Sensory and Motor Systems

Aging But Not Age-Related Hearing Loss Dominates the Decrease of Parvalbumin Immunoreactivity in the Primary Auditory Cortex of Mice

Meike M. Rogalla and K. Jannis Hildebrandt

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Department of Neuroscience, Division of Auditory Neuroscience, and Cluster of Excellence, Hearing4all, Carl von Ossietzky University, Oldenburg 26129, Germany

Abstract

Alterations in inhibitory circuits of the primary auditory cortex (pAC) have been shown to be an aspect of aging and age-related hearing loss (AHL). Several studies reported a decline in parvalbumin (PV) immunoreactivity in aged rodent pAC of animals displaying AHL and conclude a relationship between reduced sensitivity and declined PV immunoreactivity. However, it remains elusive whether AHL or a general molecular aging is causative for decreased PV immunoreactivity. In this study, we aimed to disentangle the effects of AHL and general aging on PV immunoreactivity patterns in inhibitory interneurons of mouse pAC. We compared young and old animals of a mouse line with AHL (C57BL/6) and a mutant (C57B6.CAST-Cdh23Ahl1) that is not vulnerable to AHL according to their hearing status by measuring auditory brainstem responses (ABRs) and by an immunohistochemical evaluation of the PV immunoreactivity patterns in two dimensions (rostro-caudal and layer) in the pAC. Although AHL could be confirmed by ABR measurements for the C57BL/6 mice, both aged strains showed a similar reduction of PV− positive interneurons in both, number and density. The pattern of reduction across the rostro-caudal axis and across cortical layers was similar for both aged lines. Our results demonstrate that a reduced PV immunoreactivity is a sign of general, molecular aging and not related to AHL.

Key words: age-related hearing loss; aging; mouse primary auditory cortex; parvalbumin

Significance Statement

Deficiency of sensory functions is one of the major detriments of aging. In hearing, aging affects both the periphery and inhibitory circuits in the central system, resulting in hearing loss and altered perception. Centrally, the major subclass of inhibitory interneurons [parvalbumin (PV)−] shows reduced PV immunoreactivity, which is believed to be related to altered inhibition. Identifying the factor that dominates this decline is important to understand molecular aging in the central auditory system. Here, we demonstrate that the decreased PV immunoreactivity in the primary auditory cortex (pAC) of mice is dominated by general aging rather than age-related hearing loss (AHL), suggesting that altered cortical inhibition in the auditory system may not be secondary to peripheral changes, but a consequence of aging per se worldwide, approximately affecting one third of adults above 65 years in forms of progressive loss of auditory function (Roth et al., 2011; Loughrey et al., 2018).

Introduction

Age-related hearing loss (AHL), also referred to as presbycusis, is one of the most common sensory impairments...
On the physiological level, the loss of hearing during aging is usually related to a loss of sensory hair cells or a decline of spiral ganglion cells (Frisina et al., 2016). Such age-related morphologic changes have not only been observed in humans, but also in laboratory rodents, especially in mice (Hequembourg and Liberman, 2001; McFadden et al., 2001; Li et al., 2002) which therefore have been suggested to be a suitable model of AHL (Gratton and Vázquez, 2003; Bowl and Dawson, 2015).

The decline of peripheral signal transmission has been linked to subsequent pathologic changes within the central nervous system, especially in the primary auditory cortex (pAC; Eckert et al., 2012). Alterations in inhibitory circuits of the pAC have been linked to hearing loss and the aged auditory system (Kotak et al., 2005; Takesian et al., 2012; Peelle and Wingfield, 2016). The largest class of cortical GABAergic interneurons, the parvalbumin (PV) neurons, are believed to play an important role when it comes to loss-of-input dependent changes in the neuronal inhibitory circuits of pAC. Several studies reported a relationship between AHL and a decline in PV immunoreactivity in the primary auditory cortex (de Villers-Sidani et al., 2010; Martin del Campo et al., 2012; Brewton et al., 2016), whereas others report a decline in PV immunoreactivity for several cortical areas, unrelated to any decline in sensory function (Miettinen et al., 1993; Ueno et al., 2018). Because of these contrary findings, it remains elusive whether a decline of PV immunoreactivity in pAC is a result of progressive AHL and the loss of input or if it is just a general phenomenon in the aging central (auditory) system.

In this study, we aimed to resolve the effect of aging and AHL on the PV immunoreactivity in the pAC of mice. To this end, we used a mouse line with a rapid, progressive development of AHL (C57BL/6) and the mutant C57BL/6.CAST-Cdh23<sup>ABH</sup>+, a congenic strain that carries the wild-type allele of Cdh23, producing C57BL/6 mice that are not vulnerable to AHL (Johnson et al., 1997; Keithley et al., 2004; Mock et al., 2016). We compared young and old animals of both lines according to their hearing status by using auditory brainstem response (ABR) measurements and an immunohistochemical evaluation of the PV immunoreactivity patterns in two dimensions (rostro-caudal and layer) of the pAC.

Materials and Methods

Experimental groups

All animal experiments were performed in accordance with the animal welfare regulations of Lower Saxony and with the approval from the local authorities (State Office for Consumer Protection and Food Safety/LAVES, permission number 33.9-43502-04-13/1271).

In total, 43 mice were used in this study, of which 21 were used for ABR measurements only, 15 in histology only, and seven animals in both.

Male C57BL/6J (stock number 017320; RRID: JAX:017320) mice were used to serve as an animal model with AHL. In contrast, male C57BL/6.CAST-Cdh23<sup>ABH</sup>+ (stock number 002756; RRID: JAX:002756) mice were used as an animal model without the development of AHL. Both strains were in-bred animals, originating from purchased breeding pairs (The Jackson Laboratory) and kept in small colonies with <i>ad libitum</i> access to water and food in the local animal facility under standardized conditions until terminal experiments.

The age groups were designed as follows: young animals (10–12 weeks) of both strains (young<sup>B6</sup>, n = 11) and young<sup>B6.CAST</sup>, n = 7) and aged animals (12–15 month) with AHL (aged<sup>B6</sup>, n = 13) and without (aged<sup>B6.CAST</sup>, n = 12; for details, see Table 1).

### Evaluation of hearing status, ABR

Animals were anesthetized with ketamine (initial 10 mg/kg, maintenance 2.5 mg/kg) and medetomidine (initial 0.083 mg/kg, maintenance 0.01 mg/kg). The state of anesthesia was checked in regular intervals, if needed anesthesia was topped up with the maintenance dose (typically every 1.5 h). Needle electrodes were placed subcutaneous with the recording electrode at the neck and the reference electrode at the vertex. The electrode signal was bandpass filtered (300 Hz to 30 kHz) and amplified by an ISO-80 Bio-amplifier (World Precision Instruments), before A/D-converted through a Fireface UC 24-bit sound device at a sample rate of 96 kHz. The same device was used for stimulus delivery. Stimuli were created by a custom MATLAB application. The sound was binaurally delivered directly into the ear canal of the animal through horns that were attached to Vifa/Peerless XT-300 K4 loudspeakers. The system was calibrated before recording using small microphones (Knowles FG-23329) that were inserted into replicas of mice ear canals.

In order to determine hearing thresholds at different tone frequencies, tone pips (10 ms, 2-ms cosine ramps at onset and offset) were presented binaurally at 4, 8, 12, 16, 20, 24, and 30 kHz. The intensity was varied between 35- and 95-dB SPL in steps of 5 dB. Interstimulus intervals were randomly chosen from the interval 50–150 ms. The sequence of stimuli was random. An automatic online artifact rejection algorithm discarded trials with muscle potential

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Table 1: Assignment of animals to the groups according to hearing status and age

| Group       | Age          | Histology | ABR | Double | Total |
|-------------|--------------|-----------|-----|--------|-------|
| Young<sup>B6</sup> | 10–12 weeks | 6         | 6   | 1      | 11    |
| Young<sup>B6.CAST</sup> | 10–12 weeks | 2         | 9   | 1      | 7     |
| Aged<sup>B6</sup> | 12–15 months | 8         | 7   | 2      | 13    |
| Aged<sup>B6.CAST</sup> | 12–15 months | 6         | 9   | 3      | 12    |
| Sum         | 22           | 28        | 7   | 43     |       |

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Correspondence should be addressed to Meike M. Rogalla at meike.rogalla@uni-oldenburg.de.

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artifacts according to a preset threshold. ABR data were recorded continuously and saved for offline evaluation.

**Histology**

Animals were injected with a lethal overdose of pentobarbital (Narcoren, Boehringer Ingelheim) and transcardially perfused with phosphate buffer (PB; pH 7.4) followed by fixative (4% paraformaldehyde in PB). The brains were removed and kept in immersion fixative over-night at 4°C, followed by four rinsing steps the next day (PB). For cryoprotection, brains were stored in 30% sucrose solution in PB for 2–3 d at 4°C. After complete saturation, brains were frozen using tissue freezing medium (TFM-5, TBS) and stored at −20°C.

Coronal frozen slices (25 μm) containing pAC were cut (CM 1950, Leica) in series of 4. A mark was placed subcortically into the right hemisphere using a cannula for poststaining distinctness. Slices of the first series were directly mounted on gelatin-coated object slides and air-dried for Nissl staining. Remaining slices of series 2–4 were stored in a 24-well plate/series, containing cryoprotection solution (30% ethylenglycol and 30% sucrose in PBS) and stored at −20°C until immunohistochemistry.

**Nissl staining**

The total number of cortical neurons may decrease during aging and this effect may be stronger in animals suffering from hearing loss. To account for this, an evaluation of the total number of neurons in the pAC, a standard Nissl protocol using 0.1% cresyl violet in watery solution was applied. After staining, object slides were rinsed in distilled water, followed by differentiation and complete dehydration with ascending alcohol concentrations. After clearing with xylol, object slides were coverslipped using rapid non-aqueous mounting medium (Entellan, 107960, Merck).

**PV immunohistochemistry**

The slices for visualization of PV⁺ interneurons were treated free floating. Slices of one series were rinsed 5 × 5 min in PBS (pH 7.4), followed by 10-min permeabilization in 0.5% Triton X-100 in PBS at room temperature. To prevent nonspecific binding, epitopes on the tissue were blocked with 10% normal goat serum in PBS for 1.5 h at room temperature, followed by 24-h incubation with a polyclonal primary antibody against PV produced in rabbit (1:800, rabbit anti-PV PV27, Swant Swiss Antibodies, RRID:AB_2631173) diluted in blocking solution at 4°C. After rinsing in PBS for 5 × 5 min, slices were incubated with the polyclonal secondary antibody produced in goat (1:200, goat anti-rabbit Alexa Flour 488, ab150077, Abcam) in blocking solution for 5 h at room temperature in darkness. Slices were rinsed 3 × 5 min in PBS and mounted on object slides (Superfrost Plus, Thermo Scientific) and coverslipped with anti-fade mounting medium (Vectashield H-1000, Vector Laboratories).

**Data analysis and statistics**

**ABR**

For every stimulus at least 400 artifact free trials were recorded. Thresholds at individual frequencies were determined by eye from the average stimulus-aligned traces at each frequency as the lowest level that evoked an ABR (Fig. 1A). If no response could be evoked up to 95 dB, threshold was set to 95 dB, which was the highest level we were able to present without distortions.

**Histology**

For quantitative analysis of the PV immunoreactivity in young and old animals (with and without AHL), pAC was photographed at 10 × magnification using an Axioskop 2 MOT Plus (Carl Zeiss Microscopy GmbH) equipped with a camera (Eos 7D, Canon). The pAC was identified by using anatomic landmarks (method adopted from Martin del
Campo et al. (2012) and a mouse brain atlas as reference (Allen Mouse Brain Volumetric Atlas 2012; https://mouse.brain-map.org). Images were acquired directly above the center by placing the pAC into the middle of the camera section (Fig. 2A). Images were exported as TIFF. Neurons and PV$^+$ neurons were counted within the region of interest (ROI; 400-μm height for PV slices and 50 μm for Nissl slices; Fig. 2B,C), which was placed above the center. We previously confirmed the position of auditory cortex in B6.CAST animals (Extended Data Fig. 2-1; Gothner et al., 2019). Counting was performed manually by a person who was blind to the experimental condition using the cell counter plugin in Fiji for non-automated quantification (Schindelin et al., 2012). Only neurons with a clearly identifiable soma were labeled as positive. In Nissl slices, only cells with the neuron-characteristics perikarya and soma staining were counted (Fig. 2C). Neurons which were touching the lateral (relative to cortex) border where included, whereas those touching the medial border of the ROI were excluded.

For each animal, at least eight slices (both hemispheres), distributed along the rostro-caudal axis were analyzed to calculate the mean number of PV$^+$ neurons. The depth of the cortex (from pia to white matter) was labeled in each slice to calculate the mean density of PV$^+$ neurons (neurons/mm$^2$). The total number of neurons (Nissl) was multiplied by the factor of eight and the mean served as a control for the evaluation of possible cell loss during aging and/or AHL.

All variables (number of neurons, number of PV$^+$ neurons and density of PV$^+$ neurons) were statistically analyzed (IBM SPSS Statistics version 25, IBM) for the following groups: young (including both, young$^{B6}$ and young$^{B6,CAS}$), aged$^{B6}$ and aged$^{B6,CAS}$. Data were tested for normal distribution and homogeneity of variances before the ANOVA. Effect size was calculated as $\eta^2 / 1 - \eta^2$. Power ($1 - \beta$, see Table 2) was calculated using G*Power (Faul et al., 2007). A one-sided post hoc test (Dunnett) was applied according to the hypothesis of a reduction in PV cells in aged animals of both groups.
Aging causes a reduction in PV immunoreactivity in the pAC of mice with and without AHL

In the present study, we investigated the number and density of PV+ neurons in the pAC of young B6/B6.CAST (both lines pooled) and old mice with and without the presence of AHL, previously determined by the ABR measurements. Possibly, a decline in total number of neurons may be stronger in one of the aged groups which could confound the interpretation of the total number of PV+ cells. To ensure that our results of the immunohistochemical verification were not influenced by a global decline in cortical neurons, the total number of neurons was investigated using a Nissl protocol. A mild, non-significant decrease of neurons was present in aged B6.CAST and aged B6 compared with young B6/B6.CAST (F(2,19) = 3.523; p = 0.05, ANOVA; f = 0.61, 1–β = 0.374; post hoc pair-wise comparison Dunnett, one-sided, young B6/B6.CAST > aged B6, p = 0.021; young B6/B6.CAST > aged B6.CAST: p = 0.056; Fig. 3A).

The immunohistochemical verification has been performed to investigate whether (1) a decline of PV immunoreactivity exists during aging and (2) whether it is stronger in animals suffering from progressive AHL. A significant decrease in number of PV+ neurons could be observed for aged B6.CAST and aged B6 groups (F(2,19) = 1.182; p < 0.001, ANOVA; f = 1.49, 1–β = 0.9993; Fig. 3B). The one-sided pairwise comparison to young B6/B6.CAST animals revealed a strong difference for both, young B6/B6.CAST versus aged B6, and young B6/B6.CAST versus aged B6.CAST (p < 0.001).

Additionally, we investigated the density of PV+ neurons by taking the cortical depth of each analyzed imaged into account (400-μm ROI width × cortical depth, calculated as neurons/mm2; Fig. 3C). The density of PV+ neurons/mm2 differed significantly (ANOVA, F(2,19) = 9.301; p < 0.01; f = 0.99, 1–β = 0.884). Similar to the absolute counts, we found a significant lower density in both aged B6 versus young B6/B6.CAST (p < 0.01) and aged B6.CAST versus young B6/B6.CAST (p = 0.001).
Differences in number and density of PV⁺ interneurons might not be observable in the whole sample but could be dependent of the cell position in two dimensions: layer positions defined as cortical depth and along the rostro-caudal axis. In order to receive a detailed description of the immunoreactivity pattern, we performed an analysis of PV immunoreactivity in in these two dimensions in the pAC of all animals. We observed a clear layer dependence of PV⁺ neurons revealed by the analysis of the density as a factor of cortical depth, with a strong peak in deep (sub-granular) layers (Fig. 4A). The largest density was present in the middle layers of pAC (maximum just below Layer IV). This layer dependence occurred with a nearly uniform distribution across the rostra-caudal axis of pAC (data of all animals, Fig. 4B). Subsequently, we analyzed layer dependence and rostro-caudal distribution for the factor of age and AHL. We could not observe a robust overall pattern of reduction along the rostro-caudal axis, but slightly higher reduction of PV⁺ neurons in the rostral portion of pAC in aged B6 compared with young and aged B6.CAST (Fig. 4C). In contrast, the reduction was stronger in the caudal region in aged animals without AHL (aged B6.CAST). When comparing groups according to the factor of cortical depth (Fig. 4D), the reduction across lamina was uniform in both, aged B6.CAST and aged B6. PV density in the middle layers was mildly more reduced for animals from the aged B6.CAST group than from aged B6. In summary, the combined pattern of reduction (Fig. 4C) across laminar and rostro-caudal axis appears to be very similar for aged animals with AHL (aged B6) and without (aged B6.CAST).

Discussion

We aimed to reveal whether a decrease in PV immunoreactivity during aging is stronger in the presence of AHL, as previously indicated (de Villers-Sidani et al., 2010; Martin del Campo et al., 2012; Brewton et al., 2016). On that account, we investigated the hearing thresholds and the PV immunoreactivity in the pAC of aged animals from a mouse line with progressive, early onset AHL (C57BL/6J) and from a congenic strain which is not vulnerable to AHL (C57B6.CAST-Cdh23<sup>Ahl<sup>−/−</sup>), in comparison with young animals. The main outcome of this study is a decline in PV immunoreactivity in the pAC of aged mice, regardless of AHL. Although AHL could be confirmed by our ABR measurements for the aged B6 animals (Fig. 1), both aged strains showed a similar reduction of PV⁺ positive interneurons in pAC in both number and density (Fig. 3). The pattern of reduction across the rostro-caudal axis and across cortical layers was similar in both strains (Fig. 4).

Specificity of reduction of PV cells

We investigated PV immunoreactivity in the pAC of all experimental groups by an indirect, immunofluorescent approach, using a primary antibody against PV. Additionally, as a control, the total number of neurons was evaluated to test for possible unequal cell loss between aged groups, following a standard Nissl protocol. Reduced PV immunoreactivity and thus a smaller number of positive cells may have been the results of a global loss of neurons for the aged groups compared with young animals and may be more severe in animals suffering from AHL. In our study, we found the total number of neurons in the pAC to be decreased in both aged groups, albeit not significantly (Fig. 3A). This mild, non-significant decrease in number of neurons for aged animals has been previously reported, at least for the rat auditory cortex (Burianova et al., 2015). In relation to our data from the evaluation of PV immunoreactivity (Fig. 3B,C), it can be concluded that a decreased number of PV⁺ interneurons is not solely based on a cortical neuronal decline in only one of the experimental groups. However, the identity of the lost neurons (reflected by the mild decrease in number of neurons) cannot be clearly defined with our experimental approach. It remains to be shown whether PV⁺ neurons remain intact but display a reduced protein expression or if the density of these specific inhibitory interneurons is decreased.

Reduction of PV⁺ interneurons is present in aged animals with preserved hearing thresholds

Regarding the number and density of PV⁺ neurons, aged B6.CAST and aged B6 show a decrease of both.
Indeed, a reduction in PV immunoreactivity seems to be dominated by the factor age rather than by hearing loss. This is in line with results from other studies, reporting decreased inhibitory properties in the aged auditory cortex independent of hearing status (Ueno et al., 2018; Kessler et al., 2020) and a reduction in PV neurons in other cortical areas, like somatosensory or motor (Miettinen et al., 1993).

One main reason for controversial results might be strong strain differences of PV immunoreactivity levels in animals. These differences are not only present on the molecular but also on the physiological level (Brewton et al., 2016; Bowen et al., 2019). All work that has been done so far to reveal the influence of AHL on the PV immunoreactivity pattern used either two different strains of laboratory rodents with/without the development of AHL (Ouda et al., 2008; Brewton et al., 2016) or compared old versus young animals of the same strain (de Villers-Sidani et al., 2010; Martin del Campo et al., 2012). It is possible that not only physiological properties differ between strains, but also the development of PV immunoreactivity patterns over time. In our study, the only difference between the two mouse strains used is the wild-type allele of Cdh23 in aged B6.CAST animals, eliminating the possibility of general strain differences in basal PV immunoreactivity patterns. However, it cannot be ruled out that patterns of PV immunoreactivity in the central auditory system depend on the genetic, peripherally acting cause of AHL. As the cause in our hearing impaired aged B6 animals, the missense mutation of Cdh23 is believed to result in a defective encoded protein that acts as a component of the stereocilia tip links in hair cells. The defect can cause a weakening of the tip links over time, which may result in progressively impaired mechanotransduction in the aging individual (Kazmierczak et al., 2007; Schwander et al., 2009; Johnson et al., 2010). Whether our results can be directly transferred to other mutations needs to be further investigated, e.g., in mutations of Sod1, which encodes for a defective superoxide dismutase, resulting in increased oxidative stress and therewith hair cell loss in the inner ear (McFadden et al., 2001; Jiang et al., 2007; Johnson et al., 2010). However, our results can be interpreted as a first step toward a better understanding about the central molecular alterations in Cdh23-mediated presbycusis.

Pattern of PV immunoreactivity along the rostro-caudal axis and cortical depth

To get an overall impression of the neuronal PV pattern in the pAC of young, aged, and aged mice expressing hearing loss, we provide a two-dimensional descriptive analysis of the rostro-caudal and layer-dependent distribution of PV neurons in the three groups (mean ± SEM). Positions are relative to the pia (see A). For a more fine-grained, unbiased evaluation of differences.
between immunoreactivity patterns, it should be considered to analyze multiple samples per animal along the rostro-caudal axis of the structure of interest as done in this study. Small sample sizes confined to narrow rostro-caudal sections could lead to false-positive differences that might disappear when analyzing a sample size distributed along the rostro-caudal axis or vice versa.

The PV density as a function of cortical depth peaks in middle layers, with a small population of neurons in the deeper and the fewest number in the superficial layers (Fig. 4D) as previously revealed by others (del Río et al., 1994; Martin del Campo et al., 2012; Brewton et al., 2016; Ueno et al., 2018). Regarding age and hearing status, we could not detect a difference between distributions of an aged<sup>+</sup>CAST or an aged<sup>+</sup>66 animal. Thus, an altered density of PV<sup>+</sup> neurons along the cortical depth is related to the factor age and remains unaltered by hearing status, similar to what we observed for PV immunoreactivity along the rostro-caudal axis.

**Reduced PV immunoreactivity in the pAC, a sign of central molecular aging in the mouse model**

The C57BL/6 mouse is commonly used as an animal model to study presbycusis due to its progressive, early onset AHL which is the result of the degenerating basal portion of the cochlear (Ison et al., 2007; Park et al., 2010; Martin del Campo et al., 2012). This process can be confirmed by analyzing ABRS, and it has been shown to become histologically detectable by the age of three months (Park et al., 2010). In parallel, a second aging-induced process seem to act on the central (auditory) system in form of reduced PV immunoreactivity in the inhibitory network of the cortex. Our data indicates that this process is independent of declined hearing sensitivity and should rather be interpreted as a sign of central molecular aging. The consequences of reduced intraneuronal protein levels in inhibitory system are not fully understood yet. Hence, it remains controversial to what extent a reduction of PV immunoreactivity affects inhibition and therewith central sensory processing in the (auditory) cortex.

PV itself as a calcium-binding protein is believed to serve the distinct function of buffering intracellular Ca<sup>2+</sup>, enabling the neuron to fire rapid spike trains and protecting it from toxic intracellular calcium levels (Ferguson and Gao, 2018; Fairless et al., 2019). This might support the unique function of this neuronal subtype: PV<sup>+</sup> interneurons are known for their remarkable fast spiking phenotype with a local widespread of activity regulating contacts to nearly every pyramidal neuron in their surrounding (Kawaguchi and Kubota, 1997; Packer and Yuste, 2011; Ferguson and Gao, 2018). On a functional level, this organization principle allows for strong feedback and feedforward inhibition with a precision in the millisecond range (Cardin, 2018; Hu et al., 2014). A change in the physiology of PV<sup>+</sup> cells may be particularly impactful in the auditory cortex, where precise modulation of sensory input is of great importance as it sharpens spike timing, shapes receptive fields, provides gain control and is involved in the generation of network oscillations (Wehr and Zador, 2003; Sohal et al., 2009; Moore and Wehr, 2013; Hu et al., 2014; Gothner et al., 2019). In that context, a reduced PV immunoreactivity could result in declined inhibitory properties in cortical circuits, which seems to be independent of AHL.

An additional potential role of PV that is discussed in current research is its involvement in (synaptic) plasticity: PV seems to prevent cumulative facilitation and maintains the strength of the synapse near its resting level (Caillard et al., 2000; Tripodi et al., 2018). Additionally, a few studies indicate that the PV immunoreactivity pattern cannot be interpreted as a static rather than an ongoing plastic process, influenced by environmental factors as previously demonstrated by de Villers-Sidani et al. (2010), who showed that decreased PV immunoreactivity in the aged auditory cortex of rats can be recovered by auditory training.

Given the importance of PV<sup>+</sup> inhibition and the strong reduction of PV immunoreactivity in the aging auditory cortex as shown here, further research is urgently needed to reveal the actual consequences of age-related reduction of PV immunoreactivity on inhibitory circuits on a physiological level. However, given the results presented here, physiological and possibly perceptual consequences of PV<sup>+</sup> reduction in pAC will have to be seen as a result of general, molecular aging in the auditory cortex instead of being restricted to individuals suffering from AHL.

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