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Assessing Tinnitus and Prospective Tinnitus Therapeutics Using a Psychophysical Animal Model

CAROL A. BAUER and THOMAS J. BROZOSKI

Department of Surgery, Division of Otolaryngology, Southern Illinois University School of Medicine, Springfield, IL 62794, USA

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ABSTRACT

Subjective tinnitus is a common and often debilitating disorder that is difficult to study because it is a perceptual state without an objective stimulus correlate. Studying tinnitus in humans is further complicated by the heterogeneity of tinnitus quality, severity, and associated hearing loss. As a consequence, the pathophysiology of tinnitus is poorly understood and treatments are often unsuccessful. In the present study, an animal psychophysical model was developed to reflect several features of tinnitus observed in humans. Chronic tinnitus was induced in rats by a single intense unilateral exposure to noise. The tinnitus was measured using a psychophysical procedure, which required the animals to discriminate between auditory test stimuli consisting of tones, noise, and 0 dB. Tinnitus was indicated by a frequency-specific shift in discrimination functions with respect to control subjects not exposed to noise. The psychophysical consequences of the noise exposure were best explained by a tinnitus hypothesis and could not be explained easily by other consequences of noise exposure such as hearing loss. The qualitative features of the tinnitus were determined and related to the duration of noise exposure and the associated cochlear trauma. The tinnitus was found to persist and intensify over 17 months of testing. Finally, the tinnitus was reversibly attenuated by treatment with gabapentin, a GABA agonist. It was concluded that this model reflected several features of human tinnitus, such as its tonality and persistence, and could be useful as a screen for potential therapeutics as well as a tool to help unravel the pathophysiology of the disorder of phantom auditory perception.

Keywords: tinnitus, animal model, psychophysics, gabapentin

INTRODUCTION

It has been estimated that over 35% of the U.S. population experiences tinnitus (National Center for Health Statistics (U.S.) 1967). Ten percent experience tinnitus as a debilitating condition that has a significant impact on quality of life (Cooper 1994). Despite the prevalence and morbidity of tinnitus, the pathophysiology of this phenomenon is not known; consequently, there is no generally accepted cure or treatment. Subjective tinnitus is defined as sound(s) heard exclusively by the affected individual. The subjective nature of the disorder, i.e., a perceptual state without external stimulus correlates, has made it difficult to study objectively.

We have reported a psychophysical procedure for measuring chronic salicylate-induced tinnitus in rats (Bauer et al. 1998, 1999a). Using this procedure we were able to measure tinnitus continuously for over six months and characterize qualitative features of the condition as well. These results correspond to those obtained by other researchers using different methods to assess salicylate-induced tinnitus in animals (Jastreboff et al. 1988).

Why use animals to study an objectively elusive symptom that occurs in humans? When using animals there is direct quantitative control over the causal condition, and, therefore, it is more likely that a single form of the disorder is being studied. Understanding

Correspondence to: Dr. Carol A. Bauer • Department of Surgery • Division of Otolaryngology • Southern Illinois University School of Medicine • Springfield, IL 62794. Telephone: (217) 785-4777; fax: (217) 524-0253; email: cbauer@siumed.edu

the pathophysiological features of a single disorder is a more tractable problem than understanding a heterogeneous collection of disorders having only some features in common. The latter is likely to be true for the human tinnitus population (Murai et al. 1992). In an animal model, both acute and chronic tinnitus can be readily quantified using psychophysical procedures. In contrast, reliable and reproducible psychophysical quantification of tinnitus in human patients can be surprisingly difficult (Henry et al. 1999). Psychophysical assessment is affected by intrinsic (short- and long-term fluctuations in tinnitus loudness and pitch) and extrinsic factors (loudness differences, residual inhibition, octave confusion). Penner (1983) has noted that because tinnitus pitch and loudness vary, the qualitative aspects of tinnitus are difficult to measure reliably. Stouffer and Tyler (1990) studied a self-selected sample of tinnitus patients from a clinical population and found that the characteristics of loudness fluctuated in 56% and pitch was variable in 49%. They concluded that there is a subset of the tinnitus population with stable parameters in whom tinnitus pitch and loudness can be reliably quantified. Nevertheless, a fundamental limitation of clinical studies of tinnitus, and conversely the advantage of an animal model, is the variability of the tinnitus population.

In the current animal model, the psychophysical measurements were determined by the animal's auditory discrimination ability. Discrimination should be affected by both objective stimulus conditions as well as internal physiological conditions such as tinnitus. Furthermore, discrimination behavior should be sensitive to the pathophysiological factors associated with tinnitus irrespective of their localization and/or distribution in the nervous system. Therefore, the effect of many experimental variables on tinnitus can be measured. Finally, animals can be used to screen potential therapeutic agents with greater efficiency and safety than can be done with human subjects.

The common drawback in animal studies using salicylate to induce tinnitus is that moderate to high doses are required to obtain reliable effects (Jastreboff et al. 1986; Chen and Jastreboff 1995; Eggermont and Kenmochi 1988). Concerns about salicylate toxicity are real and may be more pronounced in chronic studies. Moreover, in studies designed to screen drugs for antitinnitus activity, salicylate–drug interactions would be an unavoidable concern.

We now report an extension and refinement of our animal model of tinnitus in which unilateral noise trauma was used to induce tinnitus (Brozoski et al. 1996; Bauer et al. 1998, 1999b). In addition to avoiding problems associated with salicylate, noise trauma has several other attractive features as a method for producing tinnitus: A single unilateral exposure to noise is sufficient to induce tinnitus, and the resulting condition appears to be permanent. The method has clinical relevance because noise trauma is the second most common cause of hearing loss associated tinnitus in humans (Cooper 1994). Furthermore, noise-induced tinnitus can be particularly devastating since it usually affects younger individuals and therefore is present for the majority of the individual's life span.

Despite the attractiveness of noise trauma as a method for producing tinnitus in animals, there is an associated problem that must be addressed. It must be convincingly demonstrated that the effect of noise trauma being measured is best explained by a hypothesis of tinnitus and not by the hearing loss which is invariably associated with traumatic noise exposure. The present studies demonstrate that unilateral noise trauma in rats produced changes in discrimination that cannot be explained by hearing loss. The psychophysical effects of unilateral noise trauma are most reasonably explained by the hypothesis that animals experience a tonal tinnitus after noise exposure and the tinnitus persists and intensifies with age. Finally, it will be shown that at least one drug which facilitates activity in the gamma-aminobutyric acid (GABA) neurotransmitter system can attenuate the tinnitus associated with unilateral noise trauma.

METHOD

Subjects

Subjects in all experiments were male Long–Evans rats (Harlan, Indianapolis, IN) that were 60 days old at the beginning of each experiment. Subjects were maintained on a restricted diet with body weight at approximately 80% of free feeding weight as determined by normative weight-gain curves. Each subject was individually housed within a colony room maintained at 25°C and at a 12/12 h reversed light/dark schedule. The experimental protocol was approved by the Southern Illinois University School of Medicine Laboratory Animal Care and Use Committee. Subjects were treated humanely according to the guidelines formulated by the National Academy of Sciences.

Groups

A total of 72 animals were tested in 4 studies. Each study consisted of several successive experiments. Four groups of 6, 6, 8, and 8 rats each were exposed to unilateral noise trauma for 0 h; four groups of 6, 7, 8, and 8 rats each were exposed to unilateral noise trauma for 1 h; and two groups of 7 and 8 rats each were exposed to unilateral noise trauma for 2 h. In all experiments, control (0 h) and experimental (1 or 2

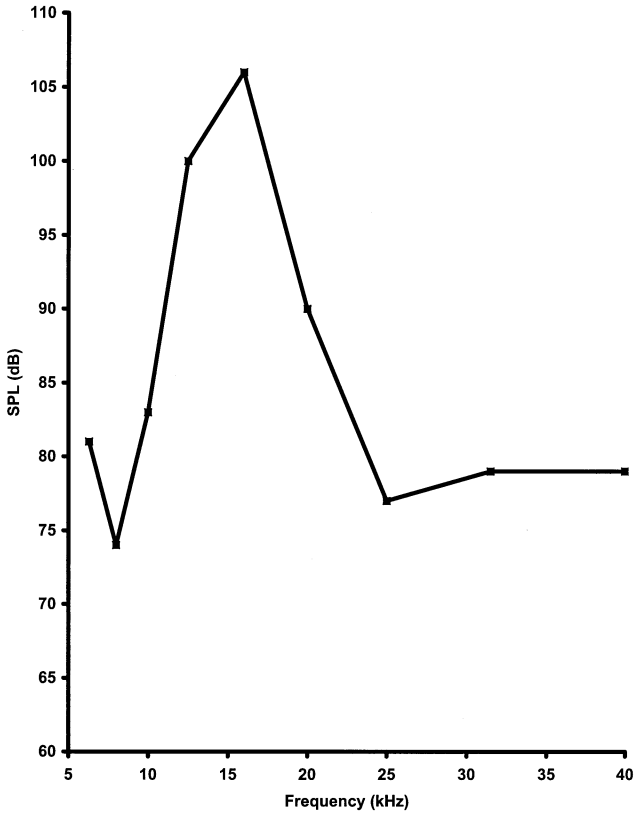

FIG. 1. Spectrum of the octave-band noise band used for trauma, as measured by a Bruel & Kjaer (Norcross, GA) 1616 sound level meter at the distal end of an artificial rat ear canal, with the speaker speculum inserted into the canal. The octave band was centered at 16 kHz (105 dB SPL).

h) groups were trained and tested in parallel, i.e., under the same stimulus conditions with each having the same experimental history.

Noise trauma

Animals were anesthetized with a ketamine HCl (50 mg/kg) and xylazine (9 mg/kg) mixture, placed in a modified stereotaxic head frame, and unilaterally exposed once to narrowband noise with a peak intensity of 105 dB centered at 16 kHz (Fig. 1) for 0, 1, or 2 h either before or after behavioral training and testing, depending on the individual experiment. Output from a high-frequency speaker (40-1398, Realistic, RadioShack Corp., Ft. Worth, TX) was directed into the left ear of the noise-exposed subjects using a 3-mm cone-shaped speculum that fit tightly into the external auditory canal. The sound field was effectively contained by the insert speculum; no additional occlusion was used in the unexposed ear. Robertson (1980) demonstrated in guinea pig that the interaural threshold difference for a unilateral auditory stimulus delivered using hollow ear bars ranged from 50 dB at 10 kHz to a minimum of 32 dB at 26 kHz. Similar levels of attenuation can be assumed in the current study, with contralateral cochlear exposure well below levels expected to induce pathological changes. Hearing levels of noise-exposed animals and randomly selected controls were measured using acoustically evoked brainstem responses (ABR) to clicks and tone bursts taken before and immediately following trauma, at 90 days after trauma, and at the conclusion of data collection. Cochleas of randomly selected animals were also subject to hair cell quantification at the conclusion of some of the experiments.

Test chambers

Subjects were trained and tested following a procedure similar to that described in prior studies (Bauer et al. 1999a). Briefly, they were behaviorally conditioned in individual commercial operant-conditioning chambers (Model 80200, Lafayette Instruments, Lafayette, IN), four of which were placed in an acoustically insulated chamber. Acoustic stimuli were digitally synthesized (DS345 SRS, Sunnyvale, CA) and played over speakers (Realistic Model 40-1398), one of which was center-mounted on the top of each conditioning chamber. Stimulus conditions and behavioral contingencies were controlled by a microcomputer running custom software. Except during test stimulus presentations, low-level (60 dB, SPL) broadband noise was played continuously over each speaker. There was no visual contact between subjects during behavioral training and testing. Stimulus conditions were presented to each subject in synchrony and simultaneously. Data collection and behavioral contingencies were monitored for each subject individually.

Behavioral training

Subjects were initially trained to lever-press for food pellets (45 mg, P.J. Noyes, Lancaster, NH) using an autoshaping procedure. Once subjects were pressing reliably, they were placed on a variable-interval (VI) schedule that was gradually increased to a VI 20 with a lower limit of 6 s and an upper limit of 30 s. VI reinforcement schedules produce moderate-to-high response rates with very low intrasubject variability within and between sessions. This steady-state behavior was necessary for efficient psychophysical testing. In the present experiments, sessions were run daily and were 60 min in duration.

Acclimation and test stimuli

The objective of acclimation was to show that none of the auditory test stimuli had a direct effect on lever-pressing. In this condition the subjects were exposed to all the auditory stimuli without any associated aversive

stimuli. Auditory stimuli were presented in pseudorandomly scheduled 60-s stimulus periods in each session. In these stimulus periods the noise was off while a test stimulus was on. Presentation order of the test stimuli was constrained so that no period occurred within 4 min of another period, or within 2 min of the start or end of the session. The frequency of test stimuli was fixed within sessions but varied randomly among several discrete values between sessions. In most experiments, five different stimulus intensities were presented, in random order, within each session, with each repeated once for a total of ten (5×2) presentations. The range of stimulus intensity levels depended upon the stimulus frequency but always included 0 dB (SPL) and always covered a range extending from near hearing threshold to clearly above threshold.

Suppression training and testing

The objective of suppression training was to condition subjects to respond in a distinctive and standard way to the auditory test stimuli. The subjects were conditioned to stop lever-pressing, i.e., suppress, during 0-dB (silent) periods. Throughout every session a suppression ratio (R) was calculated for each 1 min period:

$$R = \frac{B}{A + B}$$

where B is the number of lever presses in the current period and A is the number of lever presses in the immediately preceding period. R provided a running index of behavior and enabled a quantitative comparison of behavior during stimulus test periods to behavior during baseline noise. An R of 0.5 indicates no difference from baseline, while an R of 0.0 indicates complete suppression. It is through R that the subjects indicate what they are hearing. For graphic and statistical analyses, R was averaged for all subjects across all sessions with identical conditions. Beginning in training and thereafter, if a subject's R during a 0-dB period was ≥ 0.2, it was given a 0.5-mA foot shock, 1 s in duration, at the conclusion of the 0-dB period. All subjects quickly associated the aversive stimulus with the 0-dB period and stopped responding within the 0-dB period. When suppression ratios were <0.2 in 0-dB periods, subjects did not receive aversive stimuli. Once subjects were suppression trained, R values close to 0 were established in 0-dB periods and maintained by all subjects. Aversive stimuli were delivered very infrequently throughout the remainder of the experiment, occurring on average less than once per week.

In the initial training phase of the experiments, each test stimulus frequency was presented at an above-threshold level (i.e., 60 dB SPL of higher) in random order across sessions, for at least 8 sessions. Training conditioned animals to respond distinctively when making their discrimination between noise and tones or 0 dB. When animals lever-pressed during background noise, they received food pellets without aversive consequences. The same was true when animals lever-pressed during a test stimulus period (intensity > 0 dB SPL), i.e., they received food pellets without aversive consequences. In contrast, when animals lever-pressed during a 0-dB period, they received food pellets plus an aversive foot shock at the end of the period (final s) if they met or exceeded the R criterion level (0.2). Training continued until all animals' R values were < 0.2 for all 0-dB periods and was > 0.4 during baseline periods.

In the testing phase, which followed training, each test stimulus was presented in random order across sessions, for at least 5 sessions, with intensity levels varying randomly within sessions as previously described in the subsection "*Acclimation and Test Stimuli*." Five different intensities were presented within each session. Behavioral contingencies were identical to those in training. The test periods enabled the derivation of psychophysical discrimination functions, which characterized the animals' tone perception.

Data analysis

Lever-press rates, reinforcement rates, and derivative measures, such as R, were obtained for each subject in successive 60-s periods within each session. Runtime software determined summary descriptive statistics for each subject for each session. Three criteria had to be met for individual subject data to be included in further analysis; (a) There had to be a minimum of 200 lever presses in the session; (b) mean R for background noise periods had to be greater than 0.4; (c) the standard deviation of R for background noise periods had to be less than 0.2. Inferential statistics (mixed-design ANOVAs, independent treatment groups vs. repeated measures over stimulus conditions) were determined for all groups under identical test conditions using either Microsoft Excel® or Syntax®. The results are based on data obtained exclusively from test sessions, that followed training to criterion level. Comparisons were made exclusively between experimental and control groups with identical histories.

Cochleograms

The temporal bones of 12 animals were removed bilaterally at the conclusion of behavioral testing. At removal the animals were approximately 12 months old. Noise trauma exposure, as previously described, had occurred 5 months prior. Of the 12 animals, four each had been exposed for either 0, 1, or 2 h. Erhlich's

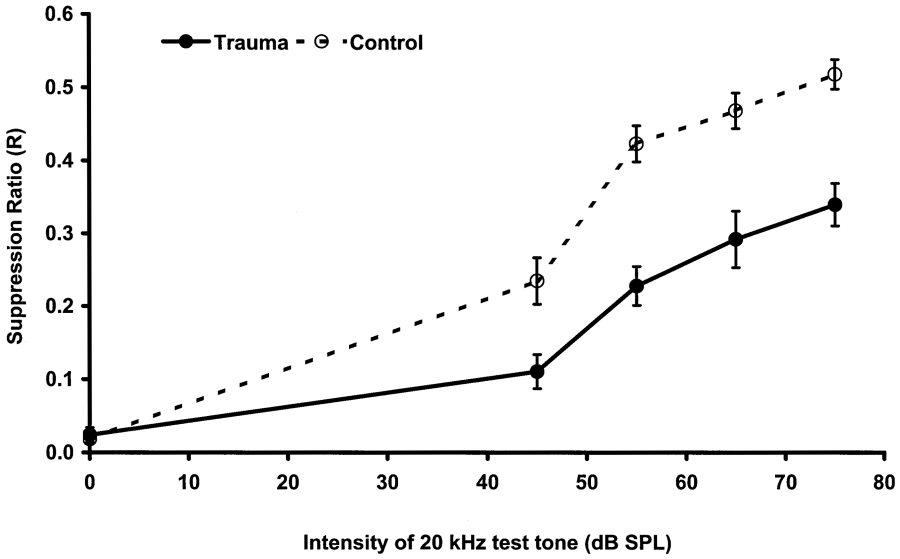

FIG. 2. Twenty-kilohertz tone intensity discrimination functions for control animals (n = 7) that were never exposed to noise, and animals (trauma) exposed once to unilateral noise for 1 h (n = 7). Noise exposure occurred 12 months prior to testing (parameters specified in Fig. 1). Error bars indicate the standard error of the mean.

hematoxilin solution was perfused through each cochlea, followed by immersion at room temperature for 5 min, followed by refrigeration. The temporal bones were shipped in fixative to the Hearing Research Lab at the University of Buffalo for processing according to procedures described in detail elsewhere (Hofstetter et al. 1997; Spongr et al. 1997). Briefly, the bone surrounding the cochlea was removed and the organ of Corti dissected out in half-turns trimmed, and mounted in glycerin on glass slides. The surface of the organ of Corti was viewed using a differential interference microscope (Zeiss Standard Zeiss, Ober-Kochen, Germany) at 400x magnification. The number of missing inner hair cells (IHCs) and outer hair cells (OHCS) were counted over 0.24-mm intervals along the length of the basilar membrane. Hair cells were counted as present if the cell body and cuticular plate were intact. The data from each cochlea were entered into a spreadsheet and the percentage of missing IHCs and OHCs was determined based on laboratory norms for age-matched animals. The percentages of missing IHCs and OHCs were plotted as a function of percent distance from the apex of the cochlea to obtain a cochleogram. The mean IHC and OHC loss from the 24 cochlea (12 rats $\times$ 2 ears) was computed over 4% intervals and plotted for each ear.

RESULTS

Unilateral noise trauma and tone perception

A typical effect of unilateral noise trauma on tone perception is depicted in Figure 2 for two groups, identical except for noise exposure. When tested with a range of intensities of a 20-kHz tone, traumatized subjects displayed more suppression of behavior than the cohort of control subjects. The difference between the two functions was highly significant ($F = 92.77$, $P = 8.2 \times 10^{-20}$). Unilateral 1-h noise exposure 12 months prior to testing shifted the psychophysical function downward.

One interpretation of this effect is that the traumatized animals had tonal tinnitus and the controls did not have tinnitus. Control animals perceived 0 dB as a negative discriminative stimulus (S^-) and both noise and above-threshold tones as positive discriminative stimuli (S^+). In contrast the traumatized animals perceived 0 dB + tinnitus as the S^- and both noise + tinnitus and tones + tinnitus as the S^+. During testing, subjects were presented with noise or a range of tone intensities extending from near threshold to above threshold and which challenged the animals' discrimination ability. The discrimination functions of all subjects, control and traumatized, slope downward with decreasing stimulus intensity because animals increasingly suppressed (i.e., R values decrease) to stimuli that approach threshold. Subjects were trained to suppress lever-pressing during 0-dB stimulus periods. However, compared with controls during test conditions, animals with tinnitus were perceptually more challenged with test stimuli that were qualitatively similar to their tinnitus. For traumatized animals with tinnitus, the perceptual difference S^- (0 dB + tonal tinnitus) and S^+ (tone + tonal tinnitus) was less than for controls, who made the same discrimination but without the presence of tinnitus. The perceptual challenge experienced by the traumatized animals was tinnitus. The perceptual challenge experienced by the traumatized animals was reflected by the downward shift of their psychophysical function (Fig. 2).

Tinnitus or hearing loss?

If the total tinnitus hypothesis is correct, two predictions follow: The first is that the psychophysical function shift for traumatized animals should be frequency dependent. The shift should be greatest when the test stimulus frequency approaches the subject's tinnitus frequency. The second is that animals with only a unilateral hearing loss and *no tinnitus* should display either no shift in their discrimination functions or at least a very different shift than that displayed by animals with tinnitus. This is important because animals exposed to unilateral noise trauma do have an immediate and persistent unilateral hearing loss (Bauer et al. 1999b). If unilateral hearing loss itself produced the same shift in psychophysical functions as that obtained following unilateral trauma, then a tinnitus explanation becomes unnecessary. The average hearing loss resulting from unilateral noise trauma, as measured by acoustically evoked brainsteam responses (ABR), is presented in Figure 3. Standard errors were between 0 to approximately 6 dB and are not shown to reduce clutter. It is important to note that while ABR thresholds were elevated by approximately 50 dB for exposed ears, thresholds were normal for unexposed ears. ABR thresholds measured 90 days post-trauma (Fig. 3B) were essentially the same as those measured immediately post-trauma (Fig. 3A).

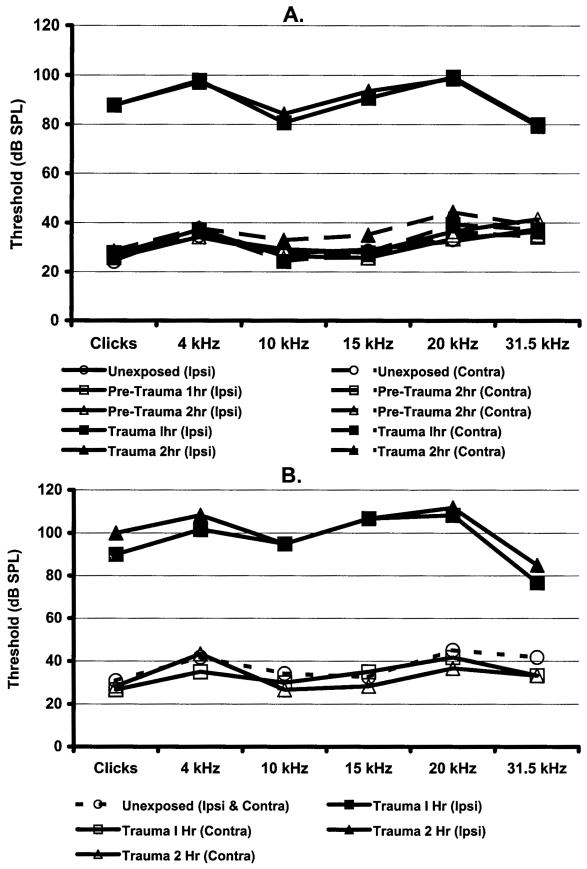

FIG. 3. Average acoustically evoked brainstem responses (ABR) of animals ($n = 7$/group) exposed for 0 (unexposed), 1, or 2 h to unilateral noise (parameters specified in Fig. 1). **A.** Immediately pre- and post exposure. There was no difference between Unexposed Ipsi and Unexposed Contra or between Pre-Trauma (1 or 2 h) Ipsi and Pre-Trauma (1 or 2 h) Contra. There were significant threshold elevations following 1- and 2-h Trauma for exposed ears only. **B.** Ninety days postexposure. Ipsi: exposed ear, Contra: unexposed ear. Thresholds of all groups were similar to those obtained immediately post-trauma (depicted in **A**). Unexposed Ipsi and Contra averaged.

Psychophysical function shift is not caused by unilateral hearing loss and is frequency specific

The two predictions of the tonal tinnitus hypothesis were supported by several experiments. Presented in Figure 4 are the psychophysical functions for control animals ($n = 7$) and those traumatized for 1 h ($n = 7$). Both groups were tested with 10-kHz (Fig. 4A) and 20-kHz (Fig. 4B) tones. The trauma group was tested under "normal" hearing conditions following trauma, while the control group was tested under two conditions: normal and with a unilateral earplug to produce a unilateral hearing loss without tinnitus. In the earplug condition, the control animals had foam earplugs [Earlink® foam eartips (Cabot Safety Corp., Indianapolis, IN) cut to fit] cemented into their left external auditory canal with cyanoacrylate. The presence of earplugs was confirmed before each test session and missing plugs were replaced as necessary. ABR thresholds for click and tone stimuli were obtained for a subset of control animals with earplugs. The earplugs produced unilateral threshold elevations (mean = 40 dB) comparable to the threshold elevations measured in unilateral traumatized animals.

In agreement with the tonal tinnitus hypothesis, the earplugs had no effect on the psychophysical functions of control animals, while trauma-exposed animals continued to display a frequency-specific psychophysical function shift. When tested with 20-kHz tones, unilateral noise trauma produced a significant ($F = 7.740$, $p = 0.008$) downward shift of the psychophysical function compared with controls without earplugs (Fig. 4B). In contrast, unilateral hearing loss alone, i.e., controls tested with earplugs, produced no significant ($F = 0.552$, $p = 0.485$) function shift when tested with 20 kHz (Fig. 4B) or with 10 kHz ($F = 0.852$, $p = 0.392$) compared with controls tested without earplugs. As predicted by a tonal tinnitus hypothesis, the effect of unilateral noise trauma was frequency specific and produced no psychophysical effect when the test stimulus was either 10 kHz (Fig. 4A) or broadband noise (Fig. 4C) ($F = 0.862$, $p = 0.358$). These results clearly support the hypothesis that unilateral noise trauma produced a tonal tinnitus in rats, with consequences

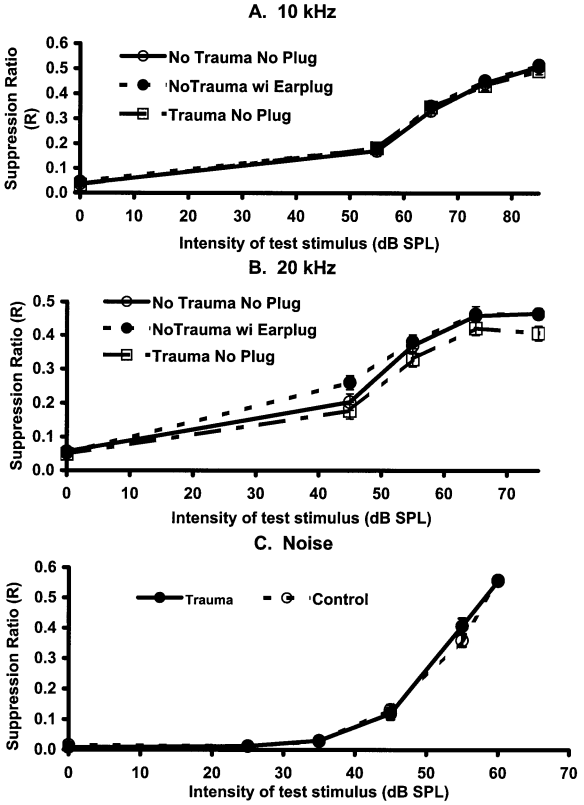

FIG. 4. Average discrimination for animals exposed to unilateral noise trauma for 1 h (n = 7) and control (no trauma) animals (n = 7) under two test conditions: normal (no earplug) and unilateral hearing deficit (with unilateral earplug). The average ABR threshold elevations for controls with earplugs were comparable to ABR threshold elevations produced by unilateral noise trauma. Error bars indicate the standard error of the mean. **A.** Ten-kilohertz tones: no significant differences between groups. **B.** Twenty-kilohertz tones: significant difference between trauma and controls (no trauma) with or without earplugs; no significant difference between controls with or without earplugs. **C.** Average broadband noise-intensity discrimination functions for unilateral noise trauma animals and control animals (n = 7/group) without earplugs.

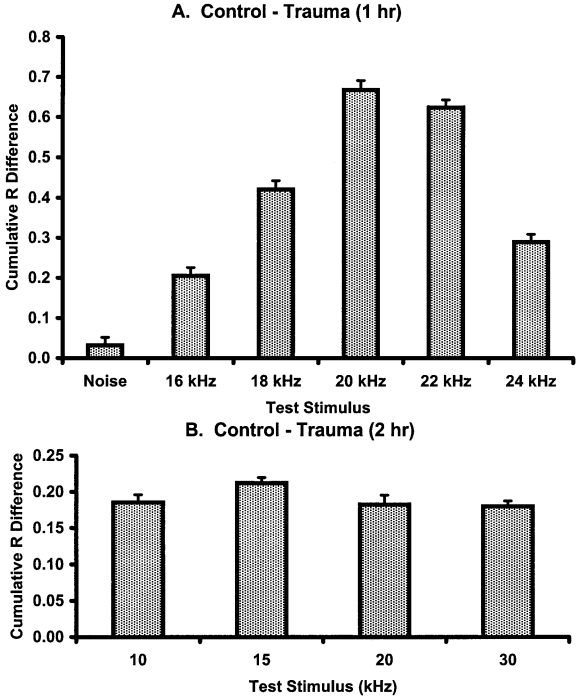

FIG. 5. Psychophysical discrimination function differences (control - trauma) for a range of frequencies and two levels (1 and 2 h) of noise trauma exposure (n = 8/group). Differences were significant for both 1- and 2-h trauma for all frequencies tested, but not for noise, with the greatest difference in functions occurring at 20 kHz for 1-h trauma, suggesting that a more tonal tinnitus results from a 1-h noise exposure. Error bars indicate the standard error of the mean.

distinct from those caused by unilateral hearing loss alone.

Frequency specificity

To further explore the effect of unilateral noise trauma on tone perception, we examined the effect of trauma level on psychophysical response to an extended frequency range of test stimuli. In these experiments, animals were exposed to 0, 1, or 2 h of unilateral noise trauma (n = 8/group). The results are summarized in Figure 5 as cumulative R (whole psychophysical function) differences between control and trauma-exposed groups. This is the cumulative difference in R between control and trauma-exposed psychophysical functions for a range of test stimuli. There was a significant interaction between trauma level, tone frequency, and tone intensity (F = 1.554, p = 0.025). Animals exposed to 1 h of unilateral noise trauma showed the greatest psychophysical function shift at 20 kHz compared with controls (F = 92.77, $p = 8.2 \times 10^{-20}$) and smaller, although still significant, shifts at surrounding frequencies (Fig. 5A). However, there was no difference in the psychophysical functions between trauma and control animals in response to noise. In contrast, animals exposed to 2 h of unilateral noise trauma showed much more moderate and equivalent shifts in the psychophysical functions for the frequencies tested (Fig. 5B). The difference between the psychophysical functions of control and 2-h trauma subjects was significant for all frequencies (F = 13.26, p = 0.0007). These results both support and qualify the frequency-specific effects of unilateral noise trauma. The effects of 1-h unilateral noise trauma were clearly frequency specific and strongly support a tonal tinnitus hypothesis. The effects of 2-h unilateral noise trauma were substantially less frequency dependent, suggesting that the tinnitus induced by the longer noise exposure may have been less tonal. In this regard it should be noted that while both 1-h and 2-h trauma groups showed comparable unilateral elevation of ABR thresholds in their trauma-exposed ears, post mortem examination revealed

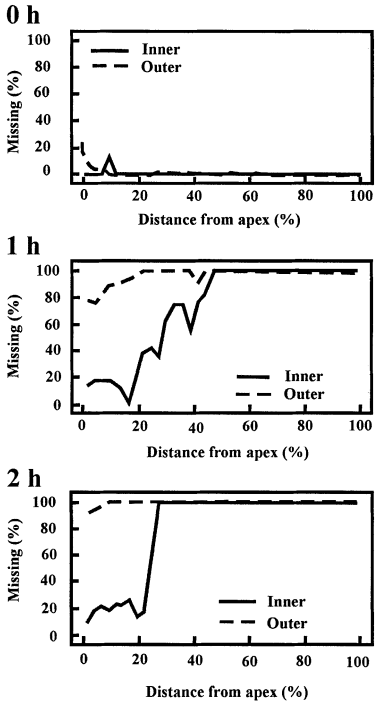

FIG. 6. Cochlear hair cell loss from representative animals; 0 h, unexposed; 1-h exposure; 2-h exposure; exposed ears only. Animals with 2-h exposure generally showed more extensive loss of inner and outer hair cells than animals with 1-h exposure. Unexposed animals showed negligible hair cell loss.

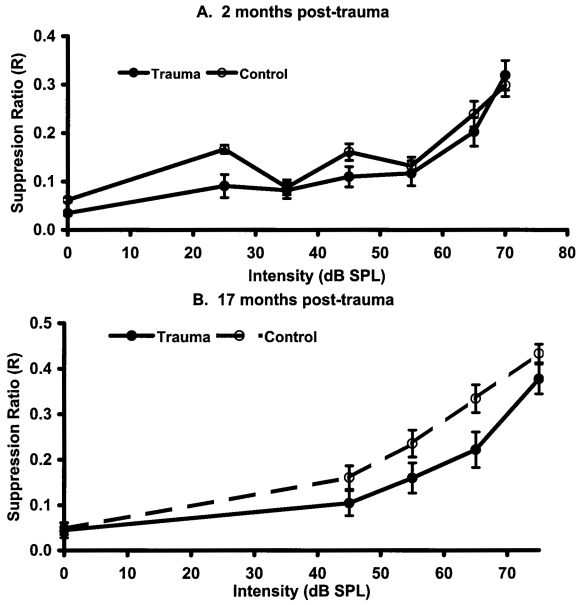

FIG. 7. Twenty-kilohertz tone-intensity discrimination functions for animals exposed 2 months prior to unilateral noise trauma for 1 h ($n = 7$) compared with control animals ($n = 8$). Trauma and control groups were trained and tested in parallel over the same time period. The difference between trauma and controls was significant. **B.** Twenty-kilohertz tone-intensity discrimination functions for the same groups 17 months postexposure. The difference between trauma and controls was larger and also significant. Error bars indicate the standard error of the mean.

more extensive cochlear hair cell loss in some members of the 2-h group (Fig. 6).

Persistence of effect

Animals were tested for up to 17 months following unilateral noise trauma. Shifts in their psychophysical functions persisted and increased over time (Fig. 7A vs. 7B). The difference between the traumatized and control groups at 17 months was significant ($F = 16.321$, $p = 0.00007$).This strongly suggests that the tinnitus in rats caused by unilateral noise trauma is permanent.

Light microscopy of traumatized cochleas revealed almost 100% loss of hair cells for 80% of the organ of Corti in the 2-h trauma group, with slightly less extensive inner hair cell damage in the 1-h trauma group. Representative cochleograms from animals 5 months post-trauma appear in Figure 6. These histological results illustrate the extensive nature of the trauma. It is unlikely there would be recovery of auditory function over the course of the experiments. It should be noted that the unexposed cochleas of traumatized animals showed no loss of hair cells and no shift in ABR thresholds, a result which has been replicated four times with independent subject groups. There are no published data on the effects of a narrowband noise exposure using a close-field technique on the cochleas of young adult rats. The current results indicate that 1- and 2-h narrowband noise exposures in rats do not result in a narrowband hearing loss but rather a loss over a much broader range of frequencies. Shorter durations of exposure may result in more focal areas of threshold elevation. It has been shown in cat, chinchilla, and guinea pig that exposure to an 8-kHz tone of increasing intensity resulted in progressive widening of the band of affected frequencies with elevated thresholds (Dancer et al. 1992).

Effect of GABAergic drugs

We have reported that chronic salicylate treatment is associated with changes in GABA receptor density and synthetic enzyme activity in the auditory midbrain (Bauer et al. 2000). These findings suggest that tinnitus may be associated with aberrant changes at GABAergic synapses in the central auditory system. Drugs that modulate GABA activity may have the potential to modulate tinnitus. Using our animal model, we tested the effect of two GABAergic drugs—gabapentin (Neurontin®, Parke Davis, Vega Baja, PR) and tiagabine (Gabitril®, Abbott, Deerfield, Il)—on the behavioral manifestations of tinnitus. The experiments were preliminary studies designed to address the feasibility of

testing the efficacy of tinnitus-modulating agents. The central mechanism of action of the GABA agonist gabapentin is still unknown. Tiagabine is a GABA agonist that selectively inhibits GABA reuptake. Each drug was tested under identical conditions at dose levels within a range indicated to be clinically effective in humans and physiologically active in animals according to the manufacturers' trials. Drug was dissolved in the animals' drinking water and delivered chronically over a 10-day period for each dose level. Animals were divided into four groups (n = 4/group): unilateral noise trauma + drug, unilateral noise trauma + no drug, control + drug, control + no drug. All groups were tested in parallel using 10-kHz and 20-kHz tones, from which psychophysical discrimination functions were derived and used as a basis for dose–response comparisons. Drug water consumption was recorded throughout the experiment. Subjects in the drug treatment groups were initially tested using the low drug concentration. After completion of behavioral testing, the higher drug dose was given and subjects retested.

It should be noted that a parenteral route of drug delivery was intentionally rejected and that drug dosing in drinking water was employed to minimize the trauma of repeated injections and the consequent disruption of behavioral performance. In other studies (unpublished data, Brozoski), both subcutaneous and intraperitoneal injections have been used prior to behavioral testing. An invariable consequence of these drug delivery routes is increased variability in behavioral performance, which we attribute to the stress of restraint and injections. Daily parenteral injections also present the risk of infection, even under the best conditions. Oral drug administration in the drinking water eliminates these problems. The inevitable variability in dose level within each treatment group, inherent to the oral method, is documented below. However, if anything, this variability should have worked against finding a significant drug effect.

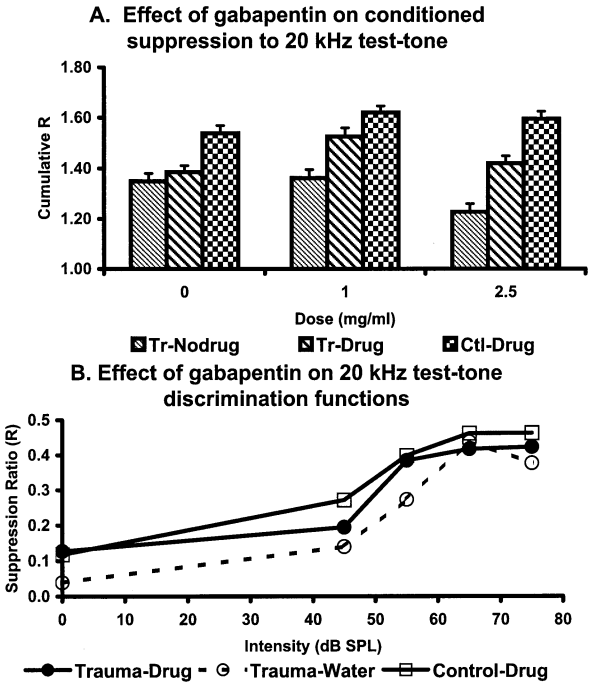

FIG. 8. Gabapentin dose–response functions for three groups (n = 4/group). Prior to drug treatment the two trauma groups were matched in terms of their mean psychophysical performance, i.e., their group discrimination functions were equivalent. Throughout the experiment, the *trauma-no-drug group* was tested *without drug*. Drug levels are shown as concentration in drinking water. **A.** Total R across all intensities levels for each group. At 0 mg/mL, both trauma groups performed identically and were significantly different than controls. At 1 mg/mL and 2.5 mg/mL, drug-treated trauma animals were significantly different than non-drug-treated trauma animals and not significantly different than controls. **B.** Twenty-kilohertz tone-intensity discrimination for the same groups at 2.5 mg/mL. See text for details. There was no difference between control subjects on drug and control subjects without drug. Only control–drug data are shown for simplicity. Error bars indicate standard error of the mean.

Gabapentin

The mean daily intake of gabapentin at a concentration of 1 mg/mL was 115 mg/kg/day (range = 90.1–180.4). The mean daily intake of gabapentin at a concentration of 2.5 mg/mL was 351 mg/kg/day (range = 196.5–551.7). These doses were well within the range shown to be effective for management of chronic pain and seizures in humans. Animals readily drank the drug water at both concentrations, consuming volumes equal to the plain water consumption of nondrug-treated subjects. There were no detectable behavioral differences between drug and nondrug animals in either their home cages or in their lever-press rates during testing. The effect of gabapentin on the psychophysical functions was as follows: Gabapentin significantly reduced the shift in the 20-kHz psychophysical function of the traumatized subjects but had no effect on the behavior of control animals in response to 20-kHz test tones (Fig. 8) (trauma: 1 mg/mL vs. 0 mg/mL, $F = 5.569$, $p = 0.019$; trauma: 2.5 mg/mL vs. 0 mg/mL, $F = 10.430$, $p = 0.001$). The drug effect was frequency specific, with no significant effect at 10 kHz for either traumatized or control animals (trauma: 1 mg/mL vs/ 0 mg/mL, $F = 2.157$, $p = 0.148$; trauma: 2.5 mg/mL vs. 0 mg/mL, $F = 0.231$, $p = 0.873$). Finally, there was no significant effect of gabapentin on the psychophysical functions of control animals at either dose or test frequency (20 kHz, control: 1.0 mg/mL vs. 0 mg/mL, $F = 3.263$, $p = 0.073$; 20 kHz, control: 2.5 mg/mL vs. 0 mg/mL, $F = 0.076$, $p = 0.0783$; 10 kHz, control: 1.0 mg/mL vs. 0 mg/mL, $F = 2.139$, $p = 0.146$; 10 kHz, control: 2.5 mg/mL vs. 0 mg/mL, $F = 0.155$, $p = 0.694$). These results collectively indicate that gabapentin significantly

reduced the evidence of trauma-induced tinnitus in rats without affecting general performance on the psychophysical task.

Tiagabine

At the two levels tested, 0.005 mg/mL and 0.0125 mg/mL, mean daily tiagabine intake was 0.56 mg/kg/day (range-0.32–1.25) at the low concentration and 1.52 mg/kg/day (range-1.12–3.21) at the high concentration. Drug-treated animals' daily water intake was equal to that of control animals. Although the doses tested were well within the clinical range suggested by the manufacturer, tiagabine had no systematic effect on the psychophysical functions of either traumatized or control animals. In addition there were no detectable behavioral differences between drug and nondrug animals in their home cages or in their leverpress rates during testing.

DISCUSSION

Validation of the tinnitus hypothesis

Is the tinnitus hypothesis, in the context of the present animal model, valid? Several lines of evidence converge to support a tinnitus hypothesis as an explanation of the psychophysical consequences of unilateral noise trauma: (a) A reliable and persistent shift in discrimination was produced by unilateral noise trauma but not by comparable unilateral hearing loss alone (from earplugs). (b) The shift in the discrimination functions produced by unilateral trauma was frequency specific, as would be predicted by tonal tinnitus but not by simple hearing loss. In this regard it should be noted that the ABR thresholds demonstrated that the unilateral hearing loss produced by unilateral trauma extended across a range of frequencies (Fig. 3). (c) The shift in the discrimination functions produced by unilateral trauma was pharmacologically reversible, an unlikely outcome if hearing loss was the critical factor causing the shift.

Useful features of the model

The current animal model has a number of useful features that derive from its foundation on steady-state discrimination behavior: (1) The model has the capacity to detect and qualify chronic tinnitus. Testing over an extended period following noise exposure supported the tinnitus hypothesis, with actually greater psychophysical function shifts obtained at 17 months than at 2 months post trauma. (2) The model can be streamlined to be quite efficient. Reliable differentiation of traumatized and control subjects can be obtained using small groups ($n = 6$), with as few as 10 test sessions post training, using two test tone frequencies at each of five intensities. (3) The model is capable of serving as an efficient screen for potential therapeutics. This was demonstrated in the present study by testing two potentially useful drugs. (4) Qualitative aspects of tinnitus can be measured in the model by manipulating the auditory test stimuli used to generate the psychophysical functions. This allows detailed examination of tinnitus characteristics that may be unique to different etiologies of tinnitus. (5) Since the model quantifies the perception of tinnitus, it should enable an analysis of the underlying pathophysiological factors responsible for tinnitus, irrespective of their location or distribution in the nervous system.

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