

Age-dependent changes of calcium related activity in the central auditory pathway



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ABSTRACT

Age-related hearing loss (ARHL) represents one of the most common chronic health problems that faces an aging population. In the peripheral auditory system, aging is accompanied by functional loss or degeneration of sensory as well as non-sensory tissue. It has been recently described that besides the degeneration of cochlear structures, the central auditory system is also involved in ARHL. Although mechanisms of central presbycusis are not well understood, previous animal studies have reported some signs of central neurodegeneration in the lower auditory pathway. Moreover, changes in neurophysiology are indicated by alterations in synaptic transmission. In particular, neurotransmission and spontaneous neuronal activity appear to be affected in aging animals. Therefore, it was the aim of the present study to determine the neuronal activity within the central auditory pathway in aging mice over their whole lifespan compared to a control group (young adult animals, ~3 months of age) using the non-invasive manganese-enhanced MRI technique. MRI signal strength showed a comparable pattern in most investigated auditory brain areas.

An increase in activity was particularly pronounced in the middle-aged groups (13 or 18 months), with the largest effect in the dorsal and ventral cochlear nucleus. In higher auditory structures, namely the inferior colliculus, medial geniculate body and auditory cortex, the enhancement was much less expressed; while a decrease was detected in the superior olivary complex. Interestingly, calcium-dependent activity reduced to control levels in the oldest animals (22 months) in the cochlear nucleus and was significantly reduced in higher auditory structures. A similar finding was also found in the hippocampus. The observed changes might be related to central neuroplasticity (including hyperactivity) as well as neurodegenerative mechanisms and represent central nervous correlates of the age-related decline in auditory processing and perception.

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1. Introduction

Age-related hearing loss (ARHL), also referred to as presbycusis, is a widespread phenomenon in modern Western societies with fast-growing elderly populations. It represents one of the most common chronic health problems facing an aging population, leading to a major impairment of communication and to other psychophysical handicaps. It has been recently described that in addition to the degeneration of sensory (cochlear) cells, central auditory structures are also involved in the ARHL process (Frisina and Rajan, 2005; Parham et al., 2013).

Although examination of presbycusis' pathologies already started in the middle of the last century, particularly through the work of Harold F. Schuknecht, there are still many open questions regarding the detailed

mechanisms involved in the development of age-related hearing loss. At the level of the inner ear, aging is accompanied by a loss of inner and outer hair cells and a degeneration of spiral ganglion cells (Bao and Ohlemiller, 2010; Spong et al., 1997). Furthermore, impairments in maintaining the endocochlear potential were attributed to reduced stria vascularis function ("strial deafness") and other, non-sensory cochlear structures (Gratton et al., 1997; Henderson et al., 2006; Sha et al., 2009). Deficits in vascular function and oxidative stress followed by energy deficiency and mitochondrial dysfunction might play a key role during ARHL development (Jiang et al., 2007; Prazma et al., 1990; Schuknecht and Gacek, 1993; Someya and Prolla, 2010). Several genes, which have been shown to play an important role in antioxidant pathways, apoptosis, or changes in ion homeostasis and neurotransmission, show large alterations in the aging cochlea and might therefore, accompany the induction of degeneration in presbycusis (Christensen et al., 2009; Tadros et al., 2007, 2008, 2014; Tra et al., 2011).

Unlike those cochlear changes listed above, the mechanisms of central presbycusis are not well understood. Some previous studies

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used mouse strains with an early onset of hearing loss. In those animal models, a reduction in cell density was shown in the dorsal and ventral cochlear nucleus (DCN and VCN, respectively) in parallel with the onset of hearing loss, accompanied by cell and tissue shrinkage within these areas (Willott et al., 1987, 1992, 1998). Normally aging mice also showed some neurodegenerative signs, particularly in the DCN (Idrizbegovic et al., 2001), but they were less markedly expressed.

Other studies have demonstrated a decline in the number of dendritic spines suggesting an impaired synaptic transmission (Browner and Baruch, 1982). This normal physiological decline in function affects in particular the inhibitory neurotransmitter release and receptor activity. Therefore, age-related hearing loss with a reduction in inhibitory neurotransmission is characterized by hyperactivity in the corresponding structures (Krenning et al., 1998; Milbrandt and Caspary, 1995; Willott et al., 1997). Another important aspect in this context is the redistribution of membrane-bound ion channels which modulate the ion conductance and, thus, the excitability of neurons (Jung et al., 2005). Recent studies have also focused on dysfunctional mitochondria during aging, usually followed by an impaired respiratory chain that could cause apoptosis (Fischel-Ghodsian, 2003; Niu et al., 2007).

Age-related changes in higher structures of the central auditory system were less prominent compared to the cochlear nucleus. In the inferior colliculus (IC), however, physiological changes are consistent with the effects as demonstrated in some brainstem structures. Inhibitory and excitatory neurotransmission and spontaneous neuronal activity in particular seem to be affected in aging animals (Milbrandt et al., 1997; Osumi et al., 2012; Willott et al., 1988; Xie and Manis, 2013), resulting in temporal processing deficits at the level of the inferior colliculus (Walton et al., 1997, 1998). Similar observations have been made in the auditory cortex (Martin del Campo et al., 2012). Further, tonotopic reorganization was shown, indicating the appearance of central neuroplasticity (Willott, 1984, 1986).

It became obvious that structural and functional changes as described above have similarities to those occurring after noise trauma (Eggermont, 2006; Gröschel et al., 2010; Kaltenbach et al., 1998; Suneja et al., 1998). Both, noise-induced and age-related hearing loss are frequently accompanied by symptoms such as tinnitus, hyperacusis or reduced speech recognition (Knipper et al., 2013). It would therefore be particularly interesting to investigate whether the observed effects are based upon similar mechanisms.

The aim of the present study was to determine the calcium-related activity within the central auditory pathway over the whole lifespan. This should provide a deeper insight into the central pathophysiological correlates of presbycusis.

2. Methods

2.1. Animals and Experimental Groups

Untreated female mice of the NMRI strain were used for the present study. Mice were bred and kept in-house at our animal facility. Recent studies demonstrated that development of ARHL differs between the sexes (Canlon and Frisina, 2009; Guimaraes et al., 2004). To keep variance of the data low as well as being much more practical, we decided to use only female mice for the present experiments.

Nineteen animals in four age groups were included in the study. To avoid any subsequent effects of the experimental treatment, different animals were used to investigate the central nervous activity at an age of 3 months (control group, $n = 7$), 13 months ("13 months group", $n = 5$), 18 months ("18 months group", $n = 3$) and 22 months ("22 months group", $n = 4$) after birth. Due to the high mortality of aged animals (18 months and older) (Gower and Lamberty, 1993), subject numbers differed between groups.

Until the day of the experiment, animals were kept in groups with free access to food and water. All efforts were made to exclude any pain or discomfort during the experimental procedures.

2.2. MEMRI

Manganese-enhanced magnetic resonance imaging (MEMRI) is a non-invasive technique to image neuronal activity in-vivo (Cory et al., 1987; Kang and Gore, 1984; Silva et al., 2004). Manganese ions are able to cross the blood–brain barrier (Takeda, 2003) substituting intracellular calcium during neuronal activation (Drapeau and Nachshen, 1984; Narita et al., 1990; Silva et al., 2004). Due to a slow clearance, manganese accumulation results in an increase of the MRI-T1 signal contrast (Lin and Koretsky, 1997). Thereby, neuronal activity is monitored using the MEMRI technique and, thus, Ca^{2+} -dependent activity can be imaged. This provides the opportunity to integrate neuronal activity, represented by the increase in signal contrast due to manganese accumulation, over a well-defined period of time before measurements, i.e., 24 h in the present experiments. This is of particular importance during the investigation of auditory-related activity inside a noisy MRI scanner (Brozoski et al., 2007; Holt et al., 2010; Yu et al., 2005). Within 24 h following manganese application, MRI contrast is steadily increased in brain regions relevant to the present study (Lee et al., 2005). The Ca^{2+} -dependent neuronal activity was integrated within this period-of-time.

On the day of the experiments, animals of the experimental groups as well as control mice received a 0.4 mM/kg dose of MnCl_2 solution (in accordance to Yu et al., 2005). Delivery was via a single intraperitoneal injection. MRI measurements were performed 24 h after the manganese treatment, when manganese accumulation reached its maximum level in the relevant brain structures (Lee et al., 2005). Between manganese treatment and MRI imaging, animals were kept in their cages and placed inside a sound proof chamber (80 × 80 × 80 cm, minimal attenuation 60 dB) to reduce environmental sound to a minimum level. Mice were still kept together in groups to avoid any stress responses due to separation from each other.

A 7 Tesla rodent MRI scanner (Pharmascan 70/16AS, BrukerBioSpin, Ettlingen, Germany) was used for scanning. It had a 16 cm horizontal bore magnet and a 9 cm (inner diameter) shielded gradient with a H-resonance-frequency of 300 MHz and a maximum gradient strength of 300 mT/m. For imaging, a 1H-RF quadrature-volume resonator with an inner diameter of 20 mm was used. Data acquisition and image processing were carried out using the Bruker software Paravision 4.0. Mice were placed on a heated circulating water blanket to ensure a constant body temperature of 37 °C. Anesthesia was induced with 3% isoflurane and maintained with 1.5–2.0% isoflurane (Forene, Abbot, Wiesbaden, Germany), delivered in 0.5 l/min of 100% O_2 via a facemask under constant ventilation monitoring (Small Animal Monitoring & Gating System, SA Instruments, Stony Brook, New York, USA).

T1-weighted 2D turbo spin-echo sequence scanning was used (TR/TE = 938/10.6 ms, RARE factor 2, 6 averages). The duration of the MRI protocol was approximately 12 min. Therefore, acute MRI noise during scanning could perhaps induce a mild temporary threshold shift in the animals. However, this should not significantly affect the results since the manganese accumulation is highly related to the time interval before MRI scanning took place. Furthermore, this procedure was similar in control and aged animals.

In total, 35 axial slices with a slice thickness of 0.3 mm, a field of view of 2.85×2.85 cm and a matrix of 256×256 resulting in an in-plane resolution of 111 μm were positioned to cover the brain from cerebellum to auditory forebrain.

Signal intensity analysis was performed using the Analyze 5.0 software (AnalyzeDirect, Inc.; Lenexa, USA) for all slices of the following auditory system brain regions: dorsal and ventral cochlear nucleus (DCN and VCN, respectively), superior olivary complex (SOC), inferior colliculus (IC), medial geniculate body (MGB), auditory cortex (AC), hippocampus (Hip) and masseter muscle (Fig. 1). These regions are supposed to be influenced during aging and appear to be involved in altered physiological processing in auditory structures referred to age-related hearing disorders. Regions of interest were marked in accordance to the mouse brain

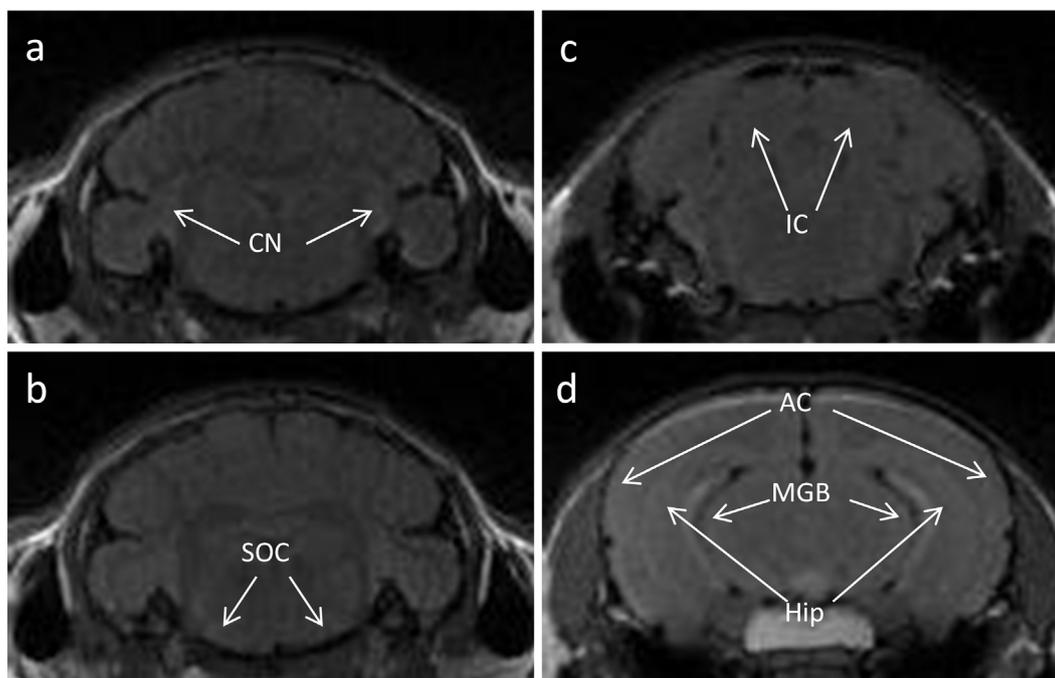


Fig. 1. Examples of MEMRI-images of a mouse brain. Arrows point to examined regions of interest (ROIs). a) (dorsal and ventral) cochlear nucleus (CN), b) superior olivary complex (SOC), c) inferior colliculus (IC), d) medial geniculate body (MGB), auditory cortex (AC) and hippocampus (Hip).

atlas (Paxinos and Franklin, 2001). Brain structures were bordered for voxel based analysis of signal intensity. The number of slices differed due to each structure's dimension. For statistical analysis the number of slices per group was as follows:

DCN: control: 39; 13 months: 31; 18 months: 19; 22 months: 26.

VCN: control: 56; 13 months: 32; 18 months: 15; 22 months: 28.

SOC: control: 28; 13 months: 20; 18 months: 13; 22 months: 12.

IC: control: 42; 13 months: 27; 18 months: 18; 22 months: 24.

MGB: control: 43; 13 months: 31; 18 months: 21; 22 months: 28.

AC: control: 44; 13 months: 38; 18 months: 25; 22 months: 29.

Hip: control: 67; 13 months: 59; 18 months: 31; 22 months: 36.

The masseter muscle was used as an objective intensity reference for each hemisphere. The relative MRI signal (Figs. 2–5) was calculated by using the mean of the measured signal strength of every slice of each analyzed structure, which was normalized in relation to the intensity of the muscle at that side. The experimenter doing this analysis was blind to the subject groups.

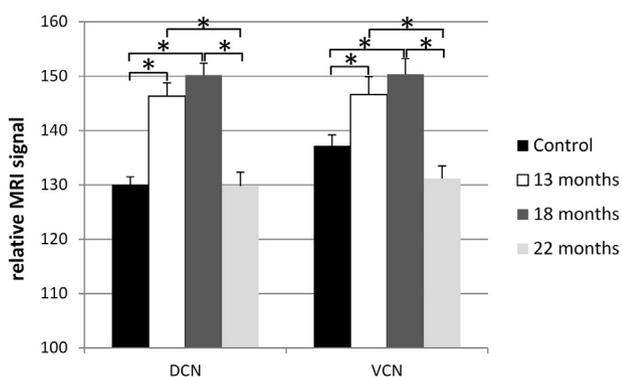


Fig. 2. Relative manganese enhanced MRI signal (mean \pm s.e.) of the dorsal (DCN) and ventral (VCN) cochlear nucleus of the central auditory pathway in control animals (black columns) compared to old mice at an age of 13 (white), 18 (dark gray) or 22 (light gray) months, respectively. Brackets and asterisks indicate significant differences between aging groups in comparison to the control group ($p < 0.05$).

The resulting relative signal intensity data from the experimental groups ("13 months", "18 months" and "22 months") were compared with each other and with the control group (young, normal hearing animals) for each brain region. For statistical analysis, ANOVA (normally distributed data) or Kruskal–Wallis-test (not normally distributed data) was applied. Data distribution was tested by applying the Kolmogorov–Smirnov test. The SPSS software (IBM SPSS Statistics Version 20, IBM Corp., Armonk, New York, USA) was used for all statistical analyses. The basic level of significance for all tests was $p < 0.05$.

3. Results

The data of the present study suggest that age-related changes in manganese accumulation are present for central structures of the auditory system. In most of the brain areas we investigated that, alterations in MRI signal strength showed a comparable pattern of calcium-dependent activity, i.e. MRI signals were increased in the middle-aged animals followed by a decline in old age compared to controls.

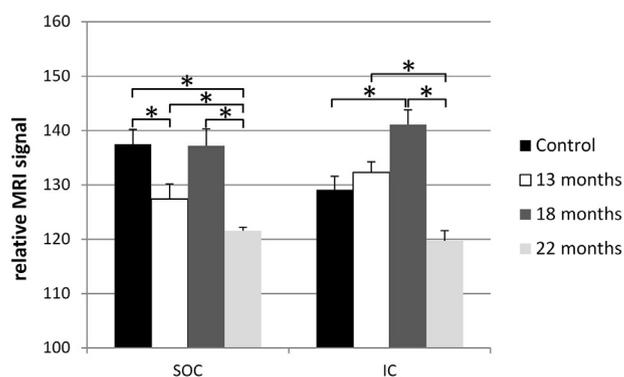


Fig. 3. Relative manganese enhanced MRI signal (mean \pm s.e.) of the superior olivary complex (SOC) and the inferior colliculus (IC) of the central auditory pathway in control animals (black columns) compared to old mice at an age of 13 (white), 18 (dark gray) or 22 (light gray) months, respectively. Brackets and asterisks indicate significant differences between aging groups in comparison to the control group ($p < 0.05$).

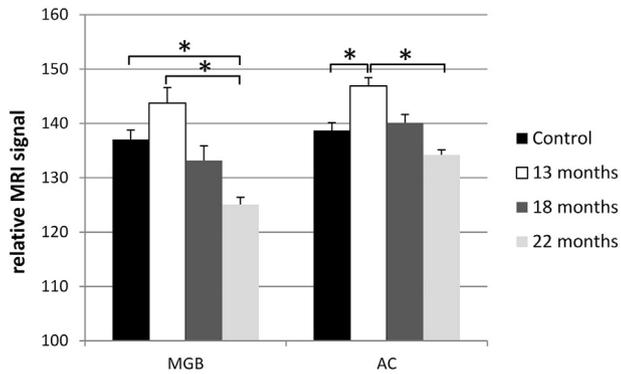


Fig. 4. Relative manganese enhanced MRI signal (mean \pm s.e.) of the medial geniculate body (MGB) and the auditory cortex (AC) of the central auditory pathway in control animals (black columns) compared to old mice at an age of 13 (white), 18 (dark gray) or 22 (light gray) months, respectively. Brackets and asterisks indicate significant differences between aging groups in comparison to the control group ($p < 0.05$).

3.1. Dorsal and Ventral Cochlear Nucleus

In the DCN, the relative MEMRI signal was significantly elevated from 130.0 ± 1.5 in controls to 146.3 ± 2.4 in the “13 months” group ($p < 0.001$; all data are given as mean \pm s.e.) and was still increased in the “18 months” group (150.2 ± 2.2 ; $p < 0.001$). After 22 months, MRI contrast declines back to control level (129.8 ± 2.5 ; n.s.).

Data in the VCN look very similar to the DCN. Again, signal intensities increased significantly when comparing the “13 months” (146.6 ± 3.3 ; $p = 0.036$) to the control group (137.2 ± 2.0) and were still elevated after 18 months (150.4 ± 2.8 ; $p = 0.021$), although changes were smaller than in the DCN. No significant differences were found between the “22 months” (131.2 ± 2.3 ; n.s.) and the control group (Fig. 2).

3.2. Superior Olivary Complex

The nuclei of the superior olivary complex showed an opposite development in the relative signal of MR images and exhibit an intensity reduction already at an age of 13 months (“13 months” group: 127.4 ± 2.7 , control group: 137.5 ± 2.7 ; $p = 0.033$) (Fig. 3). A similar observation was made for the “22 months” group (121.6 ± 0.6), where the MRI signal was also significantly lower in relation to the control ($p = 0.002$). In the group investigated at an age of 18 months (137.2 ± 3.1), however, data did not point to any significant change compared to the control group (Fig. 3).

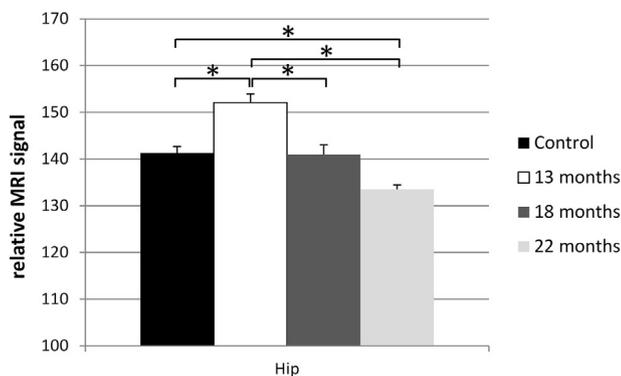


Fig. 5. Relative manganese enhanced MRI signal (mean \pm s.e.) of the hippocampus (Hip) in control animals (black columns) compared to old mice at an age of 13 (white), 18 (dark gray) or 22 (light gray) months, respectively. Brackets and asterisks indicate significant differences between aging groups in comparison to the control group ($p < 0.05$).

3.3. Inferior Colliculus

An increase in signal intensities in the middle-aged groups, followed by a subsequent decline in the “22 months” group, was also present in the inferior colliculus (Fig. 3). In the IC, relative MRI signal was 129.1 ± 2.5 in the control group and 132.3 ± 2.0 in the “13 months” group. In the “18 months” group, the mean value was elevated to 141.1 ± 2.7 and declined below control level in the “22 months” group (119.7 ± 1.9). Differences were statistically significant only in the “18 months” group in comparison to controls ($p = 0.015$; Fig. 3).

3.4. Medial Geniculate Body and Auditory Cortex

Both the MGB and AC showed comparable MEMRI signal development during aging. In the MGB, a slight signal increase was observed after 13 months (“13 months” group: 143.7 ± 2.9 ; controls: 137.0 ± 1.8), although this effect was not significant. The same was found for the “18 months” group (133.1 ± 2.7), where a small decrease was indicated. Interestingly, calcium-related activity was significantly lower in the “22 months” group (125.1 ± 1.3) in relation to control animals ($p < 0.001$; Fig. 4). In contrast to the MGB, the increase in MRI signal in the AC of the “13 months” group (146.7 ± 1.6) was statistically significant compared to the control group (138.7 ± 1.4 ; $p = 0.002$). Furthermore, relative MRI signal values did not differ in a significant manner in the older groups within this structure versus controls (“18 months” group: 140.1 ± 1.5 ; “22 months” group: 134.2 ± 1.0 ; Fig. 4).

3.5. Non-Auditory Brain Area

Significant changes between the groups were detected in the hippocampus, a structure which is not directly driven by auditory neuronal projections. Again, the manganese accumulation showed an elevation in the “13 months” group (152.1 ± 1.8) when looking for statistical differences compared to control animals (141.3 ± 1.4 ; $p < 0.001$) (Fig. 5). However, in animals at an age of 22 months, the signal intensity was even decreased significantly (133.5 ± 0.9 ; $p = 0.007$), although the values of the “18 months” group (141.0 ± 2.1) matched to the controls (Fig. 5).

4. Discussion

Manganese-enhanced MRI data from the present study demonstrate an increased calcium-dependent activity in several brain structures of the mature, but not senescent auditory system compared to normal-hearing, young animals. An activity increase was particularly obvious in the middle-aged groups (13 or 18 months), whereby the largest effects were evident in the DCN and VCN. In higher auditory structures, namely the IC, MGB and AC, the enhancement was much less expressed or had disappeared from those regions; a decrease was even detected in the SOC. Interestingly, calcium-dependent activity dropped to control levels in the oldest animals (“22 months”) in the cochlear nucleus subdivisions and showed a significant reduction in higher structures in relation to controls. A similar finding was found in the hippocampus, a structure playing an important role in neuroplasticity and memory formation and probably further involved in tinnitus and related hearing disorders (De Ridder et al., 2006; Kraus and Canlon, 2012; Kraus et al., 2010).

4.1. Cochlear Nucleus

The highest increase in manganese accumulation was shown in primary structures of central auditory processing, indicating that calcium metabolism is up-regulated in correlation with the beginning of ARHL, starting at an age of 12 to 13 months in NMRI mice. This begins in the high-frequency range, spreading towards low frequencies at 18 to

19 months of age (Ehret, 1974). In studies on CBA mice, auditory threshold shifts were already present at 14 months over a broad frequency range and animals showed a progressive hearing loss with aging (Jacobson et al., 2003). It is therefore, possible that neuronal activity is enhanced (whereby an increased inhibition would also lead to an elevated MEMRI signal) in response to a reduction of sensory input from the periphery by degeneration of neuronal cochlear and auditory nerve structures (Bao and Ohlemiller, 2010; Hinojosa and Nelson, 2011; Spongr et al., 1997; Wang and Manis, 2005). Several studies have reported changes in transmitter release, as well as receptor densities, particularly at synapses of the DCN and VCN, leading to an increase of excitatory and a reduction of inhibitory neurotransmission (Krenning et al., 1998; Shim et al., 2012; Xie and Manis, 2013).

Alterations of neuronal projections and connectivity to auditory brainstem structures in presbycusis (Frisina and Walton, 2001a, 2001b) might result in a changed calcium-dependent activity related to neuroplasticity (Mattson, 2007). Other investigations have also demonstrated a decrease in the number of neurons in DCN and VCN in aging mice and dogs (Shimada et al., 1998; Willott and Bross, 1996; Willott et al., 1992), which is followed by an up-regulation of calcium-binding proteins, perhaps to prevent further degeneration of cells in those structures (Idrizbegovic et al., 2004, 2006). Neurodegeneration during aging is supposed to be related to an increase in intracellular calcium concentrations, leading to oxidative stress and inducing apoptotic pathways (Foster, 2007). Therefore, apoptotic degeneration itself should contribute to an increase in calcium accumulation. Recent studies support the idea that alterations in auditory processing in the aging brain are due to an increase in intracellular calcium and an accompanying neurodegeneration (Hong et al., 2009). This effect could also help to explain our observed MEMRI signal enhancement during aging in higher auditory structures. A loss of neurons in central nuclei might also acutely reduce the amount of input onto the surviving cells, initiating compensatory plasticity (Cavazzini et al., 2005; Voglis and Tavernarakis, 2006; Yu et al., 2001). These effects might be induced by both, an aging brain and a loss of peripheral input (Frisina and Walton, 2006; Gourevitch and Edeline, 2011; Willott, 1996). The strength of calcium activity enhancement at the CN level in the middle-aged, 13 months' old group with a supposed onset of high-frequency hearing loss (Ehret, 1974) at this point in time might therefore, be related to the loss of primary afferent (auditory nerve) projections. This loss could be due to cochlear synaptopathy during adulthood as an early-onset event following nerve fiber loss (Sergeyenko et al., 2013) and ongoing tissue degeneration and neuroplasticity in middle-aged animals (Sharma et al., 2014).

In addition, the observed effects are also possibly related to central hyperactivity (if the observed calcium activity is due to excitatory neurotransmission). Especially in the cochlear nucleus, calcium activity remained at a significantly elevated level at an age of 18 months, although progressive central neurodegeneration has been reported (Chen et al., 2010; Shimada et al., 1998; Willott et al., 1992). Despite single cell hyperactivity (Frisina and Walton, 2006), a high level of ongoing neuroplasticity could be estimated. This assumption is supported by observed changes of synaptic properties in CN neurons (Jalenques et al., 1997; Krenning et al., 1998; Wang and Manis, 2005). If cell loss in auditory brain structures is taking place in the middle-aged groups and reduces later, neuronal reorganization is assumed to be present. This is in agreement with the presence of calcium-binding proteins (Idrizbegovic et al., 2004, 2006) and would explain the signal decrease in the "22 months" group, as both neurodegeneration as well as neuroplasticity decline. Long-duration deafness leads to a reduced expression of the calcium-binding protein calretinin. This might be in part responsible for the later decrease of calcium-dependent activity, and may be attributed to an opponent subsequent effect on calcium homeostasis (Zettel et al., 2003). Neuronal hyperactivity might still exist, as proposed by some studies, but is not reflected in MRI signal differences, although the number of neurons was diminished (Frisina and Walton, 2006; Syka, 2010).

4.2. Superior Olivary Complex

In contrast to all the other brain regions we investigated, no signal increase was observed in the SOC at any time intervals investigated. Moreover, MRI signals were even reduced in the "13 months" and the "22 months" group compared to controls. Recent studies of the olivocochlear feedback function, measuring the contra-lateral suppression of cochlear output in mice and humans, indicate an early reduction in SOC function. This decline in efferent projections to the cochlea (i.e., the peripheral part) even precedes the loss of outer hair cells, examined by distortion product otoacoustic emissions (DPOAEs), and therefore, reflects an early event during the onset of ARHL (Jacobson et al., 2003; Kim et al., 2002; Zettel et al., 2007; Zhu et al., 2007). Our present observation is possibly due not only to a loss of neurons, but also to a reduced expression of the calcium binding protein calbindin in the aging SOC and might have negative influence on the processing and localization of complex sounds and spatial acuity (O'Neill et al., 1997). An overall reduction in transmitter expression supports the idea of a general decrease in SOC activation (Shim et al., 2012), whereby an assumed age-related loss of neurons would strengthen this effect. This in turn might lead to higher susceptibility of peripheral and central structures to acoustic injury and a further auditory threshold shift, as the protective function of SOC activation reducing the gain of the cochlear amplifier and hence the cochlear output to the brain disappears (Liberman and Gao, 1995; Zheng et al., 1997). A missing function of olivocochlear projections further leads to a loss of primary afferent connections between inner hair cells and auditory nerve fibers (synaptic ribbons) accelerating ARHL (Liberman et al., 2014). Due to the fact that the SOC also plays an important role in sound filtering and the comparison of acoustic input from both ears, a loss of functional properties may lead specifically to deficiencies in directional hearing and speech comprehension in noise of human listeners (Caspary et al., 2008).

4.3. Inferior Colliculus

Comparable observations to the cochlear nucleus have been made in higher structures of the central auditory system. Changes in spontaneous activity and excitability (hyperactivity) due to changes in synaptic transmission have been shown in the inferior colliculus as well as in the auditory cortex (Syka, 2010). Data from several studies has led to the assumption that a reduced inhibition, accompanied by an increase in excitatory neurotransmission, is responsible for these effects (Burianova et al., 2009; Caspary et al., 1995, 1999; Shim et al., 2012). Moreover, an increased expression of the calcium binding proteins calretinin and parvalbumin, both of which are up-regulated during ARHL, might be due to imbalance in calcium homeostasis. This could contribute to manganese accumulation in the IC, hence leading to an impaired central processing of auditory information (Engle et al., 2014; Zettel et al., 1997, 2001).

Interestingly, the MEMRI data generated in the present study identify a different time-dependent development in calcium-related activity between the auditory structures we examined. As activity is increased in the "13 months" group in most structures, the maximum enhancement is shown in the "18 months" group for the IC. This later activity increase in the IC, perhaps reflecting an elevated neuronal excitation, could be due to an enhanced serotonin expression shown in the IC in old rats with ARHL (Shim et al., 2012).

4.4. Medial Geniculate Body

The degeneration caused by central aging of the auditory system, as well as an accompanying neuroplasticity, might be delayed within the auditory midbrain. These mechanisms could further explain the MRI signal intensities detected in the auditory thalamus, namely the MGB, where manganese accumulation was only slightly increased in the "13 months" group and below control animals in the "18 months"

group. It could be hypothesized that due to a reduced output from the IC to the MGB, together with a loss of neurons within the entire ascending pathway, calcium-dependent activity is less pronounced in this structure. Moreover, an elevation in serotonin expression was also present in the MGB, possibly leading to similar effects as in the IC (Shim et al., 2012). In the “22 months” group, the IC and the MGB show a significant reduction in calcium activity. This effect has not been observed in the cochlear nucleus and is possibly related to central neurodegeneration and a reduced input from auditory brainstem structures. Moreover, long-term changes in calcium-related activity were larger in the CN compared to higher structures after noise-induced hearing loss and might be due to an excessive deprivation-induced reorganization in the CN (Gröschel et al., 2011).

4.5. Auditory Cortex

Data from the auditory cortex of aging mice were slightly different compared to auditory midbrain and thalamic structures. Although calcium-dependent activity increased significantly after 13 months, it returned to control level until an age of 18 months. Particularly in the “13 months” group, synaptic plasticity and hyperactive disorders might be responsible for our observations, due to a loss of afferent input and intra-cortical neurodegeneration. A few studies were able to confirm a down-regulation of inhibitory neurotransmitters in the AC, possibly leading to an increase in spontaneous activity as well as acoustically driven neuronal excitability (Burianova et al., 2009; Ouda et al., 2012). Despite the changes in projections due to cell loss in lower auditory structures, cortical apoptotic degeneration and atrophy of brain structures might additionally induce neuroplasticity (Eckert et al., 2012; Profant et al., 2013, 2014; Zhong et al., 2012). Impairments in temporal neuronal responses in the AC and complex sound processing in human and animal models underline these assumptions (Chen et al., 2010; Suta et al., 2011; Tremblay et al., 2003). It could be hypothesized that central aging participates in ARHL. This is indicated by a decline in both brainstem and cortical responses in aging guinea pigs receiving auditory stimulation. Larger decreases were shown than for normal hearing animals traumatized by damaging noise, primarily affecting peripheral structures (Gourevitch and Edeline, 2011). In contrast to the IC and MGB, signals of the AC in the “18 months” and “22 months” groups were persistent and did not differ significantly to controls. Thereby, we assume an appearance of rebalancing neuronal, particularly synaptic function, as a response to changed input characteristics, representing an intrinsic cortical response in order to keep the integrated cortical activity at a constant level. An increased excitability and hyperactivity is one possible mechanism to compensate for the age-dependent cell loss and changed ascending projections. Here again, it has to be stated that a changed calcium activity could be related to both, excitatory as well as inhibitory neurotransmission, as well as further calcium-dependent mechanisms. Nevertheless, these modifications would induce profound changes in signal processing, leading to large impairments in auditory perception (Profant et al., 2013; Suta et al., 2011; Tremblay et al., 2003).

4.6. Hippocampus

In the non-auditory structure investigated during the present study, the hippocampus, calcium-dependent activity was significantly increased in the “13 months” group and declined back to control levels after 18 months. In the “22 months” group, signal strength was actually significantly below the control group level. The hippocampus is largely involved in memory formation and recognition. Atrophy of hippocampus tissue with aging has been demonstrated in several studies and is often accompanied by memory impairment and neurodegenerative diseases like Alzheimer's, Parkinson's or major depression (Bird and Burgess, 2008; Erickson et al., 2012). Direct and indirect connections between the auditory system and the hippocampus exist, and the auditory

system's activity induces hippocampal plasticity (Cenquizca and Swanson, 2007; Meng et al., 2009; O'Mara, 2005). Moreover, degeneration of hippocampal synapses has been shown in relation to ARHL, affecting cognitive performance (Yu et al., 2011). An association of hearing impairment and accelerated cognitive decline, dementia and lower physical activity or frailty was also demonstrated in older adults, whereby the mechanistic basis of this connection has not yet been investigated in detail (Gispén et al., 2014; Kamil et al., 2014; Lin and Albert, 2014; Lin et al., 2013).

The observed MRI signal increase of the middle-aged group of animals in our data might therefore, be related to an activation of the hippocampus due to the changes in activity described earlier and an ongoing neuroplasticity in the brain. However, as the hippocampus itself degenerates over time and the afferent projections from other brain regions decline, a reduction in calcium-related activity and therefore, a diminished manganese accumulation should occur. The beginning of degeneration might be additionally responsible for the signal elevation after 13 months. Furthermore, it has been shown that neurogenesis in the hippocampus is affected by noise exposure (Kraus et al., 2010) and probably during tinnitus generation, which often accompanies ARHL (Eggermont and Roberts, 2004; Landgrebe et al., 2009).

4.7. Age- and noise-related hearing loss

In accordance to our noise trauma model, the largest effects of aging have been observed at the first level of central auditory processing in the DCN and VCN (Gröschel et al., 2011). This might be due to the strong reduction of peripheral input, whereby degeneration of sensory cochlear structures represents one initial trigger at the onset of ARHL (Syka, 2010). Our recent studies on noise-induced hearing loss showed similar activity patterns (Gröschel et al., 2011), although a direct comparison between the two hearing loss models is hardly appropriate. Noise trauma reflects a profound, but short-term impact on auditory structures and thresholds, compared to a slowly, ongoing impairment during aging: differences might also rely on the normal, physiological aging in the CNS to some extent (Gourevitch and Edeline, 2011). Nevertheless, parallel findings in neuroplastic (reduced inhibition leading to hyperactivity) and neurodegenerative (loss of neurons) mechanisms are obvious (Frisina and Walton, 2006; Gröschel et al., 2010; Holt et al., 2010; Kaltenbach et al., 1998; Willott et al., 1997) and deserve further investigation to better understand the complex mechanisms of central presbycusis.

4.8. Calcium hypothesis of aging

With our present study we were able to describe changes in calcium homeostasis and uptake in brain structures participating in the processing of auditory information. However, age-related changes in calcium metabolism are not limited to the central part of the auditory system, but represent a general phenomenon in several brain structures (Das and Ghosh, 1996; Kuo et al., 2010) and might therefore, occur in all sensory modalities. It became evident that neurons in the aging brain tend to increase their activity by enhancing the probability for calcium influx into the cells due to an up-regulation of the expression and activation of (L-type) voltage-gated calcium channels (L-VGCCs) (Bissig and Berkowitz, 2014; Veng and Browning, 2002). A permanent increase in intracellular calcium in CNS neurons during aging might induce cell death pathways due to calcium toxicity and mitochondria dysfunction and hence represent a major source of age-related degeneration in the brain (Hong et al., 2009). As L-VGCCs represent a dominant influx path for manganese ions, these age-related changes would increase manganese uptake, leading to an enhancement of MEMRI signal strength (Bissig and Berkowitz, 2014; Drapeau and Nachshen, 1984).

It is still under discussion how far central presbycusis is dependent on changes in the periphery. Whereas some authors hypothesize that central effects might be related to either biological aging or peripheral

pathologies and that functional CNS deficits are not necessarily accompanied by peripheral degeneration (Willott, 1996), a recent review paper concludes that central presbycusis does not occur as an isolated entity, but manifests as a multifactorial condition that involves age-related changes in the entire (peripheral and central) auditory system and brain (Humes et al., 2012).

In essence, the results of the present study give insight into central nervous correlates of the age-related decline in auditory processing and perception. Future investigations should clarify the different contributions of central and peripheral aging to the observed modifications in calcium-related neuronal activity. They should look for pathophysiological changes at the cellular level within the auditory system during ARHL, using electrophysiological, histological and immuno-histochemical techniques.

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