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Research report

Development of the acoustic startle response in rats and its change after early acoustic trauma

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HIGHLIGHTS

- Early noise exposure on P14 elicited transient hyper-reactivity for 2 weeks.
- Startle responses in exposed rats had decreased thresholds and increase amplitudes.
- The number of ribbon synapses, but not hair cells, decreased significantly.
- Altered startle responses persisted into adulthood even in rats with normal thresholds.

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ABSTRACT

Even brief acoustic trauma during the critical period of development that results in no permanent hearing threshold shift may lead to altered auditory processing in adulthood. By monitoring the acoustic startle response (ASR), we examined the development of auditory function in control rats and in rats exposed to intense noise at the 14th postnatal day (P14). First ASRs appeared on P10–P11 to intense low-frequency tones. By P14, the range of sound intensities and frequencies eliciting ASRs extended considerably, the ASR reactivity being similar at all frequencies (4–32 kHz). During the subsequent two weeks, ASR amplitudes to low-frequency stimuli (4–8 kHz) increased, whereas the ASRs to high-frequency tones were maintained (16 kHz) or even decreased (32 kHz). Compared to controls, noise exposure on P14 (125 dB SPL for 8, 12, or 25 min) produced transient hyper-reactivity to startle stimuli, manifested by a decrease of ASR thresholds and an increase of ASR amplitudes. ASR enhancement occurred regardless of permanent hearing loss and was more pronounced at high frequencies. The hyper-reactivity of ASRs declined by P30; the ASR amplitudes in adult exposed rats were lower than in controls. The histological control did not reveal loss of hair cells in adult exposed rats, however, the number of inner hair cell ribbon synapses was significantly decreased, especially in the high-frequency part of the cochlea. The results indicate that early acoustic trauma may result in complex changes of ASRs during development.

1. Introduction

Noise exposure that occurs during the critical period of postnatal development represents a risk factor for the proper development of auditory function and may result in pathological changes in adulthood. Numerous experiments in animals have shown that the severity of the consequences of noise exposure during early ontogenesis depends on the degree of maturity of the auditory system at the time of acoustic trauma [1–3]. Alterations of the afferent neuronal activity during the sensitive developmental period (resulting, e.g., from cochlear impairment) affect the maturation of higher levels of the auditory system and may thus further aggravate primary disorders caused by noise exposure [4–6]. The rat is a suitable model for studying the effects of early noise exposure on the formation of the auditory system because rat pups are born immature and their hearing develops fully during the first several postnatal

Abbreviations: ABR, auditory brainstem response; ASR, acoustic startle response; CF, cochlear frequency; IHC, inner hair cell; OHC, outer hair cell; P, postnatal day.

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weeks. Rats begin to hear at P10–P12 and maturation of their hearing function proceeds up to the 4th–6th postnatal week [7–10]. Our previous results demonstrated that brief noise exposure (125 dB SPL, 8 min) of rat pups at the P14, producing only a temporary threshold shift, elicited permanent changes in the responsiveness of neurons in the inferior colliculus, affecting frequency selectivity and intensity coding [11,12]. The study of the auditory behavior in adult rats after such acoustic trauma demonstrated changes in the animal's startle reactivity to sounds reflecting an abnormality in loudness perception compared to unexposed rats [13]. In addition, changes in the function of the auditory system were found to be accompanied by morphological changes of neurons observed in the inferior colliculus, medial geniculate body and auditory cortex [14].

The purpose of the present study was to examine pathological changes in the rat auditory system after early acoustic trauma of varying severity using recordings of the acoustic startle response (ASR). We monitored normal development of the ASR in rat pups during the first two weeks after hearing onset and evaluated the developmental alterations of ASRs produced by an acoustic trauma occurring on P14. The ASR (a transient motor response to an intense and unexpected auditory stimulus) is a reflexive auditory behavior that does not require animal training and therefore can be used for studies of early ontogeny [15,16]. The structural basis of the ASR is formed by a short neural circuit comprising of the cochlear nerve, cochlear root neurons, posteroventral cochlear nucleus, an area ventral and medial to the ventral nucleus of the lateral lemniscus, the nucleus reticularis pontis caudalis, and motoneurons in the spinal cord [17,18]. The response to startling acoustical stimuli is readily quantifiable and easily measured. Thus, the study of the developmental and/or noise-induced pathological changes of ASRs in rat pups may provide additional information for understanding how an early sensory defect influences subsequent sensory processing. Similar to our previous studies [11–13], the rat pups were exposed on P14 to 125 dB SPL broad-band noise for either 8 min, 12 min or 25 min, with the aim to induce hearing impairments of different severity. The ASR was recorded from P10 to P30 and also in adult, 2-month old animals. In 2-month old animals, hearing thresholds, based on auditory brainstem response recordings (ABR), were measured with the aim of assessing the final level of the noise-induced hearing loss. Thereafter, cochlear histology was performed to test whether there were any peripheral pathological changes that might influence neural input to the central auditory system.

2. Materials and methods

2.1. Subjects

In total, 40 female rat pups (strain Long-Evans) were used in the experiment. The rat pups were divided into 4 groups (10 rats in each group): one unexposed group of rat pups served as controls, the other three groups were exposed to broadband noise of 125 dB SPL for either 8, 12 or 25 min on P14. In all animal groups, ASRs to tonal stimuli were measured daily during the period from P10 to P30. Additional ASR tests, the evaluation of hearing thresholds by means of auditory brainstem response (ABR) recordings and cochlear histology were performed in rats at 2 months of age. Rat pups were housed with their mother up to P30 and were then separated. Animals were kept under standard laboratory conditions in a constant environment (12/12 h light/dark cycle), with food and water available *ad libitum*. All experimental procedures were conducted during the light phase of the cycle. The care and use of the animals was approved by the Ethics Committee of the Institute of Experimental Medicine AS CR and followed the guidelines of the EU directive 2010/63/EU.

2.2. Noise exposure

Awake rat pups at P14 were exposed individually to broadband noise at 125 dB SPL for either 8, 12 or 25 min in a specially constructed anechoic box with inner dimensions $24 \times 24 \times 34$ cm, supplied with a loudspeaker (B&C Speakers DE700) coupled to a horn. During the exposure to noise, the animal was placed in a round wire mesh cage (diameter 17 cm, height 10 cm), situated in the center of the exposure box.

The broad-band noise was generated with a RFT 03 004 noise generator and amplified with a custom-made power amplifier. 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 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, measured from the center of the exposure box, was flat in the frequency range 800 Hz–20 kHz; the intensity varied by less than ± 5 dB.

2.3. Apparatus and behavioral procedure

ASRs were measured in a sound attenuated chamber (Coulbourn Habitest, model E10-21) localized in a soundproof room. During the testing procedure, the rat was confined to a small wire mesh cage on a motion-sensitive platform. Three different sizes of cages corresponding to the size of the tested rats were used (two cage sizes for growing rat pups and the third cage size for adult rats). The animal's reflex movements were detected and transduced by a piezoelectric accelerometer. An amplified voltage signal was acquired and processed using a TDT system III with Real-Time Processor RP 2 (Tucker Davis Technologies, Alachua, Fl) and custom-made software in a Matlab environment. The startle responses were evaluated in a 100 ms window beginning from 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. The startle stimuli (tone pips and noise bursts) were generated by the TDT system (Real-Time Processor RP 2) 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 4 kHz and 32 kHz by a 1/4 inch 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 at the location of the animal's head in the test cage.

The ASRs to 4, 8, 16 and 32 kHz tone pips of varying intensities (50 ms duration, 3 ms rise/fall times) were recorded. The first test session began after a 3 min acclimatization period which contained ASR recordings to a series of noise bursts with increasing intensity (from 55 to 115 dB SPL) that served also as an approximate assessment of the startle threshold level. Each test session consisted of three blocks including startle trials of the same frequency and different intensity in the range of 60–115 dB SPL and a pseudostartle trail (without the startle stimulus) for the assessment of background activity. Startling stimuli were presented in a random order with inter-trial intervals of 10–30 s; intervals between blocks were 30 s long. The reduced ASR recording procedure (each intensity was tested only three times in a session in contrast to five to ten tests usually performed) was used in order to minimize the traumatic effects of high intensity stimulation and to reduce the maternal separation of the pups as much as possible. Nevertheless, the results of our pilot study showed that in rat pups from 11 to 25 days of age, the ASRs were stable, habituation effects were very weak and no motion artifacts were observed. In addition, little or no ASR habituation was demonstrated in 18–21 day-old rats by Hamilton and Timmions [19]. ASR thresholds for each test frequency were