cannot be accounted for by any physical source. Attempting to determine the presence of tinnitus in animals, however, is not so simple. Although a variety of procedures exist for determining the ability of an animal to detect and discriminate sounds (e.g., Klump et al., 1995), they do not easily lend themselves to assessing the response of an animal to a percept over which the experimenter has little control and no objective means of measuring. However,

because an animal model is necessary for the investigation of the physiological basis of tinnitus, as well as for the assessment of potential treatments, some effort has been devoted to developing behavioral techniques for studying tinnitus in animals.

One behavioral procedure for determining if animals have tinnitus is to train them to make a response when an external sound is turned off, i.e., to respond to silence. Briefly, animals are trained to stop drinking whenever a broadband noise is turned off by pairing its absence with foot shock. The animals are then exposed to loud sound, or given drugs that cause tinnitus in humans, and tested to see how they respond during silence. Using this procedure, Jastreboff and his colleagues have shown that rats given salicylate or quinine are less likely than control animals to stop drinking when the noise is turned off. This result is taken to indicate that the treated animals still hear a sound when no external sound is present, i.e., they have tinnitus (Brennan and Jastreboff, 1991; Jastreboff and Brennan, 1994; Jastreboff et al., 1988a,b).

A second procedure involves training animals to stop lever pressing when a broadband noise is turned off in

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*Abbreviations:* ABR, auditory brainstem response; SPL, sound pressure level re 20  $\mu\text{N/m}^2$

order to avoid foot shock (Bauer et al., 1999; Bauer and Brozoski, 2001). In this case, however, the animals are tested by presenting intervals in which the noise is turned off and a pure tone is turned on. The tone is varied in frequency and intensity with the expectation that animals with tinnitus will respond to the tones differently than control animals. In one study, it was found that rats given salicylate *after* training were *less* likely than the controls to stop lever pressing during the tone intervals, because, according to the authors, the animal's tinnitus made the tones sound more noise-like (Bauer et al., 1999). In a second study, it was found that rats exposed to loud noise to induce tinnitus *before* training were *more* likely to stop lever pressing during the tone intervals. The explanation offered for this result was that the exposed animals had learned to associate shock with silent intervals in which they heard their tinnitus; as a result they were more likely to stop lever pressing to tones that resembled their tinnitus (Bauer and Brozoski, 2001).

The purpose of the present study was to determine if hamsters develop tinnitus following exposure to an intense 10-kHz tone for exposure durations from 0.5 to 4 h. The reason for choosing this particular stimulus and species is that the effect of exposure to intense 10-kHz tones has been well studied in hamsters and is known to result in an increase in spontaneous activity in the dorsal cochlear nucleus, an increase that may be related to tinnitus (Kaltenbach et al., 1992, 1998, 2000; Kaltenbach and Afman, 2000; Kaltenbach and McCaslin, 1996). The experimental procedure used to test the hamsters was a modification of the conditioned suppression technique used by Jastreboff and his colleagues (e.g., Jastreboff et al., 1988a). One important difference is that the animals in the present study received extensive training to increase their reliability so that the likelihood of tinnitus in individual animals might be assessed. In addition, both behavioral and auditory brainstem response (ABR) techniques were used to measure the hearing loss resulting from the exposure to the 10-kHz tones.

## 2. Methods

### 2.1. Subjects

A total of 61 male Syrian golden hamsters (*Mesocricetus auratus*) were used. The animals were obtained from Charles River Laboratory and ranged in age from 60 to 70 days at the beginning of training. They were housed on corn cob bedding in standard solid bottom cages with grid covers and given free access to rodent blocks occasionally supplemented with pieces of

apple. Water was available during the daily training and test sessions.

Unlike previous studies (e.g., Jastreboff et al., 1988a), no attempt was made to mask an animal's tinnitus by presenting a masking noise in the animal colony room. The ambient noise level of this room was 60 dB sound pressure level (SPL re 20  $\mu\text{N}/\text{m}^2$ ), measured with a Brüel and Kjaer (B&K) 1-in (2.54-cm) microphone and B&K 2203 sound level meter (linear setting). Analysis with an octave filter (B&K 1613) revealed that the highest background noise level was 57 dB at 63 Hz, which is below the hamster's 68-dB pure-tone threshold at this frequency (Heffner et al., 2001). To reduce exposure to extraneous loud sounds that might also cause tinnitus, a rubber gasket was attached to the lid of the metal pan in which the animals were weighed and transported each day so that placing the lid on the pan would not make a loud noise.

### 2.2. Behavioral assessment of tinnitus

#### 2.2.1. 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 acoustic 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 (35 $\times$ 21 $\times$ 24 cm) constructed of half-inch (1.27 cm) wire mesh on a supporting frame of 1/8-in (3.2 mm) brazing rods. The cage was mounted on a camera tripod 1 m above the chamber floor (for an illustration of the test cage, see Heffner et al., 2001). A water spout protruded up through the floor in the front of the cage and was adjusted to a level that permitted an animal to drink comfortably with its head facing forward. The spout consisted of 15-gauge brass tubing topped with a brass lick plate (10 $\times$ 12 mm). Water was delivered via a syringe pump with the flow rate adjusted so that an animal could satisfy its daily water needs in a single 15–20-min test session. The width of the cage was restricted by a narrow (7 cm wide) shoulder-high (2 cm) wire mesh fence that prevented an animal from standing sideways when drinking. Requiring an animal to keep its mouth on the water spout served to fix its head in the sound field, allowing precise measurement of the intensity of the sound at its ears.

A touch switch detected when an animal made contact with the spout and turned on the water. Mild shock was provided by a shock generator connected between the spout and the cage floor.

### 2.2.2. Acoustical apparatus

Sine waves were generated by a tone generator (Hewlett Packard 209A) with the frequency verified by a frequency counter (Fluke 1900A). The signal was gated with a 20-ms rise/fall time (Coulbourn S84-04), attenuated (Coulbourn S85-08), band-pass filtered (Krohn-Hite 3550;  $\pm 1/3$  octave), and amplified (Coulbourn S82-24). Broadband noise was produced by a noise generator (Coulbourn S81-02) and amplified. The electrical signal was sent simultaneously to four Motorola piezoelectric tweeters (KSN 1005A), which, unless otherwise specified, were located directly above the cage, 90° left, directly in front, and 90° right, at a distance of 1 m.

The sound pressure level (SPL re 20  $\mu\text{N}/\text{m}^2$ ) was measured using a B&K 1/4-in (0.64-cm) microphone (model 4135), preamplifier (B&K 2618), microphone amplifier (B&K 2608), spectrum analyzer (Zonic 3525), and filter (Krohn-Hite 3202) set to pass one octave above and below the test frequency. The measuring system was calibrated with a pistonphone (B&K 4230). Sound measurements were taken by placing the microphone in the position normally occupied by an animal's head when it was in contact with the spout. Because the measured intensity of a sound depends on the orientation of the measuring microphone relative to the sound source, the sound was measured by placing the speakers together and orienting the microphone directly toward them. The spectrum of the broadband noise ranged from 3 kHz to 45 kHz ( $\pm 6$  dB).

### 2.2.3. Behavioral procedure for assessing tinnitus

The animals were trained to drink from a water spout in the presence of broadband noise and/or tone and to stop drinking in the absence of these sounds (silence) in order to avoid an electric shock. They were then tested for tinnitus by determining the percentage of time they drank during noise and silent trials when shock no longer followed the silent trials. The hypothesis was that animals with tinnitus would be more likely to continue drinking during silent trials because they would now hear their tinnitus. Although we assumed that any tinnitus would be tonal, the animals were also trained using noise, making the test sensitive to non-tonal tinnitus as well.

**2.2.3.1. Training.** The animals were trained and tested in groups of 16 animals. An animal was first accustomed to drinking from the spout in the combined presence of broadband noise (32 dB SPL) and tone (10 kHz, 37 dB SPL) for three to four sessions – the noise and tone were combined to simulate the presence of tonal tinnitus in conjunction with the noise. During training, a 15-s trial was presented in which either the tone alone (tone trial) or no sound (silent trial) was presented. Initially, an animal was shocked if it contacted the spout any time after the first 2 s of a silent

trial. Once it had learned to reliably break contact with the spout, it was not shocked unless it contacted the spout during the second half of the trial. Thus, an animal had 7.5 s in which to decide whether or not to break contact. Each trial (tone or silent) was followed by a 15-s intertrial interval, in which the noise and tone were presented together after which the next trial began. An equal number of tone and silent trials were presented in a quasi-random sequence (Gellermann, 1933).

After 10–12 sessions, the animals were trained to generalize to other tones and loudspeaker locations. This was done to increase the likelihood that the animals would generalize from the training tones to any tonal tinnitus they might develop. The frequency of the tone was changed from one session to the next, but was always the same within a session. The tones and their sound pressure levels were 8 kHz (38 dB), 10 kHz (37 dB), 12 kHz (38 dB), 14 kHz (40 dB), 16 kHz (68 dB), 20 kHz (70 dB), and 24 kHz (56 dB). At these intensities, the tones ranged from 33 dB (12 kHz) to 51 dB (20 kHz) above the average threshold for hamsters (Heffner et al., 2001; thresholds available on the Internet at <http://www.utoledo.edu/psychology/animalhearing/>). These intensities were chosen because they resulted in performances well above the 0.01 level of chance for all animals. The location of the loudspeakers was also varied between sessions. In most sessions, the speakers were located directly overhead, 90° left, directly in front (midline), and 90° right so that the sounds could not be localized to one specific external location. In other sessions, all four speakers were placed either 90° to the left, directly in front, or 90° right so that an animal would learn to respond to a sound regardless of its location.

The animals were trained for 32–35 sessions and all performed well above chance levels during the last five training sessions with scores of 70% or better (Mann–Whitney *U*-test,  $P < 0.01$ ; see Section 2.2.3.2 for a description of how performance was scored).

**2.2.3.2. Testing.** For tinnitus testing, animals received 15-s trials that alternated between broadband noise and silence. The loudspeakers were located at 90° left, 0°, 90° right, and overhead. No tones were presented and no shock was given. Each animal received 25 noise and 25 silent trials per session for five consecutive sessions – limiting the number of trials meant that testing was completed before satiation could occur. The time that an animal was in contact with the water spout during the last half of each trial (7.5 s) was automatically recorded. Performance was calculated as the average percentage time that an animal was in contact with the spout during noise trials and was not in contact during silent trials. Scores could range from 100% (perfect performance) to approximately 50% (ran-

dom performance). For example, an animal that was on the spout 90% of the time during noise trials and off the spout 80% of the time during silent trials received a score of 85%.

Testing began 5 days after tone exposure. The reason for waiting 5 days after exposure was because the increase in spontaneous activity in the dorsal cochlear nucleus that follows tone exposure, which may be physiological correlate of tinnitus, reaches asymptote at about 5 days post-exposure (e.g., [Kaltenbach et al., 2000](#)).

### 2.3. Exposure to the 10-kHz tone

We attempted to produce tinnitus by exposing the left ears of hamsters to a 10-kHz tone at 124 dB SPL for 0.5, 1, or 4 h, or at 127 dB SPL for 2 h. The tone was produced by a digital signal generator (Zonic 3525), amplified (Radio Shack MPA, 100 w/channel), attenuated (L-pad), and sent to a Motorola piezoelectric speaker (KSN 1005A). A funnel (8 cm diameter) was attached to the front of the tweeter with thermo-plastic 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 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 dB and 19 dB SPL, respectively ([Heffner et al., 2001](#)).

For exposure, an animal was anesthetized (ketamine 90 mg/kg and xylazine 9 mg/kg), placed on its side, and the plastic tip of the sound tube inserted within 1–2 mm of the concha. The animal was closely observed for the duration of the exposure to ensure that the tube remained in place. Control animals were anesthetized, but not exposed to the tone.

### 2.4. Behavioral assessment of pure tone thresholds in the exposed ear

Behavioral audiograms of three hamsters were determined prior to exposing their left ears to the 10-kHz tone at 124 dB SPL for 4 h, again after exposure, and finally after surgical destruction of their unexposed (right) ears. The final audiogram was conducted 7 weeks after tone exposure and 1 week after destruction of the unexposed ear. These animals were not used in the tinnitus tests.

#### 2.4.1. Cochlear surgery

For surgery, an animal was anesthetized (ketamine 90 mg/kg and xylazine 9 mg/kg) and an incision was made

in the external auditory canal allowing the tympanic membrane to be observed through a surgical microscope. The tympanic membrane and middle ear bones were then removed, a wire was inserted into the oval window, and at least the first few mm of the basilar membrane removed. The incision was then closed and the animals allowed to recover. All three animals showed vestibular signs (chronic head tilt) which gradually disappeared.

#### 2.4.2. Behavioral procedure

The animals were tested in the previously described test cage ([Section 2.2.1](#)) with tones generated and measured as described above ([Section 2.2.2](#)). A single piezoelectric tweeter was placed 1 m in front of the cage and directed toward the position occupied by the animal's head when it was drinking from the spout. The animals were 70 days old at the beginning of testing. Thresholds were determined at 4, 5, 6.3, 8, 10, 12.5, 16, 20, 25, 32, and 40 kHz. For details of training and testing, see [Heffner et al. \(2001\)](#).

An animal was trained to make steady contact with the water spout in order to obtain a slow but steady trickle of water ([Heffner and Heffner, 1995](#)). A train of four tone pulses (400 ms on, 100 ms off, 20 ms rise/decay time) was then presented at random intervals and followed at its offset by a mild electric shock delivered between the spout and the cage floor. The animal soon learned to avoid the shock by breaking contact with the spout whenever it heard a tone. A 15-W light was mounted ~0.5 m below the cage and was turned on and off with the shock. The animals learned not to return to the spout until the light was off.

Test sessions were divided into 2-s trials separated by 1.5-s intertrial intervals. Approximately 22% of the trials contained a tone while the remaining trials contained only silence. A touch circuit detected whether an animal was in contact with the spout during the final 150 ms of every trial. If an animal broke contact for more than half of the 150-ms response period, an avoidance response was recorded. Using signal detection terminology, this response was classified as a 'hit' if the trial contained a tone or as a 'false alarm' if there was no tone, i.e., silence. The hit rate was then corrected for false alarms to produce a performance measure for that stimulus using the formula: performance = hit rate minus (false alarm rate times hit rate). This measure proportionately reduces the hit rate by the false alarm rate observed for a particular stimulus and can range from 0 (no hits) to 1 (100% hit rate with no false alarms, i.e., perfect performance). Threshold was defined as the intensity at which performance equaled 0.50 and was usually determined by interpolation.

## 2.5. Auditory brainstem response

To estimate the degree of hearing loss caused by the 10-kHz tone exposure in animals that had been tested for tinnitus, thresholds were determined using the ABR for the left and right ears of tone-exposed and unexposed (control) animals. ABR thresholds were obtained because behavioral threshold testing requires several weeks and no small-animal headphones are available to test hearing in each ear separately. To obtain a single number that reflected the overall hearing loss in an exposed ear, thresholds were determined for a single stimulus: a band-pass noise burst that spanned the range of the hearing loss found in the behavioral audiograms. Testing was conducted immediately after the tinnitus test, which was 10–11 days after tone exposure. ABR thresholds were determined for eight control, eight 0.5-h, eight 1-h, six 2-h, and eight 4-h exposed animals.

To determine the maximum intensity that could be presented to one ear without stimulating the other ear (i.e., crosstalk), the middle ear and cochlea on one side were damaged (as described in Section 2.4) in two additional (unexposed) hamsters and an ABR threshold obtained with the animal and electrodes configured for testing the damaged ear.

ABR testing was conducted in the same double-walled sound chamber used for behavioral testing. For testing, an animal 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 foam tape (approximately  $9\text{ cm}^2 \times 3\text{ mm}$  thick) to the side of the animal's head. This served to attenuate the signal in the plugged ear and to stabilize the animal'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 animal's hind leg. The speaker was positioned directly above the animal's ear at a height of 12 cm.

The noise stimulus was generated using SigGen software, Tucker-Davis Technologies (TDT), at a sampling rate of 111.1 kHz (9- $\mu\text{s}$  sampling period). The stimulus was 1 ms in duration and pulsed 27.7 times per second. The output of the DA converter (TDT, model DA3) was passed to a programmable attenuator (TDT, model PA4), two filters (Krohn-Hite, model 3550, 10–50-kHz bandpass settings providing 48-dB/octave roll-off), led to a headphone driver (TDT, model HB7), and then to a Foster ribbon tweeter (model 110T02). The maximum intensity of the stimulus, determined with the sound measuring system described in Section 2.2.2, was 77 dB SPL (Fig. 1).

Data were collected using a Nicolet model CA 2000 electrodiagnostic system. The biological signal was

## ABR Stimulus

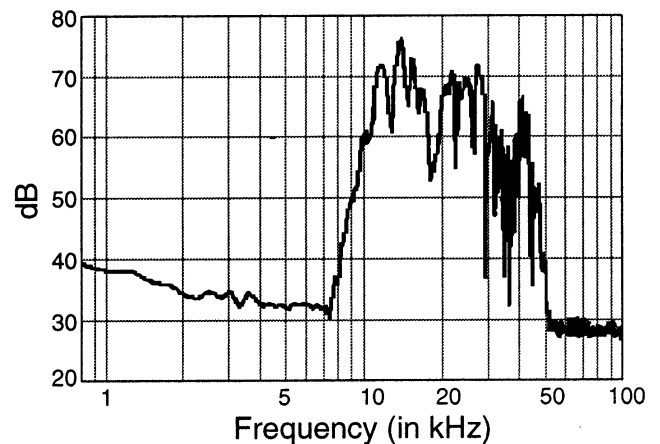


Fig. 1. Spectrum of the noise burst used to evoke the ABR. This stimulus was chosen because its energy is concentrated in the frequency range of the behaviorally-determined hearing loss produced by exposure to the 10-kHz tone, 124 dB SPL, for 4 h.

bandpass filtered (0.15–3.0 kHz) and amplified with the artifact rejection level set at 25  $\mu\text{V}$ . 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  $\mu\text{V}$  could be detected. The number of samples per average varied with the clarity of the response, ranging from a minimum of 2000 at higher stimulus intensities to between 6000 and 12000 around threshold. Both ears were tested within a session with the order of testing (e.g., right–left or left–right) varied between animals.

## 3. Results

### 3.1. Tinnitus test

The score used in the tinnitus test is the average percent time that an animal *was* in contact with the spout during noise trials, and *was not* in contact during silent trials. This was done so that the scores would be corrected for any general increase or decrease in an animal's tendency to break contact with the spout due to stress or other factors not related to tinnitus. However, this also means that a low score on the test could be caused either by a reduced tendency to break contact during silent trials, a sign of tinnitus, or by an increased tendency to break contact during noise trials, which

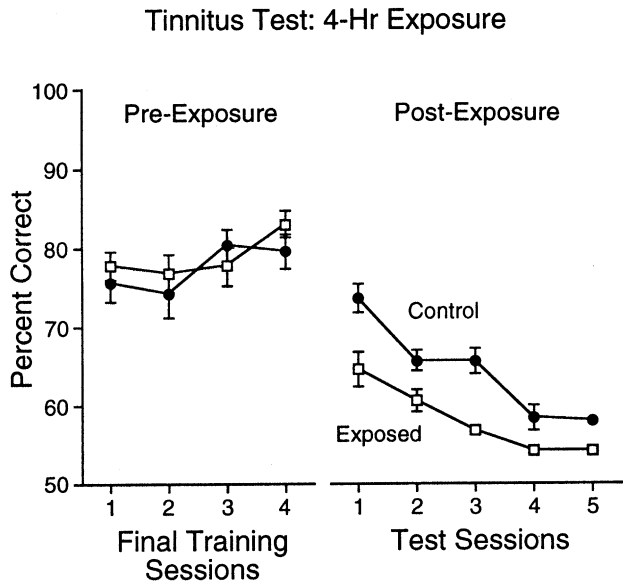


Fig. 2. Effect of exposure of one ear to 10 kHz at 124 dB SPL for 4 h. The animals in the exposed group were more likely to continue drinking during silent trials than control animals, an observation consistent with the hypothesis that they had developed tinnitus. Average scores are shown for the exposed group (open squares) and control group (filled circles) with bars indicating S.E.M. (which for some points was smaller than the symbol).

would not be a sign of tinnitus. To determine whether the results were affected by the animals' performances on noise trials, a statistical analysis of their responses on noise and silent trials was performed. This showed that there were no differences between any of the groups on their responses during noise trials [ $F(4,51) = 1.250$ ,  $P = 0.3019$ ]. Thus, any significant differences described below were due solely to the increased tendency of exposed animals to maintain spout contact during silent trials, a response indicating tinnitus.

### 3.1.1. Effect of 4-h exposure of a 10-kHz tone at 124 dB on the tinnitus test

Thirty-two animals were trained and divided into matched pairs based on the scores of their last four training sessions with one member of each pair assigned to the exposed and control group. The animals to be exposed were then anesthetized and their left ears exposed to a 10-kHz tone at 124 dB SPL for 4 h. The control animals were anesthetized, but not exposed. All animals were tested for tinnitus for five sessions beginning 5 days after tone exposure.

The mean scores of the exposed and control animals are shown in Fig. 2. Because shock no longer followed silent trials, the scores of all animals decreased with time as they extinguished. As can be seen, the exposed group scored lower than the controls because the exposed animals were more likely to continue drinking

when no external sound was present [ $F(1,30) = 21.283$ ,  $P = 0.0001$ ]. Analysis of the animals' daily scores showed that the largest difference between the groups occurred on the first test session [ $F(1,120) = 30.591$ ,  $P < .0001$ ] with  $F$  values generally declining for the four subsequent sessions as extinction progressed [ $F = 9.772$ ,  $P = 0.0022$ ;  $F = 29.219$ ,  $P < 0.0001$ ;  $F = 6.812$ ,  $P = 0.0102$ ; and  $F = 4.97$ ,  $P = 0.0277$ , respectively]. The difference between the groups is consistent with the hypothesis that the exposed animals had tinnitus.

The individual scores were examined to determine if there was some indication that the exposed animals varied in the likelihood that they had tinnitus. This was done by comparing the cumulative average for each animal as each session of 25 trials was completed. This comparison shows each animal's relative standing after the first session (where extinction has the smallest effect and the scores are most widely distributed), as well as at intermediate and final points. Inspection of the animal's average scores during testing indicates some consistency (Fig. 3). For example, there were eight exposed animals whose average scores virtually never overlapped with those of the control animals, and five control animals whose scores almost never overlapped those of the exposed animals. Overall, the scores of the exposed animals were shifted downward relative to the

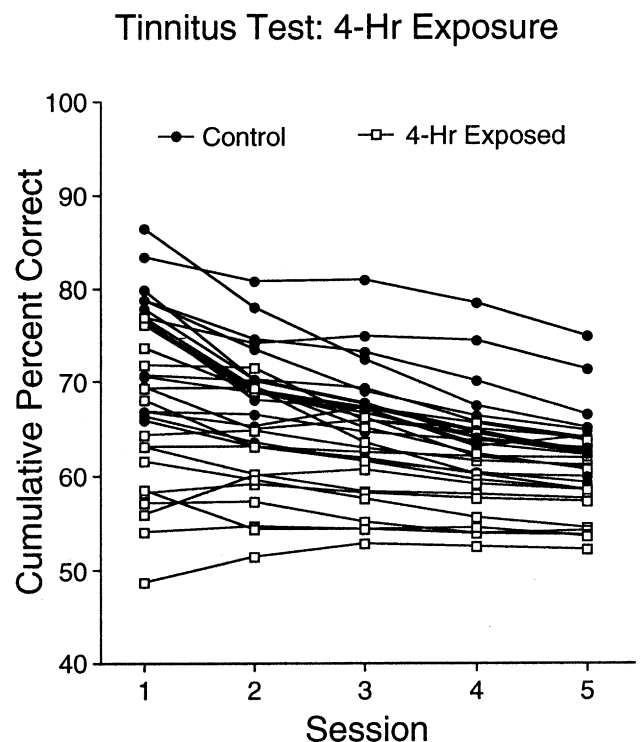


Fig. 3. Cumulative scores for 16 exposed (open squares) and 16 control animals (filled circles). Lower scores suggest a higher likelihood of tinnitus. Note that eight of the exposed animals scored consistently lower than any of the control animals.

control animals, but with some overlap. Whether those exposed animals whose scores overlapped those of the controls did not develop tinnitus or had less severe tinnitus is unknown. Nevertheless, we are more confident that those animals whose scores did not overlap those of the controls had developed tinnitus.

Analysis of the animal's daily scores indicated that the first session, which consisted of 25 trials, gave the clearest indication of group differences. Not only were the differences largest on the first session, but the first session also yielded the largest range of scores. Subsequent statistical analyses were therefore restricted to the overall (five-session) and first session results.

### 3.1.2. Effect of duration of the 10-kHz tone exposure on the tinnitus test

**3.1.2.1. 2-h exposure.** Sixteen animals were trained and divided into matched pairs based on their last four training session scores with one member of each pair randomly assigned to the exposed and control group. The animals to be exposed were then anesthetized and their left ear exposed to the 10-kHz tone at 127 dB SPL for 2 h. The control animals were anesthetized, but not exposed to the tone. Analysis indicated that the exposed animals scored significantly lower than the control animals on the first test session [ $F(1,60) = 26.98, P = 0.0001$ ] and over all five sessions [ $F(1,15) = 11.673, P = 0.0038$ ]. These results are consistent with the hypothesis that the exposed animals had tinnitus.

**3.1.2.2. 0.5- and 1-h exposures.** Sixteen animals were trained and divided into matched pairs based on their last four training session scores with one member of each pair exposed to 10 kHz at 124 dB SPL for 1 h and the other exposed to the same tone for 0.5 h. Instead of using a concurrent control group, the animals were compared with the control animals used in the 4-h test. This comparison was considered appropriate for two reasons. First, the training scores of the 0.5- and 1-h exposure animals did not differ from those of the 4-h controls, indicating that the groups did not differ prior to exposure [ $F(2,29) = 0.071, P = 0.9314$ ]. Second, the scores of the control groups used in the 2- and 4-h tests did not differ, indicating that the control groups were not changing over time [ $F(1,23) = 0.345, P = 0.5629$ ].

Analysis of variance indicated that there was no effect of the 1-h exposure on either the first test session [ $F(1,88) = 1.374, P = 0.02443$ ] or over all five sessions [ $F(1,22) = 1.504, P = 0.2330$ ]. Similarly, there was no effect of the 0.5-h exposure either on the first test session [ $F(1,22) = 3.691, P = 0.0579$ ] or over all five sessions [ $F(1,22) = 4.424, P = 0.0471$ ]. These results suggest that an exposure duration of more than 1 h at 124 dB is necessary to reliably produce tinnitus.

The cumulative scores for each of the animals in the

## Tinnitus Test: Effect of Exposure Duration

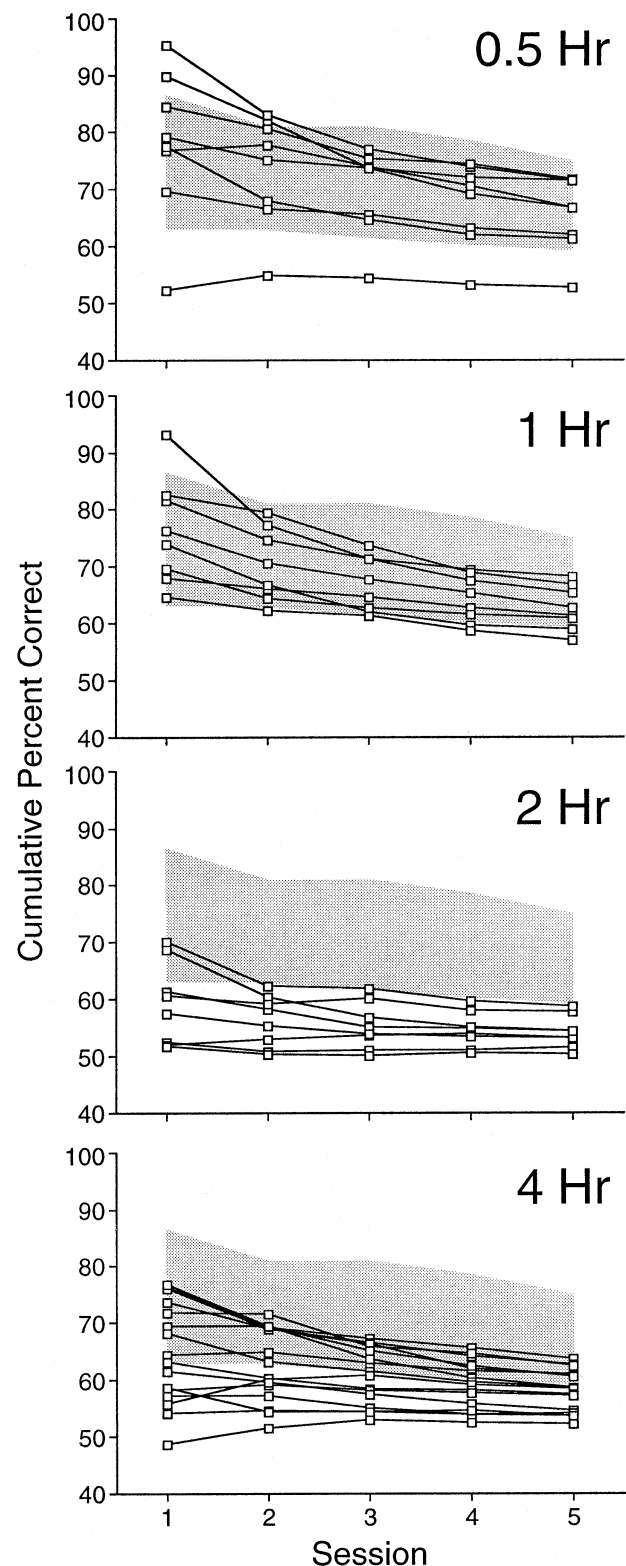


Fig. 4. Cumulative scores for animals exposed for 0.5, 1, 2, and 4 h. Shaded area in these figures indicates the entire range of performance of 16 control animals (from Fig. 3). Lower scores suggest a higher likelihood of tinnitus.

0.5-, 1-, 2-, and 4-h exposure groups are shown in Fig. 4 with the range of scores of the 16 control animals from Fig. 3 shown in gray. As can be seen, the animals in the 0.5-h exposure group overlapped considerably with the control animals. However, one animal consistently scored low, suggesting that it may have had tinnitus. It should be noted that such a score is unlikely to have been due to pre-existing tinnitus because the animal would have learned to ignore it during training. Thus, it is possible that in this one case a 0.5-h exposure was sufficient to cause tinnitus.

Turning to the other groups, the 1-h exposure shows almost complete overlap with the controls, suggesting that none of these animals had developed tinnitus. The 2-h exposure group, on the other hand, shows almost no overlap with the control group, suggesting that all of these animals developed tinnitus. This is in contrast with the 4-h exposure group which shows partial overlap with the control group. However, because the 2- and 4-h exposure groups did not differ statistically over all five sessions [ $F(1,22) = 2.879$ ,  $P = 0.1038$ ], we are reluctant to conclude that the two groups differed.

### 3.2. Behavioral audiogram

The effect on pure tone thresholds of exposing one ear to 10 kHz at 124 dB SPL for 4 h was determined behaviorally for three hamsters. Fig. 5 shows the hearing loss in the exposed ear, which was determined by subtracting the audiogram taken before destruction of the unexposed ear from that taken after. All three animals showed hearing loss with the amount of loss varying between animals. The greatest hearing losses were at 20 kHz for hamsters A (24 dB) and B (27 dB), and at 40 kHz for hamster C (28 dB).

Because exposure to the 10-kHz tone resulted in a hearing loss, it was necessary to rule out the possibility that hearing loss alone accounted for the difference between the exposed and control animals on the tinnitus test. This was the goal of the tests described in Sections 3.3 and 3.4.

### 3.3. Controlling for unilateral hearing loss

Because tinnitus is perceived as a sound originating inside the head or ear on one side, initial training of the animals was conducted with the four speakers located around the animal to prevent the sound from being perceived as having a particular locus in space. However, because the tone exposure resulted in a unilateral hearing loss, a preliminary test was conducted prior to tinnitus testing to determine if changing the relative intensity of the sound at the two ears could affect an animal's performance on the tinnitus test. This was done by training a group of 17 unexposed animals

with the speakers located in the four positions described above. The animals were then divided into matched groups and given the tinnitus test for one session. For one group, all four speakers were placed on the right side, simulating a hearing loss in the left ear; for the other group the speakers remained in their original locations. A spectrum analysis indicated that placing all four speakers on one side resulted in the broadband noise being up to 25 dB lower at the far ear with the greatest difference at frequencies  $\geq 20$  kHz. This difference is similar to the hearing loss shown by animals receiving the 4-h exposure (Fig. 5). The results of this test showed that placing all four speakers to one side

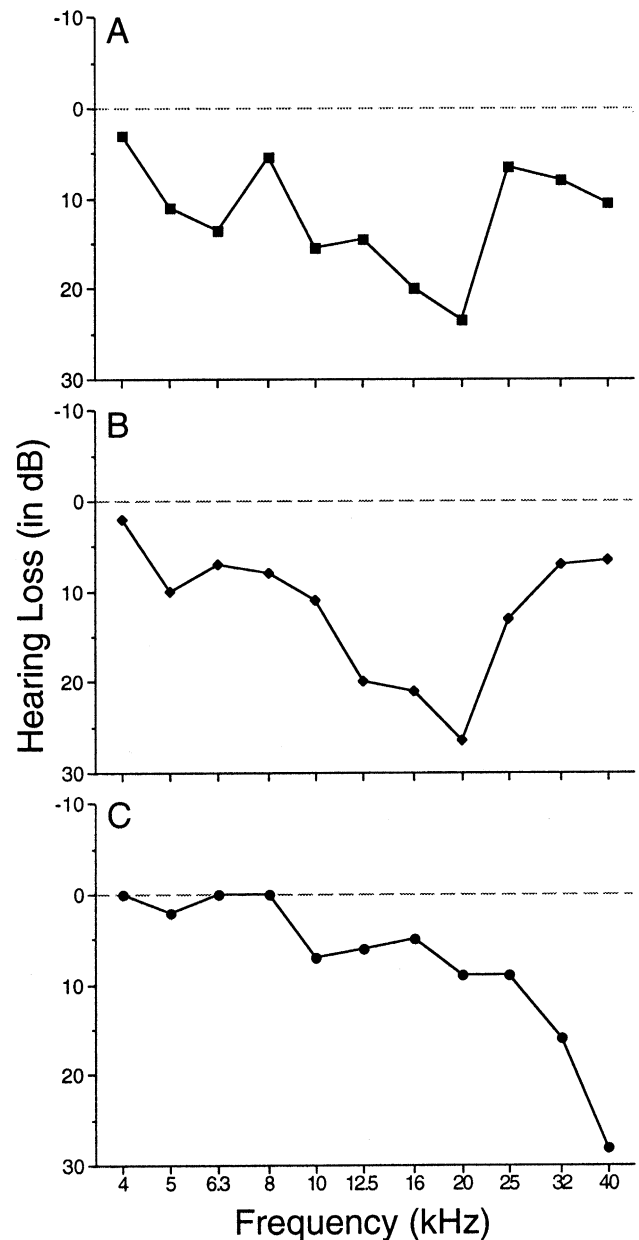


Fig. 5. Effect of exposure to 10 kHz at 124 dB SPL for 4 h on the absolute thresholds of three hamsters determined behaviorally.



caused those animals to score significantly lower than the control group [ $F(1,15) = 12.664$ ,  $P = 0.0029$ ].

Because this result indicated that the tinnitus test could be sensitive to a difference in the location of the sound, it suggested that the unilateral hearing loss produced by the tone exposure, which can shift the perceived locus of a sound, might in itself be sufficient to cause the exposed animals to score lower than the controls. As a result, steps were taken to eliminate this factor by systematically varying the location of the loudspeakers between training sessions. Thus, in addition to placing the four speakers around the animal, they were also placed all to the left, right, or front position. To determine if this was sufficient to prevent the location of the sound from affecting the results, 16 unexposed animals were trained with the speaker position varied and then given the tinnitus test for one session with the four speakers again to the right side for half of the animals. This time the results showed no difference between the two groups [ $F(1,14) = 0.002$ ,  $P = 0.9658$ ].

In summary, these results indicate that the tinnitus test could be sensitive to the location of the sound source if speaker location was kept fixed during training. Because exposure to the 10-kHz tone results in a hearing loss in the exposed ear, which can shift the perceived locus of a sound, speaker location was varied during the training of all of the animals whose results are reported here. This step reduced the possibility that the performance of the animals would be affected by a hearing loss.

### 3.4. Relation between hearing loss and behavioral scores

To further examine the possible relationship between tinnitus scores and hearing loss, the degree of hearing loss was estimated using the auditory brainstem response for animals receiving different exposure durations as well as for a control (unexposed) group. ABRs were recorded from eight animals in each group with the exception of the 2-h exposure group from which only six animals were examined. An example of a normal ABR threshold series evoked by the band-pass noise is shown in Fig. 6.

Because the exposed animals had a hearing loss in only one ear, it was necessary to determine the maximum intensity that could be presented to that ear before a response from the unexposed ear could be detected. This was done by obtaining thresholds for two hamsters that had been deafened in one ear by removing the middle ear and damaging the cochlea. Thresholds obtained with the loudspeaker and electrodes configured for testing the damaged left ear of one animal and the damaged right ear of the other were 57 and 60 dB SPL. These responses were presumed to be the re-

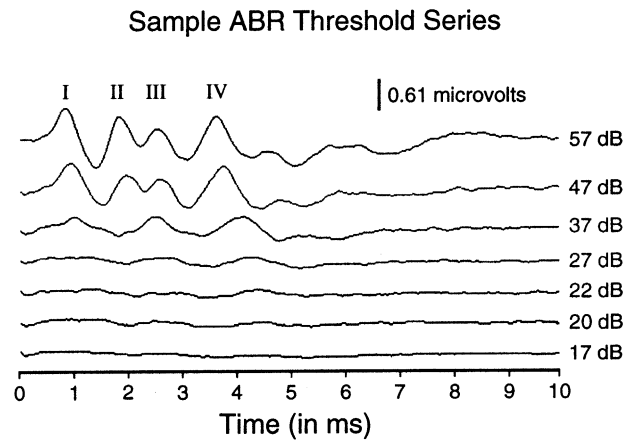


Fig. 6. ABR of a normal hamster to the noise burst (shown in Fig. 1). Threshold, defined as the lowest intensity that evoked a latency-appropriate response of  $0.05 \mu\text{V}$ , was reached in this example at 20 dB SPL.

sult of sound reaching the other ear, i.e., crosstalk. Thus, thresholds lower than 57–60 dB SPL were unlikely to have been affected by crosstalk.

ABR thresholds in the exposed ears were elevated relative to the controls [ $F(4,33) = 14.208$ ,  $P < 0.0001$ ] (Fig. 7A). Indeed, even the 0.5-h exposure was sufficient to produce a noticeable hearing loss [ $F(1,33) = 6.725$ ,  $P = 0.0141$ ]. However, not all of the exposed groups differed from each other. Specifically, post-hoc comparisons showed that although the 1-, 2-, and 4-h groups all had greater hearing losses than the 0.5-h exposure group (all  $P < 0.0001$ ), these three groups did not differ from each other (all  $P > 0.3971$ ). This observation suggests that the maximum hearing loss was reached after a 1-h exposure. On the other hand, ABR thresholds in the unexposed (right) ears of the 0.5-, 1-, 2-, and 4-h exposure groups did not differ from those of control animals (all  $P > 0.3593$ ). Thus, the hearing loss appeared limited to the exposed ear.

The observation that there was no reliable difference in the degree of hearing loss suffered by the 1-, 2-, and 4-h groups helps rule out the possibility that the results of the tinnitus test were caused by a hearing loss. Specifically, if the scores on the tinnitus test simply reflected the magnitude of the hearing loss, then the 1-h exposure group should have had the same scores as the 2- and 4-h exposure groups. However, although the 1-h exposure group had the same hearing loss as the 2- and 4-h exposure groups, it differed from both on the tinnitus test: 1-h vs. 2-h [ $F(1,204) = 29.165$ ,  $P = 0.0001$ ], and 1-h vs. 4-h [ $F(1,204) = 16.482$ ,  $P = 0.0002$ ]. Thus, we have a partial dissociation between hearing loss and tinnitus in that the 1-h exposure produced the same hearing loss as the longer exposures, but did not appear to produce tinnitus.

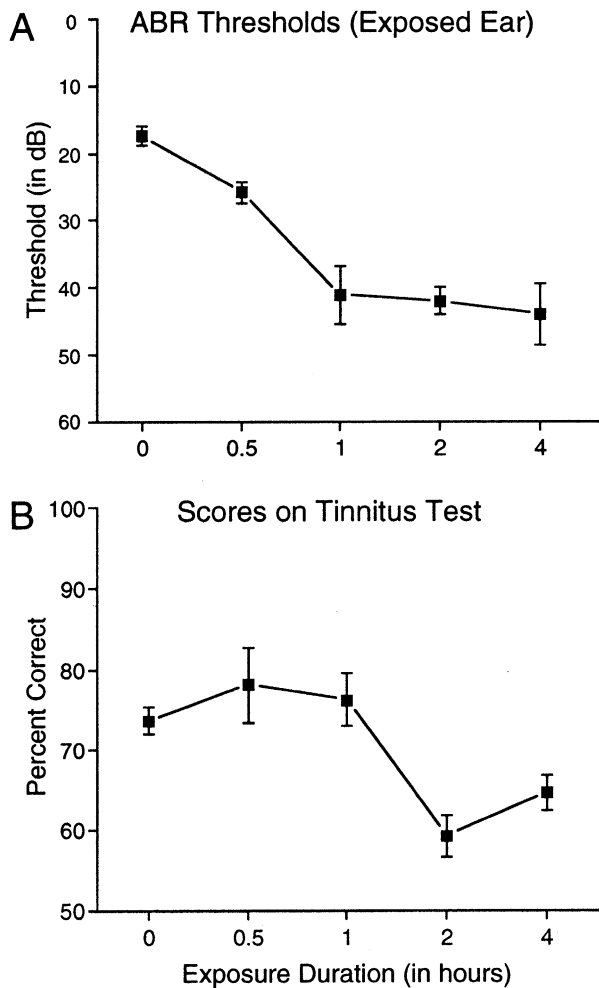


Fig. 7. (A) Effect of exposure duration on ABR thresholds for the band-pass noise. Note that the 1-, 2-, and 4-h groups have the same degree of hearing loss, indicating that the maximum hearing loss was reached after a 1-h exposure. (B) Effect of exposure duration on the behavioral scores from the first tinnitus test session. Although the maximum hearing loss was reached with the 1-h exposure, the exposed and control groups did not differ on the tinnitus test for exposure durations of less than 2 h. Bars indicate S.E.M.

## 4. Discussion

### 4.1. Evidence of tinnitus

The procedure used in this study was designed to increase the likelihood that animals would generalize from externally presented sounds to any tinnitus they might develop. This included training animals using sounds that, while clearly audible, were as low as 33 dB above threshold and varying the location of the loudspeakers so that the animals learned to respond to sound regardless of its location. The results demonstrated that hamsters trained to stop drinking during silence are more likely to continue drinking following exposure to a loud 10-kHz tone for 2 or 4 h. In other

words, they behave as though they hear a sound when no external sound is presented. The question is whether this result is due to tinnitus or can be explained by other factors.

The main alternative explanation is that the exposed animals responded differently because of hearing loss. Indeed, we found that simulating a unilateral hearing loss by training animals with the speakers placed around them and then testing them with all the speakers to one side did cause the animals to be more likely to continue drinking during silence. For this reason, the location of the loudspeakers was routinely varied during training to reduce the possibility that the hearing loss would affect the results. However, the most convincing evidence that hearing loss cannot explain the results of the tinnitus test is that the 1-h exposed animals had a hearing loss similar to that of the 2- and 4-h exposed animals, but tested negative for tinnitus. Thus, hearing loss alone cannot account for a positive score on the tinnitus test.

The procedure used here differs from those used elsewhere in two ways. First, it is possible to obtain a reliable assessment of tinnitus in a single 20-min session – other procedures require approximately five sessions to accumulate a sufficient number of trials (e.g., Jastreboff et al., 1988a). However, the ability to test in a single session comes at the cost of having to give animals extensive training. Thus, our animals are trained for approximately 30 sessions, whereas other procedures require as few as seven training sessions (e.g., Jastreboff et al., 1988a). Another difference is that the animals can be ranked as to the likelihood that they have tinnitus – although there is no reason why this cannot be done with other procedures, those procedures have restricted themselves to group data. Thus, exposed animals scoring outside the range of the controls may be more likely to have tinnitus than those falling within the control range. The ability to assess animals individually is important in searching for the physiological basis of tinnitus because not all exposed animals may develop tinnitus. Interestingly, such a comparison suggests that a 2-h tone exposure may be more likely to produce tinnitus than a 4-h exposure (Fig. 4), a result that warrants replication. However, at the very least, it suggests that increasing exposure time beyond 2 h does not increase the likelihood of tinnitus.

### 4.2. Previous studies of tinnitus in animals

#### 4.2.1. Studies by Jastreboff and colleagues

The first behavioral test of tinnitus in animals was developed by Jastreboff and his colleagues using rats (e.g., Jastreboff and Brennan, 1994; Jastreboff et al., 1988a). Although the details of their method have varied slightly, the basic procedure involves training ani-

imals to stop licking a water spout whenever a broadband noise is turned off for 60 s by presenting a brief foot shock at the end of the ‘noise off’ or silent interval. Training consists of two sessions in which the animals are presented with five silent intervals each. The entire training procedure requires as few as 7 days and is followed by five test sessions each containing five silent intervals (25 intervals altogether). Animals with tinnitus are expected to hear a sound (i.e., tinnitus) and be more likely to continue licking during silent intervals. As in the present experiment, shock is no longer given and all animals eventually learn to continue licking during silence.

Using this procedure, Jastreboff and his colleagues have found that animals given salicylate at the beginning of testing are more likely to continue drinking during silent intervals than animals given saline. Furthermore, they found that animals given salicylate at the beginning of *training* are more likely to stop drinking during silent intervals than those given salicylate at the beginning of *testing*, presumably because those given salicylate during training hear their tinnitus during the silent intervals and thus learn to associate it with shock. This is an important control because it reduces the possibility that the group receiving salicylate at the beginning of testing was less likely to stop drinking because of other effects of salicylate, such as nausea, change in motivation, etc. Jastreboff and his colleagues have also demonstrated that the effect of salicylate increases as a function of dosage (Jastreboff and Brennan, 1994). In addition, they have found that quinine also produces tinnitus and that the effects of salicylate and quinine can be abolished by nimodipine (Jastreboff and Brennan, 1988; Jastreboff et al., 1991).

Jastreboff and his colleagues have conducted a number of control tests to further explore their results. First, they addressed the question of whether animals trained to treat a broadband noise from a loudspeaker as a safe signal would generalize to a tonal signal that presumably resembled tinnitus. They showed presenting control animals with a 7-kHz tone (60 dB SPL) during the silent intervals increased the likelihood that they would continue drinking (Jastreboff et al., 1988a). Thus, the animals generalized from an external noise to an external tone presented about 60 dB above their threshold (cf. Heffner et al., 1994). However, a later study found that presenting a 10-kHz tone at levels from 32 to 62 dB above threshold had no effect on the animal’s performances and, furthermore, that presenting the 10-kHz tone at higher intensities made the animals less likely to continue drinking (Jastreboff and Brennan, 1994). Thus, the degree to which animals trained with this procedure generalize to tones is not clear.

A second question concerns whether factors such as

motivational level or stress could affect the outcome of these tests. The effect of motivational level was addressed by testing animals whose body weights were reduced to 90% ad lib weight, thus making them less thirsty than animals tested at the standard 80% weight (Jastreboff et al., 1988a). The results showed that a significant, albeit smaller, effect of salicylate could still be demonstrated with the less motivated animals. They also demonstrated that salicylate by itself does not affect an animal’s water consumption, so that the tendency of salicylate-treated animals to continue drinking during silent intervals does not appear to be due to increased thirst. With regard to stress, however, it has been noted that the introduction of a stressor, such as being handled by an inexperienced technician or being presented with a loud sound, can affect the results. In these situations, the control group may actually be more likely to continue drinking during the silent intervals than the salicylate group (Jastreboff and Brennan, 1994). Thus, the animals must be carefully handled in order to obtain reliable results.

A third question is whether the effects of salicylate are specific to auditory tasks or can also affect non-auditory discriminations. This question was addressed by training animals to stop licking when a light (instead of noise) was turned off (Jastreboff et al., 1988a). The results indicated that there was no effect of salicylate on suppressing to a light cue. Thus, salicylate did not have a general effect on an animal’s performance, but, instead, was specific to auditory tasks.

Finally, the question of the pitch of the animal’s tinnitus was addressed by administering salicylate to animals *before* training so that any tinnitus they developed would be paired with shock (Brennan and Jastreboff, 1991). The animals were then tested by turning off the noise and presenting tones ranging from 7 to 11 kHz. It was expected that the animals would be less likely to continue drinking when presented with tones similar in pitch to their tinnitus. The results showed that the animals were progressively less likely to continue licking as frequency was increased, leading the authors to suggest that the tinnitus was above 12 kHz. However, it was later demonstrated that the levels of salicylate used result in a hearing loss that begins at ~2 kHz and becomes progressively greater as frequency increases (Brennan et al., 1996). Thus, the reduced drinking may have been due to a hearing loss that made the high-frequency tones less audible.

In summary, Jastreboff and his colleagues have presented evidence that animals develop tinnitus following administration of salicylate or quinine. However, they have not yet ruled out the possibility that the results might have been due to a hearing loss resulting from the drugs. To a limited extent, showing that animals given salicylate after training differ from those given

salicylate before training suggests that hearing loss *per se* cannot account for the results. However, the possibility remains that the sudden introduction of a hearing loss caused by salicylate may affect performance by initially acting as a stressor.

#### 4.2.2. Studies by Bauer and colleagues

The technique developed by Bauer and her colleagues (Bauer et al., 1999) involves training rats to press a lever in the presence of broadband noise to obtain food, but to stop pressing the lever during silent intervals to avoid foot shock. The animal is then tested by presenting four intervals containing a tone, but no shock is given, and four silent intervals followed by shock if the animal does not stop lever pressing. The tone is varied in frequency and intensity with the expectation that animals with tinnitus will respond to the tones differently than control animals. Because the animals are always shocked if they continue lever pressing during the silent intervals, their responding does not extinguish.

Their first study, in which four different frequencies (10, 15, 20 and 30 kHz) were presented at six different intensities (25–80 dB SPL), found that rats given salicylate *after* training were *more* likely than control animals to continue lever pressing during tone intervals (Bauer et al., 1999). However, the effect was variable in that the animals given salicylate differed from the controls at only one intensity at each frequency, with the intensity at which a difference was found varying from one frequency to the next. Although the authors attributed this to the variation in the absolute sensitivity of rats, there does not seem to be any systematic relationship between these results and variation in the rat audiogram (cf. Heffner et al., 1994).

The possibility that the effect might have been due to a salicylate-induced hearing loss was addressed by noting that the animal's click-evoked auditory brainstem potentials were virtually normal, and that tone-evoked potentials conducted on other animals were not affected by salicylate. This is somewhat surprising as a previous study found that salicylate caused hearing losses of 20 dB or more in rats at frequencies above 8 kHz (cf. Brennan et al., 1996). Nevertheless, it can be argued that a hearing loss should make an animal less likely to respond to the tones, and therefore more likely to stop lever pressing during tone intervals, the opposite of the effect that was found.

The explanation for the effect of salicylate on lever pressing was that an animal's tinnitus interacts with its perception of tones to make the tones 'noisier'. In other words, the tinnitus made the tones seem noise-like and, therefore, more like the background noise. As a result, animals with tinnitus were more likely than control animals to continue lever pressing when tones were pre-

sented. However, no evidence was offered to support the idea that tinnitus distorts the perception of tones.

In their second study, Bauer and Brozoski (2001) attempted to induce tinnitus by exposing rats to a 105-dB noise band centered at 16 kHz in one ear for 1 or 2 h. The behavioral procedure in this study was different from that of their previous study in that the animals were exposed to the noise before training began and the number of test intervals was increased. The results showed that rats exposed to the loud noise *before* training were less likely to continue lever pressing during tone intervals than unexposed controls, a result opposite that of their first study. The explanation was that the exposed animals developed tinnitus which caused them to be 'perceptually more challenged' by the tones than the controls. In other words, the exposed animals had been trained to stop lever pressing whenever all they heard was their tinnitus with the result that they were more likely than the controls to stop lever pressing when presented with an external tone that resembled their tinnitus. Unlike the first study, the difference between the exposed and control groups could be found at more than one tone intensity. Moreover, the difference in performance between the exposed and control groups appeared not only to be permanent, but to increase with time, in that one of the six exposure groups showed a larger effect at 17 months than at 2 months.

If the animals were mistaking external tones for their tinnitus, we would expect them to be more likely to stop lever pressing when presented with a tone of the same pitch as their tinnitus. Analysis showed that although the performance of the animals receiving the 2-h noise exposure did not vary with frequency, leading those authors to speculate that the rat's tinnitus was noise-like, the rats with 1-h exposure showed the largest difference at 20 kHz, suggesting that this was the pitch of their tinnitus.

ABR thresholds obtained for clicks and tones at 4–32.5 kHz indicated that the hearing loss ranged from 40 to 60 dB and did not systematically vary with frequency. This is in contrast to the behavioral audiograms in the present study, which found the hearing loss to be much less at lower frequencies (Fig. 5). In addition, Bauer and Brozoski found no difference between their 1- and 2-h exposure groups, which is in general agreement with the present study, although the sounds used to produce tinnitus were different. As a control for the hearing loss caused by the noise exposure, Bauer and Brozoski tested a group of animals with an ear plug in one ear to simulate a unilateral hearing loss. The results showed that the plug had no effect on the animals' overall performance, indicating that, in their test, hearing loss could not account for the difference between the exposed and control rats.

Finally, the investigators tested the effects of drugs on tinnitus, showing that gabapentin significantly reduced the effect of noise exposure, suggesting that it suppresses tinnitus, while tiagabine had no systematic effect.

In summary, Bauer and her colleagues have presented evidence that animals develop tinnitus by showing that exposure to salicylate or loud noise affects an animal's response to tones. In their first study, involving salicylate, the intensity of a tone at which an effect was found did not vary in any orderly way (Bauer et al., 1999). A more systematic effect was found in their second study, in which the experimental animals were exposed to loud noise (Bauer and Brozoski, 2001). However, evaluation of these results is hampered by a lack of detail, in particular, how the difference between the exposed and control animals varied over time. As a result, it is not possible to determine whether the effect increased over time in all six exposure groups, whether the increase was due to changes in the performance of the exposed or the control group, or how long it took for the difference to reach its maximum. Finally, it remains to be determined whether it is reasonable to expect tinnitus to affect the perception of external tones, which is the basis of these studies.

#### 4.2.3. Conclusion

With the results of the present study, there are now three independent lines of research presenting behavioral evidence of tinnitus in animals. One common feature of all of these studies is that they show a *quantitative* difference between exposed and control animals. However, there are a number of factors that could potentially account for such differences and which must be ruled out. One obvious factor is the hearing loss that accompanies both noise- and drug-induced tinnitus. Another is any source of stress, such as differences in the handling of the animals, which can also cause groups to vary. As described above, each set of experiments has attempted to rule out some of these factors with varying degrees of success. However, it would seem desirable to develop a test in which animals with tinnitus make a *qualitatively* different response that automatically rules out alternative explanations without the need for complicated and involved control tests and speculative explanations.

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