Physiology & Behavior 102 (2011) 453–458

Contents lists available at ScienceDirect

Physiology & Behavior

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

Noise exposure during early development influences the acoustic startle reflex in adult rats

Natalia Rybalko [a,*], Zbyněk Bureš [a,b], Jana Burianová [a], Jiří Popelář [a], Jolana Grécová [a], Josef Syka [a]

[a] Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[b] College of Polytechnics, Jihlava, Czech Republic

ARTICLE INFO

Article history:
Received 16 March 2010
Received in revised form 24 September 2010
Accepted 8 December 2010

Keywords:
Auditory system
Startle reflex
Prepulse inhibition
Behavioral
Noise exposure
Critical period
Development

ABSTRACT

Noise exposure during the critical period of postnatal development in rats results in anomalous processing of acoustic stimuli in the adult auditory system. In the present study, the behavioral consequences of an acute acoustic trauma in the critical period are assessed in adult rats using the acoustic startle reflex (ASR) and prepulse inhibition (PPI) of ASR. Rat pups (strain Long–Evans) were exposed to broad-band noise of 125 dB SPL for 8 min on postnatal day 14; at the age of 3–5 months, ASR and PPI of ASR were examined and compared with those obtained in age-matched controls. In addition, hearing thresholds were measured in all animals by means of auditory brainstem responses. The results show that although the hearing thresholds in both groups of animals were not different, a reduced strength of the startle reflex was observed in exposed rats compared with controls. The efficacy of PPI in exposed and control rats was also markedly different. In contrast to control rats, in which an increase in prepulse intensity was accompanied by a consistent increase in the efficacy of PPI, the PPI function in the exposed animals was characterized by a steep increase in inhibitory efficacy at low prepulse intensities of 20–30 dB SPL. A further increase of prepulse intensity up to 60–70 dB SPL caused only a small and insignificant change of PPI. Our findings demonstrate that brief noise exposure in rat pups results in altered behavioral responses to sounds in adulthood, indicating anomalies in intensity coding and loudness perception.

1. Introduction

The effect of early experience on the development of brain sensory functions is well known [1–3]. Numerous studies have demonstrated that alterations of cochlear output resulting from a transient or permanent hearing loss during a sensitive developmental period affect the development of the central auditory system [4–6]. Consequences of neonatal hearing loss leading to auditory deprivation during early ontogenesis depend on the maturity of auditory system at the time of traumatic exposure [7–9]. To study the effects of early deprivation on the formation of the auditory system, the rat often serves as a suitable animal model, as rat pups are born with an immature auditory system. The first reflex responses to acoustic stimuli in rats appear on postnatal day 10 (P10); later on, the auditory brainstem response (ABR) thresholds improve rapidly, approaching adult values between days 24 and 36. The maturation of the rat hearing function then proceeds up to the 4th–6th postnatal week [10–12]. Anomalous processing of auditory information in the higher parts of the auditory system (alterations in the frequency tuning and tonotopy) in rats exposed to sound in early ontogeny has been demonstrated in a number of electrophysiological studies [6,13–18]. In an immunohistochemical study of Pierson and Snyder-Keller [4], it was demonstrated that a brief acute acoustic trauma (125 dB SPL noise for 8 min) in rat pups on the 14th postnatal day leads to changes in the tonotopic pattern of pure tone responses in the inferior colliculus (IC) in adult rats. Diffuse non-structured patterns of Fos immunoreactivity were observed within the ventral portion of the IC (with normally structured pattern responses within the dorsal area), indicating the immature state of the high frequency tonotopic projections in the IC. In our previous electrophysiological experiments, we used the model of Pierson and Snyder-Keller (1994) and showed that an 8 minute exposure to 125 dB SPL on P14 in rats results in permanent alterations of frequency and intensity representation in the high-frequency IC neurons [17,18]. On the basis of these findings, we investigated whether the reported alterations of neuronal responsiveness are also accompanied by abnormal behavioral responses to sound. The acoustic startle reflex (ASR) was chosen as an indicator of the behavioral responsiveness to sound stimuli. The startle reflex – a transient motor response to an intense unexpected sensory stimulus – is a primitive behavior observed in all mammals. The structural basis of the startle reflex is represented by a short neural circuit comprising the posteroventral cochlear nucleus, one or more nuclei of the lateral lemniscus, the caudal pontine reticular

* Corresponding author. Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Videňská 1083, 142 20 Prague 4, Czech Republic. Tel.: +420 24106 2689; fax: +420 24106 2787.
E-mail address: rybalko@biomed.cas.cz (N. Rybalko).

0031-9384/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2010.12.010

nucleus, spinal interneurons and spinal motor neurons [19,20]. In spite of its relative simplicity, the startle reflex shows several forms of behavioral plasticity including habituation, sensitization, and prepulse inhibition, and reveals remarkable sensitivity to a variety of experimental manipulations [20–23]. Investigations of the auditory function frequently employ the ASR and prepulse inhibition (PPI) of the ASR, i.e. the inhibition of the ASR induced by the presentation of an acoustic stimulus shortly preceding the startling sound. The PPI phenomenon is considered to be an example of sensori-motor gating, which reflects inhibitory processes that regulate sensory input to the brain. Results of animal studies have suggested that the effect of auditory prepulse stimulus on the startle circuit involves primarily the cochlear nucleus, the inferior and superior colliculus and the pedunculopontine tegmental nucleus [20,24]. PPI can be modulated by auditory cortex, thalamus, amygdala, hippocampus, striatum, ventral pallidum, and globus pallidum [24]. It was shown that evaluating the PPI of ASR is a simple yet efficient method for estimating supra-threshold auditory sensitivity [22,25,26].

With the aim of determining the behavioral consequences of acoustical trauma on the developing auditory system, the ASR and PPI of ASR were investigated in adult rats that were exposed to broad-band noise of 125 dB SPL for 8 min on P14. The obtained results were compared to those of age-matched controls. Hearing sensitivity in the exposed and control adult rats was assessed by means of auditory brainstem responses.

2. Materials and methods

2.1. Subjects

Fourteen female pigmented rats of the Long Evans strain with no primary pathology were used as experimental subjects. The animals were divided into two groups: 7 rat pups were briefly exposed to a broad-band noise of 125 dB SPL for 8 min on P14, the other 7 rats, used as controls, were manipulated similarly as the first group but without the noise exposure. All animals were housed under standard laboratory conditions in a constant environment and a 12/12 h normal light/dark cycle; food and water were available *ad libidum*. The rats were tested at 3–5 months of age in the daytime. The care and use of animals were approved by the Ethics Committee of the Institute of Experimental Medicine and followed the guidelines of the Declaration of Helsinki.

2.2. Noise exposure of rat pups

Awake rat pups at P14 were exposed individually to broad-band noise at 125 dB SPL for 8 min in a specially constructed anechoic box with inner dimensions $24\times24\times34$ cm, supplied with a loudspeaker (B&C Speakers DE700) and coupled to a horn. The broad-band noise was generated with a RFT 03 004 white 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 measured in the center of the exposure box is shown in Fig. 1. 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) to prevent the animal from occluding the ear canal.

2.3. Apparatus and procedures

The ASR and PPI of ASR were measured in seven female rats (3–5 months, 250–350 g) exposed to broad-band noise on P14, and in seven age-matched control rats. 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 ($160\times85\times90$ 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 Real-Time Processor RP 2 (Tucker Davis Technologies, Florida, USA) and custom-made software in a Matlab environment. The startle responses were evaluated in a 100 ms window beginning at 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 (tone pips or noise bursts) and prepulse stimuli (tone pips) were generated by the TDT system and presented via 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 location of the animal's head in the test cage.

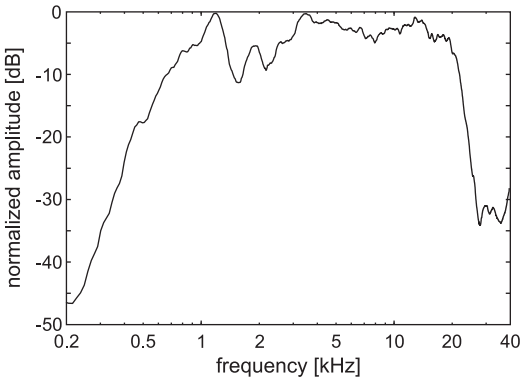

Fig. 1. Spectrum of the noise used for noise exposure.

2.3.1. Measurement of acoustic startle reflex

The ASR to 2, 4, 8, and 16 kHz tone pips and to 1.5–45 kHz broad-band noise (50 ms duration, 5 ms rise/fall times, varying levels) was recorded. Each test session contained 7 trial types: startle stimuli of different intensities (70, 80, 90, 100, 110, and 120 dB SPL) and a baseline trial without the startle stimulus, presented in a random order. Each trial type was presented ten times. The average inter-trial interval varied from 15 to 40 s. The mean ASR amplitude of each trial type was calculated as an average of all the ASR amplitudes for that given trial type with the highest and the 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 0.05 V). Thereafter, the ASR "threshold" was determined as the minimum startle intensity at which there was a startle reaction for at least 50% of the trials.

2.3.2. Measurement of prepulse inhibition of ASR

In the prepulse inhibition procedure, 10 different trial types were used: acoustic startle pulse alone (white noise at 110 dB SPL, 50 ms, 5 ms rise/fall times), 8 combinations of the startle pulse and prepulse tones (50 ms duration, 5 ms rise/fall time) at different intensities (10, 20, 30, 40, 50, 60, 70, and 80 dB SPL), and a baseline trial (without acoustic stimulus). The inter-stimulus interval between the prepulse and startle stimulus was set to 50 ms. Each of the ten trial types was presented ten times. The average inter-trial interval varied from 15 to