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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.

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; Spongr 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 densities 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 quadratur-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