

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

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H I G H L I G H T S

- 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.

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 Ca²⁺ homeostasis and for the modulation of short-lived changes in the intracellular Ca²⁺ concentration called Ca²⁺ 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 Ca²⁺ 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

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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 Ca²⁺ 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 micro-linearity 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: $PPI\% = (\text{amplitude of the ASR suppressed by the prepulse tone} / \text{amplitude of the ASR alone}) \times 100$. 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

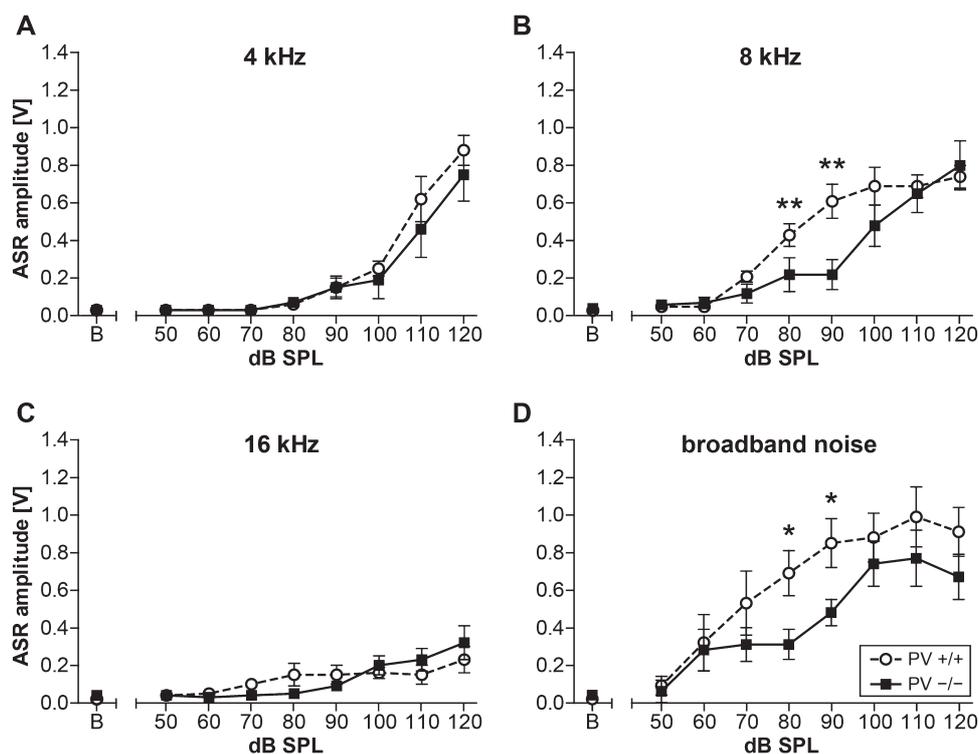


Fig. 1. Mean ASR amplitude vs. sound intensity functions in PV^{-/-} and PV^{+/+} mice obtained for tone pips of 4, 8, and 16 kHz, as well as for broadband noise bursts. Bars represent \pm SEM; "B"- baseline trial; * $p < 0.05$, ** $p < 0.01$, two-way ANOVA with the Bonferroni post-test.

PV^{-/-} and PV^{+/+} mice are shown in Supplementary material in Fig. S1.

3.2. Acoustic startle reflex (ASR)

The dependence of the ASR amplitude on the startling stimulus intensity for PV^{-/-} and PV^{+/+} mice is shown in Fig. 1.

The ASRs to broadband noise and tones of 4 and 8 kHz (Fig. 1A, B and D) were generally stronger than the ASRs to 16-kHz tones (Fig. 1C) in all mice. The differences between the ASRs in PV^{-/-} and PV^{+/+} mice were clearly evident in response to broadband noise and to tones of 8 kHz: the ASR amplitudes evoked by these startling stimuli were smaller in PV^{-/-} than in PV^{+/+} mice (Fig. 1B and D), but significant differences were only observed at 80 and 90 dB SPL ($p < 0.05$ and $p < 0.01$, respectively, two-way ANOVA with the Bonferroni post-test). In addition, PV^{-/-} mice had higher ASR thresholds than did PV^{+/+} animals, but differences were significant only at 8 kHz (78.9 ± 6.0 dB SPL vs. 68.0 ± 4.7 dB SPL, resp., $p < 0.01$, unpaired t -test) and 16 kHz (87.5 ± 4.2 dB SPL vs. 78.5 ± 6.6 dB SPL, resp.; $p < 0.05$, unpaired t -test).

The latencies of the first positive wave of the ASR evoked by 110-dB startle stimuli were analyzed (Table 1). PV^{-/-} mice exhibited longer ASR latencies in comparison to PV^{+/+} mice. Despite the fact that their amplitudes, evoked by 110 dB SPL startle stimuli, were similar, the differences in latencies were significant at 4 and

Table 1

Average latencies of the ASR in PV^{-/-} and PV^{+/+} mice (mean \pm SEM). * $p < 0.05$, ** $p < 0.01$, unpaired t -test.

Startle stimulus (110 dB SPL)	PV ^{-/-}		PV ^{+/+}
Broadband noise	25.0 \pm 0.6 ms	ns	25.1 \pm 0.6 ms
4 kHz	26.2 \pm 0.5 ms	**	25.3 \pm 0.4 ms
8 kHz	25.9 \pm 0.9 ms	*	24.7 \pm 0.7 ms
16 kHz	26.4 \pm 0.7 ms	ns	25.5 \pm 1.4 ms

8 kHz (* $p < 0.05$ and ** $p < 0.01$, resp., unpaired t -test). The ASR latencies evoked by broadband noise were similar in both experimental groups of mice.

3.3. Prepulse inhibition (PPI) of ASR

The effect of modifying the ASR amplitudes by a preceding stimulus was tested using PPI of ASR in mice of both genotypes. Fig. 2 shows the changes in the average relative ASR amplitudes evoked by 115 dB SPL broadband noise stimuli, using prepulse stimuli at 4, 8 and 16 kHz at an intensity of 70 dB SPL in PV^{-/-} and PV^{+/+} mice; a lower relative ASR amplitude corresponds to stronger PPI (from no inhibition at 100% to complete ASR inhibition at 0%). The results document a significantly less effective PPI in PV^{-/-} mice than in PV^{+/+} controls at all tested PPI frequencies (* $p < 0.05$, *** $p < 0.001$, unpaired t -test).

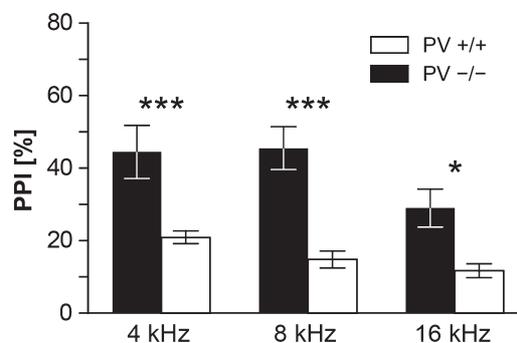


Fig. 2. The efficacy of PPI of ASR in PV^{-/-} and PV^{+/+} mice. The efficacy of the PPI of ASR was expressed as: PPI = (ASR amplitudes evoked by 115 dB SPL broadband noise stimuli and suppressed by the prepulse tone at 4, 8 or 16 kHz at an intensity of 70 dB SPL/amplitude of the ASR alone) \times 100%. A PPI of 0% corresponds to complete ASR suppression. * $p < 0.05$, *** $p < 0.001$, unpaired t -test, bars represent \pm SEM.

4. Discussion

The results of our study demonstrate that the absence of PV in PV^{-/-} mice altered ASR parameters and that these mice showed significantly less efficient prepulse inhibition of the ASR in comparison to PV^{+/+} mice with normal PV levels. The average hearing thresholds in PV^{-/-} mice were, however, comparable with those found in PV^{+/+} mice. The calcium-binding protein PV is present in both the inner and outer hair cells in the cochlea, and its role is to modulate Ca²⁺ entry to the hair cell cytoplasm during mechano-electrical transduction and afferent and efferent synaptic activity [10]. Due to the similarity of the hearing thresholds in PV^{-/-} and PV^{+/+} mice, the role of PV in Ca²⁺ buffering does not seem to be essential for the subject's hearing sensitivity near the threshold.

The ASR, as an unconditioned reflex reaction to an abrupt loud sound, is represented by a short neural circuit comprising the posteroventral cochlear nucleus, one or more nuclei of the lateral lemniscus, the caudal pontine reticular nucleus and spinal interneurons and motor neurons [5,15]. In spite of its relative simplicity, the ASR shows several forms of behavioral plasticity (habituation, facilitation, prepulse inhibition and modification by prior associative learning) that reflect the processing of acoustic information at higher levels of the auditory system than the neural circuit of the ASR itself [14,15,20]. These features of the startle reflex allow the study of not only the processing of auditory stimuli, but also of complex brain function, including movement control or cognitive disorders.

Our results indicate that the absence of PV induces "abnormalities" in the neural processes that finally result in changes of the startle reflex formation. The significantly longer ASR latency, found in our study in PV-deficient mice compared with PV^{+/+} mice, might be related to the prolongation of the time required to attain peak force and the increased force produced during a single muscle twitch [23], or it could be linked to a mild impairment in locomotor coordination that has been observed in PV^{-/-} mice [7]. The most distinct differences in ASR parameters, i.e. significantly higher ASR thresholds, lower ASR amplitudes and longer ASR latencies in PV^{-/-} mice in comparison with PV^{+/+} mice, were observed using 8-kHz tones. This is not surprising given that the hearing sensitivity was highest and the reflex response was more pronounced (especially in comparison with 16 kHz) in both genotypes at this frequency (Fig. S1, Fig. 1). These results suggest that ASR differences reflect not only parvalbumin deficiency in the skeletal muscles, but most likely also specific changes in the auditory neuronal circuits.

The PPI of ASR represents a basic inhibitory process regulating sensory inputs, and the magnitude of PPI can serve as a measure for the behavioral salience of perceptually-relevant stimuli [9,29]. Previous animal studies have shown that for PPI of ASR, several pathways are implicated including direct glutamatergic projections from the cochlear nuclei, inhibitory cholinergic projections from the pedunclopontine tegmental nucleus and indirect inputs from the superior and inferior colliculi [15]. Electrophysiological recordings of cortical, hippocampal and cerebellar neuronal activity in PV^{-/-} mice in vitro showed that the absence of PV in a subpopulation of GABA-ergic interneurons resulted in modification of the dynamics of inhibitory control (for reviews, see [22]), enhancement of the severity of pharmacologically-induced seizures [25], alterations of Purkinje cell-firing [26], and modulation of the firing properties of the reticular thalamic nucleus bursting neurons [1]. Fast-spiking PV-positive inhibitory cells, which represent the largest inhibitory subpopulation in the auditory cortex of the cat, are involved in the sharpening of frequency and intensity tuning and the shaping of spectral modulation preferences [27,31]. However, little is known so far about the influence of the missing parvalbumin on neuronal activity in the central auditory nuclei in PV^{-/-} mice. The results of animal studies have suggested that for

the PPI of ASR, the cochlear nucleus, the superior and inferior colliculi and the pedunclopontine tegmental nucleus are important [15,29], and PPI can be modulated by the auditory cortex, amygdala, hippocampus and striatum [29]. Our data clearly demonstrate that the effect of PPI on ASR is much weaker in PV^{-/-} mice than in controls. This could be linked to the decreased density of PV-positive inhibitory interneurons, possibly as the result of reduced PV expression levels, in the auditory nuclei of some mouse and rat strains during aging [13,19]. In line, in dystonic hamsters showing a transient decrease in PV-positive neurons and/or PV levels at approximately 30 days of age, ASR is decreased [11]. The low efficiency of PPI in PV^{-/-} mice could be also related to altered function of the cortico-tegmental pathways, which are under the strong influence of parvalbumin interneurons in the auditory cortex [6].

At the behavioral level, the testing of PPI of ASR has been used for detecting inhibitory alterations in various sensori-motor systems. A deficiency or even the absence of PPI of ASR was observed in mice with GABA_A receptor γ 2 subunits removed from the PV-positive neurons [16], in GAD65 knockout mice [12] and in dystonic hamsters [11].

In the present study, a reduced PPI was observed in PV^{-/-} mice at all prepulse stimulus frequencies, i.e. 4, 8 and 16 kHz. Because the hearing thresholds were similar in both genotypes, the observed differences with respect to auditory behavior (ASR and PPI of ASR) are not directly related to peripheral hearing sensitivity. Our data support the idea that the absence of PV in the central auditory nuclei is responsible for changes in the inhibitory system resulting in the reduction of PPI of ASR. For investigating specific changes in the central auditory system of PV^{-/-} mice, electrophysiological experiments and more sophisticated behavioral tests using conditioning procedures should be performed.

In conclusion, the results of our study demonstrate no effect of PV deficiency on the auditory periphery, resulting in unaltered hearing thresholds. The frequency-specific differences in the ASR and the significant reduction of PPI of ASR in PV-deficient mice likely reflect specific changes of neuronal circuits and/or circuit function, mainly inhibitory, in the auditory centers in PV-deficient mice.

Conflict of interest

The authors have declared that no conflict of interest exists.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version

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