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Effects of noise exposure on development of tinnitus and hyperacusis: Prevalence rates 12 months after exposure in middle-aged rats

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ABSTRACT

Fischer Brown Norway (FBN) rats (n = 233) were unilaterally exposed to 12 different combinations of noise intensity, duration, and spectrum, while 46 rats served as sham-exposed controls. Rats were behaviorally tested for tinnitus and hyperacusis using gap-induced inhibition of the acoustic startle reflex (Gap) and prepulse inhibition (PPI) using 60-dB SPL before noise-exposure and at regular intervals for 12 mo. 12-mo after noise exposure the middle-aged rats were then tested again for tinnitus and hyperacusis before collecting Auditory Brainstem Response (ABR) thresholds. Collapsing across all noise exposure conditions a significant tinnitus-like deficit in responding to silent gaps was observed, with the most likely tinnitus pitch around 16 kHz. Rates of tinnitus 12-mo after noise exposure were greatest in groups receiving the four least intense noise doses (110-dB for 30, 60 and 120 min, and 116-dB for 30 min), while some of the greatest rates of hyperacusis occurred in groups receiving more intense or longer exposures. The results suggest that rates for developing tinnitus in animal models may not be easily predicted based upon noise exposure dose, but that low-to-moderate noise exposures may result in the greatest likelihood for producing tinnitus.

1. Introduction

Data from humans suggest that noise exposure early in life can be associated with a delayed development of tinnitus much later. While many patients report tinnitus immediately after a noise insult, the majority of tinnitus sufferers seeking treatment identify no recent, acute trigger (Meikle et al., 2004). Many report their first experience with chronic tinnitus in middle or late adulthood (mean age of 54 years), presumably after the impacts of earlier noise trauma and age compound to result in tinnitus. A large number of tinnitus sufferers report “no onset factors.” But very often, the patients who report no onset factors are found to have a noise exposure history indicative of damaging levels of noise (Griest and Bishop, 1998). In their study to estimate the influence of early noise on tinnitus, Rosenhall and Karlsson (1991) found significant correlations between tinnitus and an earlier exposure to occupational noise, and Rosenhall (2003) discovered that aged men with a history of occupational noise exposure had a 2.5 times greater prevalence rate of continuous tinnitus. In a discussion of the medical/legal issues of tinnitus, Coles and colleagues suggested that although noise-induced tinnitus sometimes appears suddenly it actually develops gradually until it, and the related hearing loss, can no longer be ignored (Coles et al., 2000). Thus, tinnitus, like noise-induced hearing loss, can interact with the aging process and arise years after the noise exposure has ceased (Gates et al., 2000).

Delays in chronic tinnitus development are also often seen in laboratory animals. These delays can be frustrating for the researcher who wants to develop an efficient tinnitus model. For these reasons, we decided to explore, in rats, the causal factors that play a role in tinnitus development. Other studies have allowed experimental animals to age after noise exposure to explore the temporal development of tinnitus over many months or up to a year (Longnecker et al., 2014; Turner et al., 2012), but none have done so while systematically exploring specific features of the noise exposure including intensity, duration and spectral content.

The reflex-based gap-induced inhibition of the acoustic startle

Abbreviations: Gap, Gap-induced inhibition of the acoustic startle reflex; PPI, Prepulse inhibition; ABR, Auditory Brainstem Response; OBN, Octave band noise; mo, Month; min, Minute; wk, Week; y, Year; SPL, Sound Pressure Level; F344/BNF1, Fischer 344/Brown Norway F1; NIH, US National Institutes of Health; SD, Standard deviation

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reflex (Gap) approach to measuring tinnitus (Turner et al., 2006; Fig. 1) and hyperacusis (Turner and Parrish, 2008) can be used to assess the longitudinal development of chronic tinnitus and hyperacusis. The Gap method hypothesizes that the fundamental deficit in tinnitus is an inability to hear silence. Animals or humans with tinnitus should then be deficient in processing a silent gap cue embedded in a background sound (compare Fig. 1B with Fig. 1D), and that the more similar the test stimulus background is to their internal tinnitus, the less difference or "signal-to-noise" there is for detecting the silent gap cue (Turner et al., 2006; Turner, 2007; Turner and Parrish, 2008; Yang et al., 2007). The method requires no prior training, no food or water restriction or aversive shock, and allows several animals to be tested simultaneously in a short session. Because a reflex is used in Gap testing, there is no trained response to learn or maintain and the measure can be repeatedly collected across the lifespan, lending itself well to longitudinal aging studies. Our study takes advantage of these features of Gap testing and collects longitudinal data in rats for 12 mo following noise exposure. Specifically, this study explores the relative impacts of noise exposure duration, intensity, and spectrum by systematically manipulating these variables in different groups of rats and measuring the likelihood of developing tinnitus and hyperacusis 12 mo later in middle-aged animals.

2. Methods

2.1. Subjects

A total of 233 male Fischer 344/Brown Norway F1 (F344/BNF1) rats, 5–6 mo of age at the start of the experiments, were used. They have a lifespan of 36–44 mo and are generally a healthy strain (Turner and Caspary, 2005). FBN rats have commonly been used by aging studies as they have been recommended by the US National Institutes of Health's (NIH) National Institute on Aging for studies because of their hardiness and their relative resistance to the tumor growth that is often seen in many other strains. They demonstrate relatively flat ABR threshold shifts of approximately 20-dB SPL across all frequencies from young to old age (32 mo of age). Aged rats lose few if any inner hair cells but have significant outer hair cell losses starting at the cochlear apex (~75% loss), which taper to moderate losses in middle-frequency regions (~50% loss), to minimal losses in the base (~25% loss; Turner and Caspary, 2005). This pattern of loss was evident in the current study, as expressed in ABR thresholds.

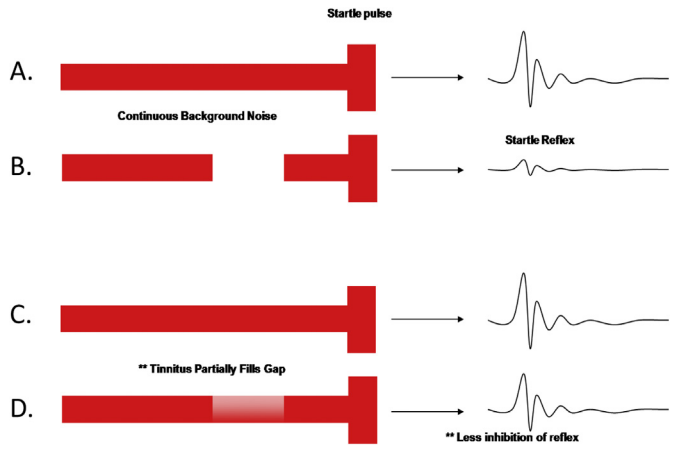

Fig. 1. In Gap testing a continuous background noise followed by a startle pulse elicits a "full startle" response (A). A silent gap, inserted before the startle pulse serves as a warning signal (B). Normal subjects can detect this signal and their response is inhibited. Subjects with tinnitus presumably cannot detect the silent gap as well due to their internal ringing, so their response is less inhibited (C and D).

The animals were maintained on an *ad libitum* diet and individually housed at ~25 °C with a 12 h/12 h light/dark cycle. All protocol procedures were approved by the Southern Illinois University School of Medicine Laboratory Animal Care and Use Committee (P190-06-005), and conformed to the NIH *Guide for the care and use of laboratory animals* as well as the guidelines established by the Society for Neuroscience.

2.2. Part 1 of study: noise duration and intensity

A total of 167 subjects were used for the first half of the study and were introduced in groups of 30 per mo. After acclimating in the animal facility for 1 wk, two short-duration Startle-Only sessions were conducted to acclimate them to the procedure and equipment. Subjects were then behaviorally pretested twice (one day apart) using the full Gap and prepulse inhibition (PPI) procedure, to establish a baseline. Subjects ($n = 137$) were matched as evenly as possible and assigned to one of nine noise conditions (14–18 subjects each). The conditions corresponded to the nine possible combinations of three noise intensities (110, 116, and 122-dB SPL) and three noise durations (30, 60, and 120 min) of an exposure to a 16-kHz, octave band noise (OBN). Thirty additional subjects were divided among three sham-treated control conditions ($n = 9–11$ rats) corresponding to the three durations used for noise (30, 60, and 120 min).

Pre-exposure ABR thresholds were determined bilaterally for all subjects. Subjects were noise/control-exposed with all subjects in each session receiving the same exposure. The exposure ear was randomly chosen beforehand and resulted in an approximately equal number of left and right ears for each condition. Sham-treated control subjects were treated exactly as noise subjects, except that the OBN exposure was never turned on. Immediately following the exposure, all subjects underwent post-exposure ABRs and were allowed to recover until the following day. Subjects were then Gap and PPI tested on post-OBN exposure days 1, 3, 7, 14, 21 and 28 and monthly thereafter over the course of 1 y until they reached middle age at ~18 mo of age. Once behavioral testing ended, final ABR measurements were conducted.

2.3. Part 2 of study: noise spectrum

A total of 66 naive subjects were used for the second half of the study and were introduced at a rate of 33 per mo. They were acclimated and behaviorally tested in the same manner as the rats of Part 1 of the study. A total of 48 subjects were matched, divided among three different noise conditions (16 each) and were unilaterally exposed to an 8- or 32-kHz OBN, or to a broadband noise (BBN) at 110-dB for 30 min. This particular noise intensity and duration led to the most predictable, robust tinnitus in Part 1 of the study. A total of 18 sham-treated controls were exposed to "silence" for 30 min. Following the noise/control exposures, subjects were Gap tested on post-exposure days 1, 3, 7, 14, 21, 28, and monthly thereafter over the course of 1 y until they reached middle age at ~18 mo of age. As in Part 1, once behavioral testing ended, final ABR measurements were conducted.

2.4. ABR threshold and noise/sham-exposure apparatus

A four-station, rodent isoflurane anesthesia system (Vet Equip Inc, RC2) was used to anesthetize up to four subjects at a time for noise and sham exposures. During Part 1 of the study, we found that our isoflurane-anesthetized subjects produced very poor ABR waveforms and unreliable thresholds (e.g., Ruebhausen et al.,