Tinnitus in hamsters following exposure to intense sound

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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

order to avoid foot shock (Bauer et al., 1999; Bauer and Brozoski, 2001

2.2.2. Acoustical apparatus

Sine waves were generated by a tone generator (Hewlett Packard 209A) with the frequency veri¢ed 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 ¢ltered (Krohn-Hite 3550; □1/3 octave), and ampli¢ed (Coulbourn S82-24). Broadband noise was produced by a noise generator (Coulbourn S81-02) and ampli¢ed. The electrical signal was sent simultaneously to four Motorola piezoelectric tweeters (KSN 1005A), which, unless otherwise speci¢ed, 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 $\mu N/m^2$) was measured using a B&K 1/4-in (0.64-cm) microphone (model 4135), preampli¢er (B&K 2618), microphone ampli¢er (B&K 2608), spectrum analyzer (Zonic 3525), and ¢lter (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 measurem[kwiq7/BuPW)B7()VnphTnonphTnB¢k%je5□WWdVnph-WkTsV)fWat()□ctqPC)(O)OciBVPWSO7g□ctqPC)(g
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dom performance). For example, an animal that was on the spout 90% of the time during noise trials and o¡ 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.,

2.5. Auditory brainstem response

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

caused those animals to score signi¢cantly 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 di¡erence 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 su⁄cient 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 su⁄cient to prevent the location of the sound from a¡ecting 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 di¡erence 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 ¢xed 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 a¡ected 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 di¡erent 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 bandpass 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 de-

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 di¡erently 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 a¡ect the results. However, the most convincing evidence that hearing loss cannot explain the

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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

mals to stop licking a water spout whenever a broadband noise is turned o¡ for 60 s by presenting a brief foot shock at the end of the 'noise o¡' or silent interval. Training consists of two sessions in which the animals are presented with ¢ve silent intervals each. The entire training procedure requires as few as 7 days and is followed by ¢ve test sessions each containing ¢ve 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, Jastrebo¡ 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 e¡ects of salicylate, such as nausea, change in motivation, etc. Jastrebo¡ and his colleagues have also demonstrated that the e¡ect of salicylate increases as a function of dosage (Jastrebo¡ and Brennan, 1994). In addition, they have found that quinine also produces tinnitus and that the e¡ects of salicylate and quinine can be abolished by nimodipine (Jastrebo¡ and Brennan, 1988; Jastrebo¡ et al., 1991).

Jastrebo¡ 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 (Jastrebo¡ et al., 1988a). Thus, the animals generalized from an external noise to an external tone presented about 60 dB above their threshold (cf. He¡ner 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 e¡ect on the animal's performances and, furthermore, that pre-

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 a¡ect 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 di¡erently than control animals. Because the animals are always shocked if they continue lever pressing during the silent intervals, their responding does not extinguish.

Their ¢rst study, in which four di¡erent frequencies (10, 15, 20 and 30 kHz) were presented at six di¡erent 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 e¡ect was variable in that the animals given salicylate di¡ered from the controls at only one intensity at each frequency, with the intensity at which a di¡erence 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. He¡ner et al., 1994).

The possibility that the e¡ect 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 a¡ected 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 e¡ect that was found.

The explanation for the e¡ect 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 presented. However, no evidence was o¡ered 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 di¡erent 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

Finally, the investigators tested the e¡ects of drugs on tinnitus, showing that gabapentin signi¢cantly reduced the e¡ect of noise exposure, suggesting that it suppresses tinnitus, while tiagabine had no systematic e¡ect.

In summary, Bauer and her colleagues have presented evidence that animals develop tinnitus by showing that exposure to salicylate or loud noise a¡ects an animal's response to tones. In their ¢rst study, involving salicylate, the the intensity of a tone at which an e¡ect was found did not vary in any orderly way (Bauer et al., 1999). A more systematic e¡ect 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 di¡erence between the exposed and control animals varied over time. As a result, it is not possible to determine whether the e¡ect 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 di¡erence to reach its maximum. Finally, it remains to be determined whether it is reasonable to expect tinnitus to a¡ect 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 behavior-