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Age-related changes in the acoustic startle reflex in Fischer 344 and Long Evans rats

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ABSTRACT

The behavioral consequences of age-related changes in the auditory system were studied in Fischer 344 (F344) rats as a model of fast aging and in Long Evans (LE) rats as a model of normal aging. Hearing thresholds, the strength of the acoustic startle responses (ASRs) to noise and tonal stimuli, and the efficiency of the prepulse inhibition (PPI) of ASR were assessed in young-adult, middle-aged, and aged rats of both strains. Compared with LE rats, F344 rats showed larger age-related hearing threshold shifts, and the amplitudes of their startle responses were mostly lower. Both rat strains demonstrated a significant decrease of startle reactivity during aging. For tonal stimuli, this decrease occurred at an earlier age in the F344 rats: middle-aged F344 animals expressed similar startle reactivity as aged F344 animals, whereas middle-aged LE animals had similar startle reactivity as young-adult LE animals. For noise stimuli, on the other hand, a similar progression of age-related ASR changes was found in both strains. No significant relationship between the hearing thresholds and the ASR amplitudes was found within any age group. Auditory PPI was less efficient in F344 rats than in LE rats. An age-related reduction of the PPI of ASR was observed in rats of both strains; however, a significant reduction of PPI occurred only in aged rats. The results indicate that the ASR may serve as an indicator of central presbycusis.

1. Introduction

Hearing loss associated with the aging process (presbycusis) is the most common cause of chronic, permanent hearing disability in both animals and humans (Gates and Mills, 2005; Syka, 2002; Willott, 1991). Pathological changes occur in presbycusis in both the inner ear and in the central auditory system. The peripheral component of presbycusis, which comprises mainly alterations of the inner and outer hair cells and/or the stria vascularis, is relatively well understood (Harding et al., 2005; Parham, 1997; Schuknecht and Gacek, 1993; Spongr et al., 1997). The central component of presbycusis is thought to be especially associated with age-related decline in the processing of complex acoustical stimuli including human speech (Gordon-Salant, 2005; Willott, 1991). In the human population, a loss of speech understanding with aging constitutes an important health and social impairment (Frisina and Frisina, 1997; Gordon-Salant et al., 2007; Mazelová et al., 2003).

Rodents with a life span of two to three years represent a suitable laboratory model to study the processes associated with the age-related deterioration of the auditory system. In our previous studies (for review see Syka, 2010) two rat strains were used as models of normal and fast aging: Long Evans (LE) rats — an outbred strain with normal aging and preserved hearing function up to late senescence, and Fischer 344 (F344) rats — an inbred strain with a frequent occurrence of pathologies, including an early deterioration of hearing function. Age-related alterations in the function of the inner ear in F344 rats, including pathology of the hair cells, stria vascularis and ligamentum spirale, have been described in detail and compared systematically with the age-related changes in LE rats (Buckiová et al., 2006, 2007; Popelář et al., 2003, 2006). Age-related changes in the function of the inner ear were found to be accompanied in both strains by pathologies in the central part of the auditory system, which comprised a significant decline in glutamic acid decarboxylase (GAD) levels and changes in the occurrence of parvalbumin-immunoreactive neurons (Burianová et al., 2009; Ouda et al., 2008). Another of our recent studies also demonstrated the existence of age-related changes in the temporal processing of acoustical signals in LE rats (Šuta et al., 2011).

In the present study, we decided to use the same model of fast aging F344 rats and normally aging LE rats for assessing the influence of aging on behavioral reactions to auditory stimuli. Therefore, the acoustic startle reflex (ASR) (a transient motor response to an intense unexpected stimulus) was used as an indicator of the behavioral responsiveness to sound stimuli. As the ASR is an unconditioned reflex reaction, there is no need for animal training, and the ASR can be measured at any age. The structural basis of the ASR is represented by a short neural circuit comprising the cochlear nucleus, the caudal pontine reticular nucleus and spinal motor neurons (Davis et al.,

Abbreviations: F344, Fischer 344; LE, Long Evans; ASR, acoustic startle response; PPI, prepulse inhibition; ABRs, auditory brainstem responses; WN, white noise.

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1982; Koch, 1999). In spite of its relative simplicity, the ASR shows several forms of behavioral plasticity (habituation, facilitation and prepulse inhibition) that are functionally significant and reflect the processing of acoustic information at higher levels of the auditory system than the ASR itself (Ison and Hammond, 1971; Koch, 1999; Young and Fechter, 1983).

The ASR may be used to assess behavioral responses only to louder auditory stimuli (higher than approximately 70 dB SPL). To determine the auditory function in the range of intensities below the startling threshold, the measurement of the prepulse inhibition (PPI) of ASR is widely used in both human and animal studies. In the PPI procedure, the startle reaction is inhibited by a low-level sound that shortly precedes the intense startle stimulus. Animal studies have shown that auditory PPI is associated with the function of the cochlear nucleus, the inferior and superior colliculi and the pedunculopontine tegmental nucleus (Koch, 1999). The efficacy of the PPI undergoes a considerable modulating influence of the hippocampus, amygdala and the medial prefrontal cortex (Swerdlow et al., 2001). The PPI of ASR, which represents a basic inhibitory process regulating sensory inputs (Geyer and Braff, 1987; Swerdlow et al., 2001), may be used for estimating the age-related changes in inhibitory function.

The aim of the present study was to evaluate age-related changes in auditory behavior in rat strains with fast and normal aging and to estimate the behavioral correlates of auditory system aging in animals with the presence or absence of hearing loss. The hearing threshold in rats was assessed by recording of auditory brainstem responses (ABR). The strength of the acoustic startle response and the efficiency of the prepulse inhibition of ASR to noise and tonal stimuli were measured and compared in young-adult (approximately 3 months old), middle-aged (approximately 12 months old), and aged (older than 23 months) animals of the F 344 and LE rat strains.

2. Methods

Behavioral and electrophysiological testing of hearing function in Fischer 344 and Long Evans female rats of different ages was performed in this study using measurement of the ASR and its suppression by acoustic prepulse stimuli and the assessment of the rat's hearing threshold on the basis of the recording of ABR.

2.1. Subjects

The measurements were performed in three age groups: young-adult rats (3–5 months old: 10 rats of the F344 strain, mean body weight 198 ± 15 g, and 9 rats of the LE strain, mean body weight 246 ± 14 g), middle-aged rats (12–14 months old: 6 rats of the F344 strain, mean body weight 277 ± 13 g, and 5 rats of the LE strain, mean body weight 334 ± 14 g) and aged rats (24–26 months old: 7 rats of the F344 strain, mean body weight 291 ± 18 g, and 24–34 months old, n = 9 rats of the LE strain, mean body weight 338 ± 9 g). The range of animal ages in the aged F344 group was narrower than in LE rats due to differences in their lifespan (the life span for F344 rats is about 6–8 months shorter than for LE rats (Sass et al., 1975; Hoffman, 1979). For this reason, the aged LE group was divided into two subgroups that were evaluated separately: group LE-A1 with 24–26-month-old rats (n = 5, mean body weight 339 ± 14 g), and group LE-A2 with 29–34-month-old rats (n = 4 mean body weight 337 ± 37 g). This allowed us to compare both age-matched F344 and LE rats, and also the oldest animals of both strains. Animals in all age groups were in good health conditions; the testing period for each rat did not exceed three weeks. In each rat the normal otoscopic status of the outer ear canal and middle ear was controlled periodically using HEINE mini 2000 otoscope to exclude a middle ear infection.

Fischer 344 rats were purchased from Charles River Deutschland (Sulzfeld, Germany), while Long Evans rats were obtained from a local facility. All animals were housed in age-matched groups of two or three per cage under standard laboratory conditions in a constant environment and a 12/12 h normal light/dark cycle; food and water were available ad libitum. The care and use of animals and all experimental procedures were performed in compliance with the guidelines of the Ethical Committee of the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, and followed the guidelines of the EU Directive 2010/63/EU for animal experiments.

2.2. Apparatus and procedures

All behavioral tests were performed in a sound attenuated chamber (Coulbourn Habitest, model E10-21) located in a soundproof room. During the testing procedure, the rat was confined to a small wire mesh cage (160 × 85 × 90 mm) on a motion-sensitive platform. The animal's reflex movements were detected and transduced by a piezoelectric accelerometer. The amplified voltage signal was acquired and processed using a TDT system III with Enhanced Real-Time Processor RP2.1 (Tucker Davis Technologies, Florida, USA) and custom-made software in the Matlab environment (The MathWorks, Inc.). The startle responses were evaluated in a 100 ms window beginning at the onset of the startle stimulus. The magnitude of the ASR was given by the maximal peak-to-peak amplitude of transient voltage occurring in the response window. Acoustic startle stimuli (tone pips or noise bursts) and prepulse stimuli (tone pips) were generated by the TDT system and presented via a loudspeaker (SEAS, 29AF/W) placed 12 cm above the platform inside the chamber. Stimulus presentation and data acquisition were controlled by a custom-made application in the Matlab environment. Calibration of the apparatus was performed for frequencies between 1 kHz and 32 kHz by a 1/4 in. Brüel & Kjaer 4939 microphone connected to a Brüel & Kjaer ZC 0020 preamplifier and a B&K 2231 sound level meter. The calibrating microphone was positioned in the location of the animal's head in the test cage.

The ASRs to 4, 8, and 16 kHz tone pips and white noise (WN) bursts (50 ms duration, 5 ms rise/fall times, varying intensity levels) were recorded. Each test session contained 7 trial types presented in a random order: a baseline trial (–10 dB SPL stimulus intensity) and 7 startle stimuli of different intensities (60, 70, 80, 90, 100, 110, and 120 dB SPL). Each trial type was presented ten times. The inter-trial interval varied from 15 to 30 s. The mean ASR amplitude of each trial type was calculated as the average of all the ASR amplitudes for that given trial type with the highest and lowest ASR amplitudes excluded. A trial was considered to have evoked a startle reaction if the mean ASR amplitude for that trial exceeded the average amplitude of the baseline trial (0.03 ± 0.008 V) by more than twice the standard deviation (i.e., it was more than approximately 0.05 V). Thereafter, the ASR threshold was determined as the minimum intensity at which there was a startle reaction for at least 50% of the trials.

In the prepulse inhibition procedure, 3 different trial types were used: a baseline trial without any stimulus, an acoustic startle pulse alone (white noise at 115 dB SPL, 50 ms, 5 ms rise/fall times), and a combination of the startle pulse and prepulse tone pips (50 ms duration, 5 ms rise/fall time) at frequencies of 4, 8, and 16 kHz at 75 dB SPL. The inter-stimulus interval between the prepulse and the startle stimulus was set to 50 ms. Each of the trial types was presented ten times. The inter-trial interval varied from 15 to 30 s. The efficacy of the PPI of ASR was expressed as:

$$\mathrm{PPI} = [1 - (\text{amplitude of ASR suppressed by prepulse tone} / \text{amplitude of ASR alone})] \times 100\%.$$

Thus, a PPI of 100% corresponds to complete suppression of the ASR, and higher percentages indicate stronger PPI.

To assess the hearing thresholds in rats, ABRs to tonal stimuli were recorded in anesthetized rats with an intramuscular injection of 38 mg/kg body weight of ketamine (Calypsol, Gedeon Richter Ltd.)