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The effect of parvalbumin deficiency on the acoustic startle response and prepulse inhibition in mice

Jiří Popelář[a,*], Natalia Rybalko[a], Jana Burianová[a], Beat Schwaller[b], Josef Syka[a]

[a] Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Department of Auditory Neuroscience, 14220 Prague, Czech Republic
[b] Anatomy, Department of Medicine, University of Fribourg, CH-1700 Fribourg, Switzerland

HIGHLIGHTS

- PV−/− mice had smaller ASR amplitudes in response to relatively weak startling stimuli (80–90 dB SPL) in comparison with PV+/+ mice.
- PPI of the ASR in PV−/− mice was less effective than in PV+/+ mice.
- Mean ABR audiograms were found to be similar in both genotypes.

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ABSTRACT

The strength of the acoustic startle response (ASR) to short bursts of broadband noise or tone pips (4, 8 and 16 kHz) and the prepulse inhibition (PPI) of the ASR elicited by prepulse tones (4, 8 and 16 kHz) were measured in parvalbumin-deficient (PV−/−) mice and in age-matched PV+/+ mice as controls. Hearing thresholds as determined from recordings of auditory brainstem responses were found to be similar in both genotypes. The ASRs to broadband noise and tones of low and middle frequencies were stronger than the ASRs in response to high-frequency tones in both groups. In PV−/− mice, we observed smaller ASR amplitudes in response to relatively weak startling stimuli (80–90 dB sound pressure level (SPL)) of either broadband noise or 8-kHz tones compared to those recorded in PV+/+ mice. For these startling stimuli, PV−/− mice had higher ASR thresholds and longer ASR latencies. PPI of the ASR in PV−/− mice was less effective than in PV+/+ mice, for all tested prepulse frequencies (4, 8 or 16 kHz) at 70 dB SPL. Our findings demonstrate no effect of PV deficiency on hearing thresholds in PV−/− mice. However, the frequency-specific differences in the ASR and the significant reduction of PPI of ASR likely reflect specific changes of neuronal circuits, mainly inhibitory, in the auditory centers in PV-deficient mice.

1. Introduction

Parvalbumin (PV) belongs to a large family of EF-hand calcium-binding proteins, which comprises more than 240 members in man [21]. Studies on calcium-binding protein functions indicate that these proteins are essential in Ca^{2+} homeostasis and for the modulation of short-lived changes in the intracellular Ca^{2+} concentration called Ca^{2+} transients. For PV, this is the case in fast-twitch muscles [23] and in a specific subpopulation of neurons [2], where PV is implicated in the subtle regulation and timing of Ca^{2+} signals pre- and postsynaptically. In the brain, PV is almost exclusively present in subpopulations of inhibitory GABAergic interneurons in different brain regions including the neocortex, cerebellum, hippocampus and the reticular nucleus of the thalamus [2–4,18]. In the auditory system PV is expressed in cochlear hair cells [11] and in globular bushy cells' endings in the large calyx of Held on the principal cells in the medial nucleus of the trapezoid body [8]. A large number of PV-immunoreactive neurons are present in all subnuclei of the inferior colliculus, PV-positive neurons are scattered throughout layers II–VI in the auditory cortex and sparse small, oval PV-positive neurons are scattered in both the dorsal and ventral divisions of the medial geniculate body [19]. In the case of the medial geniculate body the distribution of GABA-ergic neurons is highly species-specific: the proportion ranges from <1% in the bat and rat to about 25% in the cat and monkey [30]. However, only limited information exists on the role of PV in the auditory system. Several recent studies have reported age-related and region-specific changes in the expression of PV-positive neurons in a variety of mammalian species: an age-related decline of calcium binding protein-positive neurons in the dorsal cochlear nucleus of CBA/CaJ mice [13], a

* Corresponding author at: Institute of Experimental Medicine AS CR, Department of Auditory Neuroscience, Videnska 1083, 14220 Prague 4, Czech Republic. Tel.: +420 241062689; fax: +420 241062787.
E-mail addresses: jpopelar@biomed.cas.cz (J. Popelář), rybalko@biomed.cas.cz (N. Rybalko), burianova@biomed.cas.cz (J. Burianová), Beat.Schwaller@unifr.ch (B. Schwaller), syka@biomed.cas.cz (J. Syka).

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decrease in the percentage of PV-expressing neurons in the superficial layers of the auditory cortex of C57Bl/6 mice [17] or an increase of PV-positive neurons in the inferior colliculus of old Long Evans rats and a pronounced decline in the number of PV-positive neurons in the auditory cortex of aged Fischer 344 rats [19]. The assessment of the function of PV in mice has been facilitated by using genetically modified mice deficient for this protein [23]. The results obtained in in vitro studies using PV−/− mice suggest that PV has evolved as a functionally distinct, physiologically relevant modulator of intracellular Ca^{2+} transients [22,24,25]. Behavioral tests performed in PV−/− mice revealed subtle alterations of locomotor function in comparison with PV+/+ mice, characterized by a slightly increased motor activity, decreased exploratory activity and increased microlinearity of movements [7]. However, the functional consequences of PV deficiency in terms of auditory perception have not been studied until now.

In the present study, PV−/− mice were used to investigate, whether the loss of PV has an effect on auditory behavior, in which both sensory and motor components play important roles. The strength of the acoustic startle reflex (ASR) and the auditory prepulse inhibition (PPI) were evaluated in PV−/− mice and compared with those observed in age-matched PV+/+ mice. The ASR, defined as a transient motor response to an intense, unexpected stimulus, was used as an indicator of the behavioral responsiveness to loud sounds. The assessment of auditory PPI, i.e. the suppression of the ASR by a prepulse preceding the startling stimulus, was used as an indicator of possible changes in inhibitory function. PPI is considered to be a form of sensorimotor gating that reflects a basic inhibitory process that regulates sensory input to the brain [28]. In addition, measurements of the auditory threshold, which can influence ASR and PPI, were performed by recording auditory brainstem responses.

2. Materials and methods

2.1. Animals

Parvalbumin-deficient mice (PV−/−) were generated by homologous recombination [23] and backcrossed to C57BL/6 mice for >10 generations and are considered to be congenic to C57Bl/6 mice [18]. PV−/− mice ($n = 9$, mean body weight 29.0 ± 1.6 g) obtained directly from the animal facility of the University of Fribourg were genotyped by PCR [23] to validate the inactivation of the *Pvalb* gene. Animals were tested at five months of age and the results were compared with age-matched C57BL/6 wild type males (PV+/+; $n = 10$, mean body weight 30.1 ± 1.4 g).

Mice were housed under standard conditions on a 12 h/12 h light/dark cycle. 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 European Community Directive 86/609/EEC.

2.2. Behavioral tests

All behavioral tests were performed in a ventilated sound-attenuated chamber (Coulbourn Habitest, model E10-21) located in a soundproof room. During the testing procedure, mice were confined to a small wire mesh cage ($85 \times 45 \times 45$ mm) on a motion-sensitive platform. The whole-body startle responses were detected and transduced by a piezoelectric accelerometer (Coulbourn E45-11B). The amplified voltage signal was acquired and processed using a RP2.1 enhanced real-time processor (Tucker Davis Technologies systemIII, Alachua, FL). The startle responses were evaluated in a 100-ms window beginning at the onset of the startle stimulus. Acoustic startle stimuli (tone pips or noise bursts) and prepulse stimuli (tone pips) were generated by the RP2.1 enhanced real-time processor and presented via a loudspeaker (SEAS, 29AF/W) placed 12 cm above the platform. ASRs to 4, 8 and 16 kHz tone pips and broadband noise bursts (with a relatively flat frequency spectrum between 2 and 35 kHz) of 50-ms duration and varying intensity levels were recorded. In the ASR experiments, each session contained 9 trial types: a baseline trial without any stimulus and 8 startle stimuli of different intensities (50–120 dB SPL with a step size of 10 dB) presented in a random order at intervals of 10–30 s; each trial type was presented ten times. The ASR threshold, the ASR amplitude and the latency of the ASR to 110 dB SPL sound were analyzed.

In the PPI testing, three trial types were used: a baseline trial without any stimulus, a startling pulse alone (115 dB SPL broadband noise burst of 50 ms), and a combination of the startle pulse and 50-ms prepulse tones of 70 dB SPL, at frequencies of 4, 8 and 16 kHz. The interval between the prepulse and startling stimuli was 50 ms. All trial types were presented 10 times in pseudo-random order separated by 15–30 s. The efficacy of the PPI of ASR was expressed as: PPI% = (amplitude of the ASR suppressed by the prepulse tone/amplitude of the ASR alone) × 100. Thus, a PPI of 100% means no ASR suppression, a PPI of 0% corresponds to complete suppression of the ASR.

2.3. Auditory brainstem response recordings

The hearing threshold in mice was assessed on the basis of auditory brainstem response recordings using subcutaneous needle electrodes placed on the vertex (an active electrode) and in the neck muscles (ground and reference electrodes). Auditory brainstem responses were elicited using short-tone bursts (3-ms duration, 1 ms rise/fall times, frequency range 4–32 kHz) generated with a RP2.1 enhanced real-time processor. Acoustic stimuli were delivered in free-field conditions via a two-way loudspeaker system [Jamo® woofer (Denmark) and SEAS® T25CF 002 tweeter (Norway)] placed 70 cm in front of the animal's head. The acoustic system was calibrated with a Bruel&Kjaer® 4939 microphone, a ZC0020 preamplifier and a B&K 2231 Sound Level Meter. The frequency–response curve of this system was relatively flat and varied by less than ±9 dB between 0.15 and 40 kHz. The signal from the electrode was amplified 10,000-times, band-pass filtered over the range of 300 Hz–3 kHz, processed with a RX5-2 Pentusa Base Station (Tucker Davis Technologies systemIII, Alachua, FL) and analyzed using BioSig software. The response threshold to each frequency was determined as the minimal tone intensity that still evoked a noticeable potential peak in the expected time window of the recorded signal.

2.4. Statistical analysis

The differences between hearing thresholds and ASR amplitudes in PV−/− mice and PV+/+ mice were tested using two-way ANOVA with the Bonferroni post-test. The differences between ASR thresholds, ASR latencies and PPI of ASR at individual frequencies were tested using an unpaired *t*-test.

3. Results

3.1. Hearing thresholds

Hearing thresholds in PV−/− mice tended to be slightly lower (on average by 5–10 dB) than the thresholds in PV+/+ mice, but the differences were not significant ($p > 0.05$, two-way ANOVA, Bonferroni post-test). The average hearing thresholds in 5-month-old