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Descending projections from the inferior colliculus to medial olivocochlear efferents: Mice with normal hearing, early onset hearing loss, and congenital deafness

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ABSTRACT

Auditory efferent neurons reside in the brain and innervate the sensory hair cells of the cochlea to modulate incoming acoustic signals. Two groups of efferents have been described in mouse and this report will focus on the medial olivocochlear (MOC) system. Electrophysiological data suggest the MOC efferents function in selective listening by differentially attenuating auditory nerve fiber activity in quiet and noisy conditions. Because speech understanding in noise is impaired in age-related hearing loss, we asked whether pathologic changes in input to MOC neurons from higher centers could be involved. The present study investigated the anatomical nature of descending projections from the inferior colliculus (IC) to MOCs in 3-month old mice with normal hearing, and in 6-month old mice with normal hearing (CBA/CaH), early onset progressive hearing loss (DBA/2), and congenital deafness (homozygous Shaker-2). Anterograde tracers were injected into the IC and retrograde tracers into the cochlea. Electron microscopic analysis of double-labelled tissue confirmed direct synaptic contact from the IC onto MOCs in all cohorts. These labelled terminals are indicative of excitatory neurotransmission because they contain round synaptic vesicles, exhibit asymmetric membrane specializations, and are co-labelled with antibodies against VGlut2, a glutamate transporter. 3D reconstructions of the terminal fields indicate that in normal hearing mice, descending projections from the IC are arranged tonotopically with low frequencies projecting laterally and progressively higher frequencies projecting more medially. Along the mediolateral axis, the projections of DBA/2 mice with acquired high frequency hearing loss were shifted medially towards expected higher frequency projecting regions. Shaker-2 mice with congenital deafness had a much broader spatial projection, revealing abnormalities in the topography of connections. These data suggest that loss in precision of IC directed MOC activation could contribute to impaired signal detection in noise.

1. Introduction

The central auditory system is composed of ascending and descending pathways. Olivocochlear (OC) efferents of the brain stem comprise the final stage of these descending pathways as they communicate directly with the sensory receptors of the auditory periphery. In mouse, medial olivocochlear efferents (MOCs) reside bilaterally in the ventral nucleus of the trapezoid body (VNTB) and dorso-medial periolivary nucleus (Brown et al., 2013). MOCs send myelinated axons that terminate on outer hair cells (OHCs). MOC activation effectively decreases cochlear gain, which facilitates signal extraction from background noise, known as 'antimasking'

Abbreviations: ABC, avidin biotin complex; ABR, auditory brainstem response; ANF, auditory nerve fiber; BDA, biotinylated dextran amine; CAP, compound action potential; CM, cochlear microphonic; CNIC, central nucleus of the inferior colliculus; CTB, cholera toxin subunit B; DPOAE, distortion product otoacoustic emission; IC, inferior colliculus; LSO, lateral superior olive; MNTB, medial nucleus of the trapezoid body; MOC, medial olivocochlear; Ni-DAB, nickel intensified diaminobenzidine; NR, no response; OC, olivocochlear; OHC, outer hair cell; VNTB, ventral nucleus of the trapezoid body

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(Winslow and Sachs, 1987; Kawase et al., 1993; Kumar and Vanaja, 2004), and promotes protection from acoustic trauma (Rajan, 1988b; a; 1990, 1995; Brown et al., 2003; Maison et al., 2013; Tong et al., 2013; Liberman and Maison, 2014).

Previous anatomical and electrophysiological experiments suggest that the OC system receives descending input from higher auditory centers, including the inferior colliculus (IC; Faye-Lund, 1986; Caicedo and Herbert, 1993; Thompson and Thompson, 1993; Vetter et al., 1993; Mulders and Robertson, 2000a, 2002; Mulders et al., 2010) and auditory cortex (AC; Mulders and Robertson, 2000b; Xiao and Suga, 2002; Perrot et al., 2006; Dragicevic et al., 2015). Electrical stimulation of the IC results in voltage changes reminiscent of both sound and electrical activation of MOC efferents (Mulders and Robertson, 2000a, 2002; Popelar et al., 2002; Groff and Liberman, 2003; Ota et al., 2004; Zhang and Dolan, 2006). Anatomically, descending inputs appear to be arranged in a topographic manner where dorsally placed injections in lower frequency regions label projections more laterally in VNTB, and ventrally placed injections in higher frequency regions label projections progressively more medially (Caicedo and Herbert, 1993; Malmierca et al., 1996). This relationship is consistent with the idea that the MOC filtering operation is optimized by frequency-specific suppression that enables spectral differentiation between signals and noise. In the presence of multiple voice streams, selective spectral filtering directed by descending connections from higher centers would be crucial for speech intelligibility.

Impaired signal extraction in noisy environments is a hallmark feature of age-related hearing loss. We hypothesize that hearing loss may result in an organizational disintegration of descending projections from the IC to auditory efferents. In this study, we have shown that the IC provides direct synaptic input to MOC efferents in hearing, hearing loss, and deafness whose morphology is indicative of excitatory transmission. We have mapped the topographic descending projection from the IC to the region containing MOC efferents, the VNTB, in the normal hearing, adult CBA/CaH mouse. We then investigated this organization of descending IC input in mouse models of early onset hearing loss (DBA/2) and congenitally deaf (Shaker-2) mice.

2. Materials and methods

All methods used for this report followed the guidelines established by the NHMRC and were approved by the Animal Ethics Committee for the Garvan Institute of Medical Research and St. Vincent's Hospital, University of New South Wales.

2.1. Mouse models of hearing, hearing loss and deafness

Three well characterized mouse strains were used in this study. Normal hearing, 3-month old and 6-month old CBA/CaH mice (Zheng et al., 1999) were used as baseline controls from which descending projections from IC to VNTB were investigated. The DBA/2 mouse exhibits early onset hearing loss at around 3 weeks of age due to a mutation in the Cadherin-23 gene (*Cdh23*), which affects the tip links of stereocilia atop hair cells (Willott et al., 1984; Hultcranz and Spangberg, 1997; Johnson et al., 2000; Wang and Manis, 2006). The homozygous Shaker-2 (*sh2/sh2*) mouse exhibits congenital deafness due to a point mutation in the gene, *Myo15*, on chromosome 11, whose expression is limited to the inner ear and pituitary gland (Probst et al., 1998; Liang et al., 1999). Functionally, the shortened stereocilia atop hair cells affect both auditory and vestibular systems producing phenotypic deafness and circling behavior (Beyer et al., 2000; Lee et al., 2003).

2.2. Auditory Brainstem Response (ABR) testing

ABR testing was performed prior to experimentation to verify that the degree of hearing ability in each cohort matched that reported in the published literature. A total of 31 animals were used to compile ABR data from the 3 mouse strains at 1, 3 and 6 months of age (Fig. 1). A subset of this ABR cohort was examined in the course of this study (Table 1). Animals were anaesthetized with ketamine/xylazine (50 mg/kg and 10 mg/kg, respectively) and placed in a double walled, sound attenuated chamber (Sonora Technology Co., Yokohama, Japan), on a battery operated, infrared heat pad. Needle electrodes were inserted into the skin at the vertex and pinna, with a ground reference inserted into the muscle of the hind leg (biceps femoris). Free field click (0.1 ms rectangular pulse, repetition rate of 10/sec) and tone stimuli (4, 8, 16, 24, 32 and 40 kHz; 5 ms duration; 0.5 ms rise-fall) were generated with a signal processor (RZ6; Tucker Davis Technologies [TDT], Alachua, FL) controlled by BioSigRZ software (v5.3; TDT), preamplified (Medusa RA16PA; TDT) and delivered using a magnetic speaker (MF1; TDT). Stimuli were presented at 10 dB decreasing steps from 90 dB SPL to 0 dB SPL. Averaged responses to 512 stimuli presentations were filtered (0.5–3 kHz; notch at 50 Hz), plotted and used to determine threshold.

2.3. Neuronal tract tracing

Discrete deposits of anterograde biotinylated dextran amine (BDA) tracer were made into the central nucleus of the IC (CNIC) to label descending axon projections and their terminals in the VNTB. Injections were guided by *in vivo* electrophysiological recordings of multi-unit responses to sound in awake 3 month (n = 6) or 6 month (n = 2) CBA/CaH mice, or placed stereotaxically in mice with hearing loss (6 month old DBA/2; n = 2) or congenital deafness (6 month old *sh2/sh2*; n = 3). Animals were prepared for awake electrophysiological recordings using published methods to install a head restraint under anaesthesia (Muniak et al., 2012). Briefly, the procedure involved securing the animal in a stereotaxic frame under general anaesthetic (isoflurane; 1.5–2.0% in ~600 cc/min O_2). A midline incision was made on the superior surface of the calvaria for installation of a head post and ground pin. Using a #11 scalpel blade to etch through the bone overlying the inferior colliculus, a

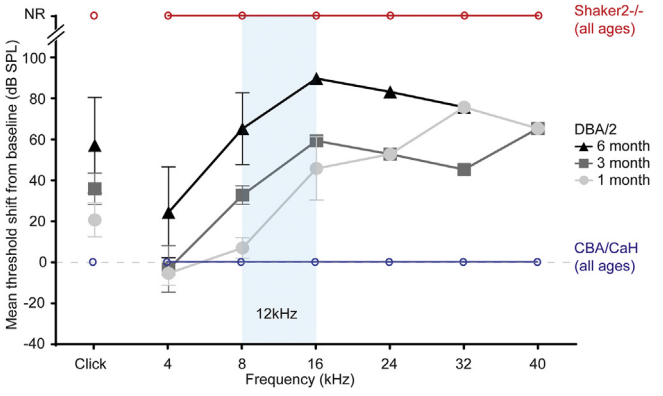

Fig. 1. Auditory Brainstem Response (ABR) thresholds shifts in DBA/2 compared to baseline, normal hearing CBA/CaH mice. At all ages, DBA/2 mice show significant threshold shifts in frequency regions greater than 16 kHz ($p < 0.0001$). One-month old DBA/2 mice start life with normal hearing between 4 and 8 kHz. Threshold shifts for DBA/2 mice are greatest at the 8–16 kHz region, and occur in a progressive manner. Because of the dynamic change in hearing status at this octave band, we targeted this 12 kHz frequency region (blue highlight) for our stereotaxic injections and anatomical analyses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)