ELSEVIER

Neurobiology of Aging 33 (2012) 2892–2902

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Efferent synapses return to inner hair cells in the aging cochlea

Amanda M. Lauer[a,*], Paul A. Fuchs[a], David K. Ryugo[a,b], Howard W. Francis[a]

[a] The Center for Hearing and Balance, Department of Otolaryngology-HNS, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[b] Hearing Research Division, Garvan Institute, Darlinghurst, Sydney, New South Wales, Australia

Received 12 December 2011; received in revised form 9 February 2012; accepted 10 February 2012

Abstract

Efferent innervation of the cochlea undergoes extensive modification early in development, but it is unclear if efferent synapses are modified by age, hearing loss, or both. Structural alterations in the cochlea affecting information transfer from the auditory periphery to the brain may contribute to age-related hearing deficits. We investigated changes to efferent innervation in the vicinity of inner hair cells (IHCs) in young and old C57BL/6 mice using transmission electron microscopy to reveal increased efferent innervation of IHCs in older animals. Efferent contacts on IHCs contained focal presynaptic accumulations of small vesicles. Synaptic vesicle size and shape were heterogeneous. Postsynaptic cisterns were occasionally observed. Increased IHC efferent innervation was associated with a smaller number of afferent synapses per IHC, increased outer hair cell loss, and elevated auditory brainstem response thresholds. Efferent axons also formed synapses on afferent dendrites but with a reduced prevalence in older animals. Age-related reduction of afferent activity may engage signaling pathways that support the return to an immature state of efferent innervation of the cochlea.

Keywords: Cochlear innervation; Age-related hearing loss; Olivocochlear; Efferent; Neural plasticity

1. Introduction

It is estimated that 25% of people aged 65–75 years and 70%–80% of people older than age 75 suffer from hearing loss associated with aging (Lin et al., 2011; Sprinzl and Riechelmann, 2010). Cumulative structural alterations in the cochlea affecting information transfer from the auditory periphery to the brain may contribute to age-related hearing deficits. Changes in the proportion of low spontaneous rate auditory nerve fibers observed with age (Schmiedt et al., 1996) may be linked to afferent synapse alterations at the inner hair cell (IHC) including loss of afferent terminals; enlarged synaptic terminals, mitochondria, postsynaptic densities (PSDs), and synaptic bodies; flattened PSDs; increased prevalence of PSDs associated with multiple or missing synaptic bodies; and increased synaptic vesicle density (Stamataki et al., 2006).

Changes in efferent feedback to the cochlea also may occur during age-related hearing deficits. The mammalian olivocochlear efferent system consists of 2 distinct pathways: a medial olivocochlear (MOC) component that projects bilaterally from the superior olivary complex to the outer hair cells (OHCs) and a lateral olivocochlear (LOC) component that projects ipsilaterally from the superior olivary complex to the auditory nerve dendrites contacting IHCs (summarized in Fig. 1; recently reviewed by Brown, 2011). These projection patterns reflect the mature innervation of adult animals. Activation of the MOC component causes hyperpolarization of OHCs by acetylcholine release (Blanchet et al., 1996; Evans, 1996), resulting in modulation of the active mechanical properties of the cochlea (Russell and Murugasu, 1997). The resulting suppression of the cochlear response modulates the auditory nerve's dynamic range (e.g., Dolan and Nuttall, 1988; Galambos, 1956; Kawase and Liberman, 1993; Kawase et al., 1993; Liberman and Brown, 1986; Winslow and Sachs, 1987), enhances the representation of transient signals in noise (Dolan and Nuttall, 1988; Kawase and Liberman, 1993; Winslow and

* Corresponding author at: 514 Traylor Building, 720 Rutland Avenue, Baltimore, MD 21205, USA. Tel.: +1 443 287 6336; fax: +1 410 955 1299.
E-mail address: alauer2@jhmi.edu (A.M. Lauer).

0197-4580/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
10.1016/j.neurobiolaging.2012.02.007

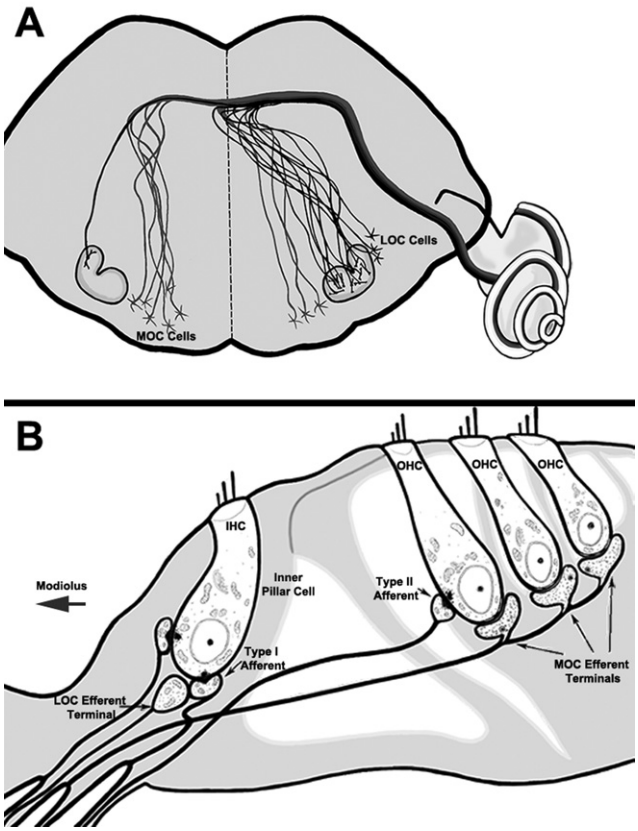

Fig. 1. (A) Illustration of the olivocochlear efferent pathways originating in the brainstem. Lateral olivocochlear (LOC) fibers project predominantly to the ipsilateral cochlea. Medial olivocochlear (MOC) fibers are both crossed and uncrossed. (B) LOC fibers primarily contact type I auditory nerve dendrites below inner hair cells (IHCs) in young adult animals. MOC fibers contact the outer hair cells.

Sachs, 1987), protects the auditory system from acoustic trauma (e.g., Kujawa and Liberman, 1997; Lauer and May 2011; Maison et al., 2002; Rajan, 2000). Deficient MOC function has been reported in older human listeners and mice (Jacobson et al., 2003; Zettel et al., 2007). The functional role of the LOC system is poorly understood, but it appears to modify afferent activity through various neurotransmitter systems and may protect the ear from acoustic overexposure and/or balance the sensitivity of the 2 ears to sensory stimulation (Darrow et al., 2006, 2007; Le Prell et al., 2003, 2005).

Efferent innervation of the cochlea undergoes extensive modification early in development (Simmons et al., 2011), including the loss of efferent contacts from IHCs near the onset of hearing. It is not known if these changes are permanent, or if they vary with acoustic damage, aging, or both. C57BL/6J mice show age-related loss of MOC terminals contacting OHCs (Fu et al., 2010), but virtually nothing is known about age-related changes to efferent innervation in the IHC area. In the present study, we investigated changes to efferent innervation in the vicinity of IHCs in aged mice.

2. Methods

2.1. Subjects

Adult female C57BL/6J (C57) mice were studied at 2–3 months of age ($n = 3$) and 8–11 months of age ($n = 3$). The efferent innervation of the 22 kHz region of the cochlea was examined for a total of 10 cells from young animals and 8 cells from old animals using transmission electron microscopy. A total of 3 axosomatic efferent synapses and 348 axodendritic efferent synapses were observed in young animals, and 35 axosomatic efferent synapses and 60 axodendritic synapses were observed in old animals. Auditory brainstem response (ABR) thresholds and age-related alterations to afferent synapses were previously quantified in the same mice (Stamataki et al., 2006), and the number of afferent synapses per IHC reported here are from this dataset. Behavioral hearing deficits and patterns of hair cell loss were also characterized in the older mice (Francis et al., 2003; Prosen et al., 2003). In Table 1 we summarize the pure tone thresholds measured by Stamataki et al. (2006) by averaging across the frequencies tested. This average provides a single summary measure of hearing status, similar to what is often reported by audiologists testing human listeners.

No outer or middle ear pathology was observed in any of the subjects at the time of tissue harvest. All procedures were conducted in accordance with protocols approved by

Table 1
Subject age, hearing status, and histological characteristics of the cochlear region under study

Subject	Age, mo (wk)	Pure tone average (dB SPL)	Cochlear location studied (% related to base)	OHC loss, %	Average axosomatic efferent synapses per IHC, *n*
Young 1	2 (9)	NA	40	0	0.25
Young 2	3 (12)	22.5	48	0	0.25
Young 3	3 (13)	35.2	47	0	0.5
Old 1	8 (35)	71.5	45	40.5	1.33
Old 2	8 (35)	82.4	47	70.1	3.3
Old 3	11.5 (46)	91.85	47	100	7

Age, hearing thresholds, cochlear location, and % outer hair cell (OHC) loss from Stamataki et al. (2006).
Key: IHC, inner hair cell; NA, not applicable; SPL, sound pressure level.