Neuroscience 310 (2015) 176–187

VARIATION IN ACOUSTIC OVERSTIMULATION CHANGES TINNITUS CHARACTERISTICS

L. KIEFER, A. SCHAUEN, S. ABENDROTH, B. H. GAESE AND M. NOWOTNY*

Institute of Cell Biology and Neuroscience, Goethe University Frankfurt am Main, Max-von-Laue-Strasse 13, D-60438 Frankfurt am Main, Germany

Abstract—Tinnitus often occurs after exposure to loud noise. This raises the question of whether repeated exposure to noise increases the risk of developing tinnitus. We thus studied tinnitus development after repeated acoustic overstimulation using startle and auditory brainstem-response techniques applied to Mongolian gerbils. Noise with bandwidths ranging from 0.25 up to 0.5 oct were used for repeated acoustic overstimulation. Auditory brainstem response measurements revealed similar threshold shifts in both groups of up to about 30 dB directly after the acoustic overstimulation. We identified an upper limit in threshold values, which was independent of the baseline values before the noise exposure. Several weeks after the acoustic overstimulation, animals with the noise bandwidth of 0.25 oct showed a permanent threshold shift, while animals of the group with the 0.5-oct noise band featured only a temporary threshold shift. We thus conclude that the threshold shift directly after noise exposure cannot be used as an indicator for the upcoming threshold level several weeks later. By using behavioral measurements, we investigated the frequency-dependent development of tinnitus-related changes in both groups and one group with 1-oct noise bandwidth. The number of animals that show tinnitus-related changes was highest in animals that received noise with the bandwidth 0.5 oct. This number was, in contrast to the number of animals in the 0.25-oct bandwidth, not significantly increased after repeated overstimulation. The frequency distribution of tinnitus-related changes ranged from 4 to 20 kHz. In the group with the narrow-band noise (0.25 oct) changes center at one frequency range from 10 to 12 kHz. In the group with the broader noise band (0.5 oct), however, two peaks at 8–10 kHz and at 16–18 kHz were found, which suggests that different mechanisms underlie the tinnitus development.

Key words: acoustic startle response, PPI, GPIAS, overstimulation, ABR, Mongolian gerbil.

*Corresponding author. Tel: +49-69-798-42063; fax: +49-69-798-42050.
E-mail address: Nowotny@bio.uni-frankfurt.de (M. Nowotny).
Abbreviations: ABR, auditory brainstem response; ASR, acoustic startle response; GPIAS, gap-in-noise inhibition of acoustic startle; IC, *inferior colliculus*; IHC, inner hair cells; Oct, octave; PPI, pre-pulse inhibition; SPL, sound pressure level.

INTRODUCTION

Worldwide, millions of people hear sounds without a detectable source, a phenomenon called tinnitus. The phantom noise sensation of a subjective tinnitus occurs without internal or external sound source and it is believed that one of the main causes of subjective chronic tinnitus is damage to the inner ear from acoustic overstimulation or Menière disease (Spoendlin and Schrott, 1987; Davis and Rafaie, 2000). However, tinnitus is not considered to be a causative disease, but rather a syndrome of different disorders presumably localized, both, in the inner ear and in the central auditory pathway (Noreña and Farley, 2013).

Initiation and early causes of tinnitus are mostly related to peripheral damage. Acoustic overstimulation, for example, initially affects different structures in the inner ear. Inner hair cells (IHC) are in the central focus of investigations, given their central role for transmitting the input signal to the brain (e.g., Liberman 1984; Lenarz et al., 1993). It has been observed that increased glutamate release from the IHCs after an acoustic trauma led to a reversible swelling of the dendrites of the cochlear nerve (Spoendlin, 1971; Robertson, 1983). Overstimulation with very loud noise can lead to a permanent shift in the hearing threshold and irreversible damage to hair bundles and destruction of hair cells (Wang et al., 2002; Kujawa and Liberman, 2009), often followed by the degeneration of auditory nerve fibers and loss of spiral ganglion cells (Pujol and Puel, 1999; Kujawa and Liberman, 2009; Lin et al., 2011). Chronic tinnitus, on the other hand, is of central origin, as the phantom noise sensation remains even after transection of the auditory nerve (House and Brackmann, 1981; Wigand et al., 1982). This form of tinnitus is related to brain plasticity responding to the pathological activity patterns following inner ear damage (Okamoto et al., 2010; Engineer et al., 2011).

The mechanisms underlying the development of tinnitus are largely unknown and only some models (e.g., Schaette and Kempter, 2006; Parra and Pearlmutter, 2007; Zeng, 2013) give suggestions how input loss and the development of tinnitus are related. Manifestation of plasticity-related changes during tinnitus development can be found in the dorsal cochlear nucleus (DCN), *inferior colliculus* (IC) and the auditory cortex (AC) (e.g., Eggermont and Komiya, 2000; Valentine et al., 2004; Scholl and Wehr, 2008; Koehler and Shore, 2013; Manzoor et al., 2013; Niu et al., 2013). These changes are accompanied by changes in limbic structures (Kraus

http://dx.doi.org/10.1016/j.neuroscience.2015.09.023

and Canlon, 2012), which probably relate to the influence of attention and emotions such as stress in tinnitus manifestation (Melcher et al., 2000; Lockwood et al., 2001). The syndrome-like characteristics of tinnitus are especially related to the large variety of phantom percepts between patients (Eggermont, 2012). Tinnitus of mainly tonal appearance can have very different pitches ranging from 0.5 up to 16 kHz (e.g., Noreña et al., 2002; König et al., 2006; Roberts et al., 2008). Non-tonal tinnitus is often described in the categories of noise, whistling, hissing, buzzling and chirping. All these different types of tinnitus cannot be easily related to the cause and circumstances of induction (Nicolas-Puel et al., 2006; Kreuzer et al., 2012), which makes the search for underlying mechanisms rather complicated.

Research using animal models can help to disentangle such mechanisms underlying tinnitus induction and development. The induction of tinnitus through acoustic overstimulation is in this context a very useful model, as tinnitus following noise trauma is in the majority of cases described as "tonal" by tinnitus patients (Kreuzer et al., 2012). This suggests that a rather localized and defined damage in the central auditory pathway is substantially contributing to the pathology. The gerbil model of trauma-induced tinnitus is well compatible with the notion mentioned above. Several weeks after noise trauma the finally developing tinnitus, as characterized at the behavioral level, has a narrow, spectrally well-defined characteristic at or slightly above the frequency range of the inducing noise (Nowotny et al., 2011).

This rather simple relationship is not the common situation in rodent models for tinnitus. Tinnitus in rats and mice was described at different positions and of different spectral width (e.g., Longenecker and Galazyuk, 2011; Turner et al., 2012; Lobarinas et al., 2013; Rüttiger et al., 2013; Singer et al., 2013). While inducing stimuli (pure tone, noise band) were somewhat different in these studies, the relationship with the induced tinnitus was not simple. The only consistency was that in almost all cases the induced tinnitus was at higher frequencies (e.g., Longenecker and Galazyuk, 2011; Dehmel et al., 2012; Turner et al., 2012). In addition, it should be noted that also in the gerbil model the situation regarding the spectral content of the tinnitus sensation is less clear in the first phase of tinnitus development when the startle-based detection of tinnitus finds more broadband and unstable pattern of tinnitus (Nowotny et al., 2011).

In order to determine possibly underlying mechanisms of tinnitus induction it is therefore necessary to investigate the dependence of tinnitus on details of the inducing stimulation. This is the aim of the present study. By systematically controlling the width and repetition pattern of the traumatizing noise we investigate important factors of tinnitus induction. The severity, width and spectral pattern of the resulting percept was investigated and revealed interesting patterns and a systematic relation to the parameters of tinnitus induction.

EXPERIMENTAL PROCEDURES

Animals

Twenty-nine Mongolian gerbils (*Meriones unguiculatus*), obtained from the institute's breeding colony, weighing on average 47 ± 18 g and with starting age between 3 and 5 months were used in these experiments. Gerbils were housed in small groups, males separated from females, in a light–dark cycle of 12:12 h with food and water accessible *ad libitum*. Experimental procedures were approved according to federal regulations (approved by Regierungspraesidium Darmstadt, number F104/53). To compare effects of noise-induced hearing loss and tinnitus development, we measured auditory brain stem responses (ABRs) and startle response-based behavior in the same animals. Since in earlier studies (Nowotny et al., 2011; Serra et al., 2015) no correlations between outer hair cell damage and tinnitus were observed, distortion product otoacoustic emissions (DPOAEs) were not measured.

General anesthesia

Animals were anesthetized for ABR measurements and acoustic overstimulation with a mixture of 100 mg/ml ketamine (Ketavet®, Pfizer), 2% xylazine (Rompun®, Bayer) diluted in physiological saline solution (0.9%, Braun) at a ratio of 4.5:1:4.5. Anesthesia was induced with an initial dose of 0.25 ml per 100 g body weight (Goss-Sampson and Kriss, 1991; Nowotny et al., 2011; Althen et al., 2012). In literature, the use of ketamine/xylazine does not alter ABR amplitudes (Smith and Mills, 1989). When data on ABR threshold shift directly after the noise trauma were compared between the anesthetized and the awake states, no effect of ketamine use was found (e.g., Popelár et al., 1987; Syka et al., 1994). Depth of anesthesia was controlled with the toe-pinch reflex and by checking for movement of the vibrissae. The animal was placed on a heating pad to maintain body temperature at 38 °C and a micropump (WPI, sp100i syringe pump, World Precision Instruments, Sarasota, FL, USA) was used for continuous infusion of anesthetics (0.04 ml/h, i.p.) during the entire experiment.

ABRs and acoustic overstimulation

ABR measurements are used to obtain information about auditory sensitivity at the levels of the auditory nerve up to the IC. Pure-tone-evoked ABR responses can be registered at the skull surface with the help of the far-field technique (Jewett et al., 1970). We used short pure tones (10 ms, 0.5 ms r/f) with stimulation frequencies from 2 to 20 kHz (2 kHz steps) and sound pressure levels from 0 to 80 dB SPL (sometimes 90 dB SPL) in 5-dB steps. The recording experiment including stimulus generation was run by a custom-built computer program written in MATLAB (The MathWorks, Inc.; Version 2007b, Natick, MA, USA). Computer-generated waveforms were transferred to an internal sound card (ESI Juli@24-bit/192 kHz, Leonberg, Germany, in some experiments. DAP 3000A-212). Signals were amplified (Rotel RB 1510,