Experimental Gerontology 84 (2016) 61–70

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

# Behavioral auditory thresholds and loss of ribbon synapses at inner hair cells in aged gerbils

Otto Gleich *, Philipp Semmler, Jürgen Strutz

*ENT-Department University of Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany*

ARTICLE INFO

*Article history:*
Received 7 June 2016
Received in revised form 29 July 2016
Accepted 24 August 2016
Available online 26 August 2016

Section Editor: Christian Humpel

*Keywords:*
Auditory
Cochlea
Presbyacusis
Synaptopathy
Sensorineural hearing loss

ABSTRACT

The potential contribution of auditory synaptopathy to age dependent hearing loss was studied in groups of young and old gerbils. The analysis of the number of inner hair cell ribbon synapses in aged gerbils (37.9 ± 3.3 months of age) revealed only a relatively small (11–17%) loss in the basal two thirds of the cochlea, while a more pronounced reduction was identified towards the apex (almost 40%) when compared to a group of young gerbils (9.5 ± 3.2 months of age). Mean threshold elevation in the old gerbils was around 25 dB at 2 and 10 kHz. Frequency-specific behavioral thresholds and ribbon synapse counts were not significantly correlated for the middle and basal regions of the cochlea, despite thresholds varying over a 45 dB SPL range. This suggests that besides a small age-dependent loss of ribbon synapses, additional cochlear pathologies, most likely a decreased endocochlear potential, contribute to peripheral hearing loss in old gerbils.



## 1. Introduction

In recent years, auditory synaptopathy has been recognized as one important mechanism contributing to sensorineural hearing loss (reviewed by Moser and Starr, 2016; Wan and Corfas, 2015). Ribbon synapses form the basis for information transfer between inner hair cells and the spiral ganglion cells. The availability of anti-CtBP2 antibodies for immunohistochemical staining of inner ear ribbon synapses led to the analysis of synaptopathy in different models of cochlear pathology including sound damage (e.g. Kujawa and Liberman, 2009; Lin et al., 2011; Liu et al., 2012; Shi et al., 2013, 2015; Wang et al., 2015) and presbyacusis (e.g. Sergeyenko et al., 2013; Jiang et al., 2015). An initially paradoxical observation in some studies of temporary threshold shift was that thresholds recovered completely despite a persistent loss of up to 50% of the ribbon synapses (Kujawa and Liberman, 2009; Lin et al., 2011). Specifically, Furman et al. (2013) found that synapses and auditory fibers with low spontaneous rates were lost even after thresholds recovered. The population of low spontaneous rate auditory nerve fibers has high thresholds and a wide dynamic range, while the fibers with a high spontaneous rate have low thresholds and a narrow dynamic range (Liberman, 1978). Consequently, the loss of fibers with a low spontaneous rate leads to a reduction of the amplitude of supra-threshold auditory evoked potentials while thresholds remain low. Thus, the loss of low spontaneous rate fibers would impair the processing of supra-threshold stimuli beyond expectations based on threshold measurements and may contribute to the observation in patients who report that they can hear, but cannot understand (Moser and Starr, 2016).

Among small rodents, in contrast to mice, sensitive hearing in gerbils includes frequencies below 5 kHz and substantially overlaps with the hearing range of humans (Ryan, 1976; for a direct comparison of audiograms see Gleich and Strutz, 2012). Although other rodents such as guinea pigs and chinchillas are also sensitive in the frequency range that is important for human communication (Heffner et al., 1971; Heffner and Heffner, 1991), gerbils have a shorter life span of 3–4 years (Cheal, 1986) making them an appropriate model for studying presbyacusis. Following an initial paper by Henry et al. (1980), different aspects of peripheral and central age dependent hearing loss have been investigated in gerbils (for review see Gleich and Strutz, 2012). Thresholds, as determined by auditory evoked potentials, begin to deteriorate beyond 2 years of age (Mills et al., 1990). Behaviorally-determined auditory thresholds, however, clearly show hearing loss in gerbils older than 3 years (Hamann et al., 2002; Gleich et al., 2006, Fig. 1). The amplitudes of auditory brainstem responses (ABRs) and the slopes of the respective growth functions were reduced in a frequency-dependent manner, even in a subgroup of old gerbils with near normal thresholds (Boettcher et al., 1993). The reduction of ABR amplitude was more pronounced at low frequencies between 1 and 4 kHz and less pronounced at 8 and 16 kHz. Based on the hypothesis that a loss of ribbon synapses and corresponding nerve fibers might cause the

*Abbreviations:* ABR, auditory brainstem response; CAP, compound action potential; CF, characteristic frequency; DAPI, 4′,6-Diamidin-2-phenylindol; EP, endocochlear potential; PB, phosphate buffer; PBS, phosphate buffer with 0.9% NaCl; PBST, PBS with 0.05% Triton X 100; TTS, temporary threshold shift.

* Corresponding author.
*E-mail address:* otto.gleich@klinik.uni-regensburg.de (O. Gleich).

http://dx.doi.org/10.1016/j.exger.2016.08.011
0531-5565/

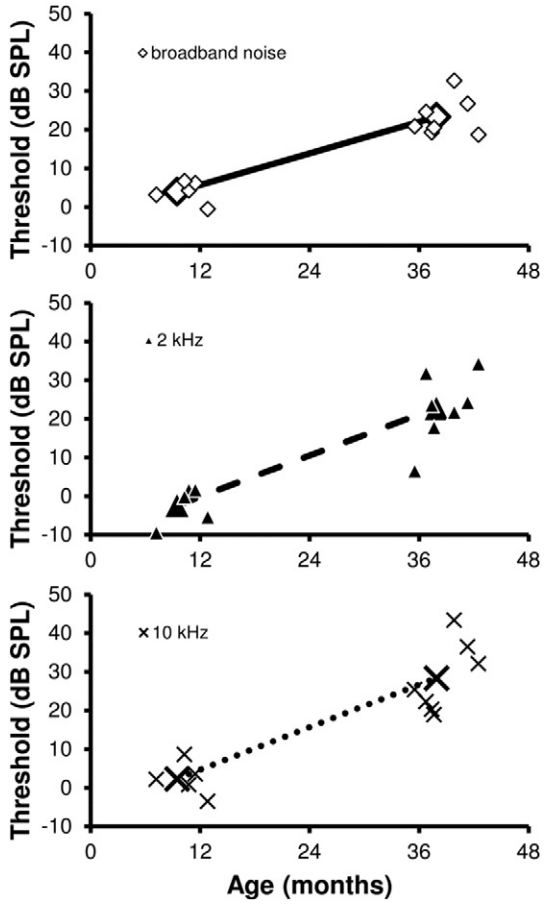


**Fig. 1.** The degree of age dependent hearing loss is illustrated by plotting thresholds for broadband noise (open diamonds), 2 kHz (filled triangles) and 10 kHz (x) as a function of age. The larger symbols represent group means of the young and old groups and are connected to indicate the systematic increase of threshold with age for the 3 different stimuli.

reduction of supra-threshold ABR amplitude, one would predict that the apex should show a more pronounced loss of ribbon synapses in old gerbils. In contrast, Schmiedt et al. (1996) found in recordings from single auditory nerve fibers that the proportion of low spontaneous rate fibers with a characteristic frequency above 6 kHz dropped significantly from 57% in young gerbils to 29% in older gerbils, while this proportion was around 30% for fibers with a characteristic frequency below 6 kHz in both age groups. This suggests that a loss of ribbon synapses might be more prominent at the base of the cochlea. Many additional data show an age-dependent loss of the endocochlear potential and associated pathology of the stria vascularis in gerbils (Schmiedt, 1993, 1996; Gratton et al., 1996, 1997; Schulte and Schmiedt, 1992; Spicer and Schulte, 2002; Spicer et al., 1997).

To investigate the potential contribution of synaptopathy to age-dependent hearing loss in gerbils, we assessed behavioral thresholds in groups of young and old gerbils for correlations with the corresponding number of ribbon synapses.

## 2. Methods

In the present experiments we determined behavioral thresholds for broadband noise as well as 2 and 10 kHz pure tones, in groups of young and old gerbils. Subsequently, the number of ribbon synapses at apical, middle, basal and very basal regions of the cochlea was assessed and correlated with the animals' age and hearing status.

### 2.1. Behavioral testing

The methods to measure behavioral detection thresholds for broadband noise and pure tones in gerbils followed the procedures reported previously (e.g. Hamann et al., 2002; Gleich et al., 2006) and are only briefly summarized.

The loudspeaker for stimulus presentation, a test cage with an observation platform and a feeder for rewarding correct responses were located in a sound shielded box lined with acoustic foam panels to reduce acoustic reflections. A light barrier formed by an infrared diode in the floor of the observation platform and a photo sensor mounted above allowed the registration of when a gerbil was sitting on the platform and interrupting the infrared light. The experiment, including stimulus generation and presentation, as well as registering the presence of a gerbil on the platform, was controlled by the setup outside the box.

Gerbils were trained to jump onto the observation platform to start a test trial and wait and listen for a stimulus. Stimuli were presented with a random delay of 2 to 6 s after the start of a trial. When gerbils jumped off the platform within 0.3 to 1.2 s after the onset of a stimulus, a correct response was registered, and the gerbils were rewarded with a 20 mg food pellet. If animals failed to respond, this was registered as a miss, and a new trial was initiated. In addition, 30% of all trials were "sham trials" without the presentation of a stimulus. These were used to determine the false alarm rate.

Stimuli consisted of 800 ms noise or tone pulses presented at 7 stimulus levels varying in 3 dB steps over an 18 dB range. Based on 2 sessions, a psychometric function for the response probability at each level was determined for 20 presentations, and the false alarm rate was obtained for 60 "sham trials". Based on training and pilot sessions, the level of the test stimuli was adjusted to include levels both well below and above threshold, covering the steep portion of the psychometric function. Additional criteria for obtaining robust threshold data were ≥80% correct responses for the two highest stimulus levels and ≤20% responses to shams. Thresholds from the resulting psychometric functions were derived using signal detection theory and a d'-value of 1.8.

Training began in all animals with broadband noise followed by 2 kHz and 10 kHz pure tones. When reliable testing was established for broadband noise and the two pure tones, sampling continued until the cochleae were harvested for the analysis of ribbon synapses. All threshold data reported in the results were obtained within 2 weeks of the histological analysis of the cochlea.

### 2.2. Histological processing

Following behavioral testing, animals were anesthetized by the gradual fill method with carbon dioxide (AVMA, 2013) and killed by decapitation following respiratory arrest. Beginning with the right side, the bulla was exposed from ventral and opened with a forceps to gain access to the cochlea. At this stage of dissection the status of the external ear canal and of the middle ear was assessed to identify potential pathologies (e.g., obstruction of ear canal or middle ear infection; see Results). A fine 45° hook was used to sever the stapes artery and remove or dislocate the stapes from the oval window. A small hole was generated in the bony wall of the apex, and the round window membrane was ruptured with the hook. Fixative (4% paraformaldehyde in 0.1 M phosphate buffer pH 7.6 [PB]) was gently flushed through the perilymphatic spaces by application to the oval and round windows as well as the opening at the apex, with a fine-tipped pipette. The fixation of the left cochlea followed immediately. Both cochleae were removed from the skull and fixed for 2 h on a shaker at 4 °C. Each cochlea was transferred to a 50 ml falcon tube in 0.1 M EDTA in PB and decalcified for 5 days at 4 °C on a shaker.

After decalcification, excess tissue was trimmed from the cochlea. Using a razor blade, the cochlea was either cut into 2 halves along the