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Frequency discrimination in rats exposed to noise as juveniles

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HIGHLIGHTS

- We analyzed frequency discrimination in adult rats exposed to noise as juveniles.
- We recorded and evaluated prepulse inhibition of the acoustic startle reflex.
- In exposed animals discrimination was altered at moderate intensities (70 dB SPL).
- Altered discrimination was found even in rats with normal hearing thresholds.
- Deficit in exposed rats disappeared at higher intensities (85–90 dB SPL).

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ABSTRACT

Sound exposure during the early postnatal period can significantly influence the function of the auditory system in rats during adulthood. In the present study, rat pups (strain Long–Evans) were exposed to broad-band noise at 125 dB SPL for 8, 12 or 25 min on postnatal day 14 and then at the age of 3–5 months their frequency discrimination at 4 and 16 kHz was assessed using a modified method of the prepulse inhibition of the acoustic startle reflex. In all groups of exposed rats, an altered frequency discrimination of the tonal stimuli was observed, in comparison with controls, at 70 dB SPL. A worsening of frequency discrimination was observed even in animals exposed for 8 min, the auditory thresholds of which were almost identical to that of control animals. The individual auditory thresholds did not correlate with frequency discrimination. The difference in frequency discrimination between the exposed and control animals disappeared at 85–90 dB SPL. Our data suggests that brief noise exposure during the critical period of development results in the altered frequency discrimination at moderate sound intensities in adult rats, which may appear even in individuals with normal hearing thresholds.

1. Introduction

The auditory system undergoes extensive refinement during the early postnatal period [17,18]. Specifically in rats, pups are born with an immature auditory system with the onset of hearing function in the second postnatal week. The maturation of hearing function then proceeds up to the 6th postnatal week. Development of auditory function can be monitored with behavioral as well as electrophysiological methods, such as acoustical startle reflexes (ASR) or auditory brainstem responses (ABRs).

It has previously been demonstrated that exposure of rats to different sound stimuli during the first postnatal weeks (in the so called 'critical period') may significantly influence the quality of the auditory system in the adult animal. Several studies have documented alterations in frequency tuning and tonotopy in rats exposed to sound during the early postnatal period [5,9,23,31]. As shown by [14] brief acute acoustic trauma in rat pups (broad-band noise exposure 125 dB SPL for 8 min) can result in changes of the tonotopic pattern in the central auditory system in adult rats. A similar model of early sound exposure was used in our previous studies, showing that 8 min of exposure to 125 dB SPL on postnatal day 14 (P14) in rat pups resulted in permanent alterations of frequency and intensity representations in the high-frequency neurons of the inferior colliculus (IC) without influencing their hearing thresholds [3,9]. In these experiments widening of the excitatory response areas and a decrease of the inhibitory sidebands in neurons localized in the high-frequency regions of the IC were observed in exposed rats. We assumed that the missing or displaced inhibitory sidebands of these neurons indicate the deterioration of frequency selectivity in these animals. Altered perception of the sound stimuli was demonstrated behaviorally using ASR and prepulse inhibition of the ASR [19,20]. In these experiments brief noise exposure in rat pups

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resulted in altered behavioral responses to sound in adulthood indicating anomalies in the intensity coding and loudness perception.

The aim of this study was to assess behaviorally frequency discrimination in adult rats exposed to noise as juveniles. To achieve this aim we used the ASR and prepulse inhibition (PPI) of the ASR, i.e. the inhibition of the ASR induced by the presentation of an acoustical stimulus shortly preceding the startling sound. It was shown by several authors that the evaluation of the PPI of ASR is a simple yet efficient method for estimating supra-threshold auditory sensitivity [10,11,28]. We used a modified method of the PPI of ASR that can serve to analyze the discrimination ability of animals [1,6,7].

2. Materials and methods

2.1. Subjects

Female pigmented Long–Evans rats with no primary pathology were used as experimental subjects. The animals were divided into four groups: three groups consisted of animals, which were exposed as pups to broad-band noise for different times: 8 min (n = 9), 12 min (n = 6) or 25 min (n = 3). The fourth group (n = 9) consisted of control animals, which were manipulated in a similar way to that of the exposed animals with the exception of there being no noise exposure. All groups of animals were housed in the same room under standard laboratory conditions in a constant environment, involving a 12/12 h normal light/dark cycle with food and water available ad libitum. The rats were tested at the age of 3–5 months.

The care and use of animals were approved by the Ethics Committee of the Institute of Experimental Medicine and followed the guidelines of the EU directive 2010/63/EU.

2.2. Noise exposure

When awake, individual rat pups were exposed to a broad-band noise at an intensity of 125 dB SPL for 8, 12 or 25 min on postnatal day 14 (P14). Noise exposure was performed in a specially constructed anechoic box with inner dimensions of 24 × 24 × 34 cm. During the exposure to noise, the animal was placed in a round wire mesh cage (diameter of 17 cm, height of 10 cm, situated in the center of the exposure box) to prevent them from occluding the ear canal.

Broad-band noise was generated using a RFT 03 004 white noise generator, amplified with a custom-made power amplifier and presented via a loudspeaker (B&C Speakers DE700) coupled to a horn. The sound field within the cage was measured with a B&K 4939 microphone, a ZC0020 preamplifier and a B&K 2231 Sound Level Meter. Measurements of the sound intensity obtained at five points within the cage were found to vary by less than 1.5 dB. The frequency spectrum of the noise, which is measured in the center of the exposure box, was flat in the frequency range of 800 Hz–20 kHz, varying by less than ±5 dB [19].

2.3. Measurement of ABR hearing thresholds

The auditory brainstem responses (ABRs) to tonal stimuli (3 ms duration, 1 ms rise/fall times) were recorded in 9 animals of the control group, 6 animals exposed for 8 min, 5 animals exposed for 12 min and 3 animals exposed for 25 min. ABR was measured at frequencies 2, 4, 8, 16 and 32 kHz with the aim to assess hearing thresholds of animals which were previously tested behaviorally using the startle procedure. The procedure of ABR recording was described in detail previously [15]. To summarize, the recording was performed in animals lightly sedated using an intramuscular injection of 0.03 mg/kg of medetomidin hydro-chloride (Domitor, Farmos). ABRs were recorded with subcutaneous needle electrodes placed at the vertex (active electrode) and inside the neck muscles (reference electrode); the signal was processed with a TDT system III setup using BioSig software.

Tonal stimuli were presented at a repetition rate of 10 Hz and at least 250 responses were collected at each sound intensity, changing by a step size of 5 dB. The threshold at each frequency was determined independently by two or three of the authors of the paper (with the exception of one, all were blinded to the animal group while evaluating ABRs) as the minimal tone intensity that still evoked a visually noticeable potential peak within the time window of 10 ms after tonal stimulation.

2.4. Startle apparatus and procedures

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 placed in a small wire mesh cage (160 × 85 × 90 mm) positioned on a motion-sensitive platform. The animal's reflex movement was detected and transduced by a piezoelectric accelerometer. The amplified voltage signal was acquired and processed using a TDT system III with Real-Time Processor RP 2 (Tucker Davis Technologies, Florida, USA) and controlled by a PC computer. The startle responses were evaluated in a 100 ms window beginning from the onset of the startle stimulus. The magnitude of ASR was given by the maximal peak-to-peak amplitude of transient voltage occurring in the response window. Acoustic startle stimuli and prepulse stimuli were generated by a TDT system and presented via a loudspeaker (SEAS, 29AF/W) placed inside the chamber. Stimulus presentation and data acquisition were controlled by a custom-made application in a 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 test cage where the animal's head would be during the experiment.

Behavioral testing was performed in rats aged between 3 and 5 months (250–350 g). During one session, a continuous background tone of a constant frequency (either 4 or 16 kHz) and intensity (70, 80 or 90 dB SPL in the case of 4 kHz and 70, 80 or 85 dB SPL in the case of 16 kHz) was presented. A brief exposure to a pure tone with a modified frequency served as the prepulse stimulus and was followed by a startle stimulus (broad-band noise burst, 50 ms duration, 115 dB SPL) with a delay of 50 ms relative to the end of the frequency change. During the prepulse stimulus, the frequency of the background tone F increased to the value of $F + \Delta F$ for 50 ms with the rise time of 1.5 ms and then returned to its original value F with the fall time of 1.5 ms while the intensity remained unchanged (Fig. 1).

Every session started with an acclimation period of at least 2 min while background noise was presented, which was followed by 3–5 trials with only startle stimuli without any PPI (i.e. $\Delta F = 0$; those trials were not included into results) and then responses to startle stimuli preceded by PPI were collected. Individual values of ΔF = 0, 2, 5, 10, 15 and 30% were presented in a random order and also with a random inter-trial interval, which varied from 15 to 40 s. Each ΔF was tested at least 8 times in one session (with the exception of one session in 2 animals with only 5 repetitions in a session).

The magnitude of the startle response obtained for $\Delta F = 0$ (i.e. no prepulse stimulus) served as the reference value (100%) of the startle response. The mean ASR amplitude of each trial type was calculated as an average of all the ASR amplitudes for that given trial type. The efficacy of the PPI of ASR was expressed as: PPI = [1 − (amplitude of ASR inhibited by the prepulse) / (amplitude of ASR alone)] × 100%.

A two-way RM ANOVA and Bonferroni's multiple comparisons test were used to determine if the differences among PPI curves and ABR thresholds were significant. The minimal frequency changes that caused significant inhibition of the ASR were identified using a one sample t-test against zero. All statistical tests were performed in GraphPad Prism (GraphPad Software, La Jolla, CA).

In behavioral studies the frequency resolution is characterized by the difference limen, which is typically determined as the frequency