Neuroscience 325 (2016) 50–62

REDUCED SENSORY STIMULATION ALTERS THE MOLECULAR MAKE-UP OF GLUTAMATERGIC HAIR CELL SYNAPSES IN THE DEVELOPING COCHLEA

M. BARCLAY,[a] R. CONSTABLE,[a] N. R. JAMES,[a] P. R. THORNE[a,b] AND J. M. MONTGOMERY[a]*

[a] *Department of Physiology and Centre for Brain Research, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*

[b] *Section of Audiology, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*

Abstract—Neural activity during early development is known to alter innervation pathways in the central and peripheral nervous systems. We sought to examine how reduced sound-induced sensory activity in the cochlea affected the consolidation of glutamatergic synapses between inner hair cells (IHC) and the primary auditory neurons as these synapses play a primary role in transmitting sound information to the brain. A unilateral conductive hearing loss was induced prior to the onset of sound-mediated stimulation of the sensory hair cells, by rupturing the tympanic membrane and dislocating the auditory ossicles in the left ear of P11 mice. Auditory brainstem responses at P15 and P21 showed a 40–50-dB increase in thresholds for frequencies 8–32 kHz in the dislocated ear relative to the control ear. Immunohistochemistry and confocal microscopy were subsequently used to examine the effect of this attenuation of sound stimulation on the expression of RIBEYE, which comprises the presynaptic ribbons, Shank-1, a postsynaptic scaffolding protein, and the GluA2/3 and 4 subunits of postsynaptic AMPA receptors. Our results show that dislocation did not alter the number of pre- or postsynaptic protein puncta. However, dislocation did increase the size of RIBEYE, GluA4, GluA2/3 and Shank-1 puncta, with postsynaptic changes preceding presynaptic changes. Our data suggest that a reduction in sound stimulation during auditory development induces plasticity in the molecular make-up of IHC glutamatergic synapses, but does not affect the number of these synapses. Up-regulation of synaptic proteins with sound attenuation may facilitate a compensatory increase in synaptic transmission due to the reduced sensory stimulation of the IHC.

Key words: cochlea, spiral ganglion neurons, development, conductive deafness, synapses, glutamate.

INTRODUCTION

The precise innervation of the inner hair cells (IHC) by the primary auditory neurons (type I spiral ganglion neurons, SGN) permits the accurate coding of timing and intensity information that is required for accurate transmission of sound information. This innervation, comprising specialized glutamatergic synapses between the IHC and type I SGN play a primary role in transmitting acoustic information from the inner ear to the brainstem (Geisler, 1998). Ribbon structures (Matthews and Fuchs, 2010) on the basolateral membrane of the IHC provide a pool of glutamate-containing vesicles that are rapidly released following stimulation (Khimich et al., 2005; Moser et al., 2006). This activates glutamate receptors, found within large post-synaptic densities on the terminal ending of the type I SGN fibers (Knipper et al., 1997; Furness and Lawton, 2003), and induces current influx into the type I SGN fibers (Glowatzki and Fuchs, 2002) and subsequently, the firing of an action potential.

Synapse consolidation in rodents occurs during the first 2 weeks of postnatal development and prior to the initiation of sound-mediated stimulation of the sensory hair cells and primary auditory neurons, which occurs around postnatal day 12 (Shnerson and Pujol, 1981; Song et al., 2006). During this period half of the synapses that form immediately after birth are pruned and there is an increased association between pre- and postsynaptic structures (Meyer et al., 2009; Huang et al., 2012). This period is associated with adenosine triphosphate (ATP) signaling in supporting tissues surrounding the IHC (Tritsch et al., 2007, 2010b; Dayaratne et al., 2015) and with spontaneous depolarizations in the IHC (Housley et al., 2006; Johnson et al., 2011) that drives activity in the primary auditory neurons (Jones et al., 1987, 2001; Tritsch et al., 2007; Tritsch et al., 2010a; Johnson et al., 2011) and ceases around the onset of hearing (Tritsch and Bergles, 2010) when sound-borne stimuli are first able to initiate a response in the sensory epithelium. It has been proposed that this intrinsic signaling contributes to the refinement of neural circuitry (Jones et al., 2001, 2007; Tritsch et al., 2010a). Subsequently, in the week

*Corresponding author. Tel: +64-9-923-9828.
E-mail address: jm.montgomery@auckland.ac.nz (J. M. Montgomery).
Abbreviations: ABR, auditory brainstem response; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; BSA, bovine serum albumin; EPSP, excitatory postsynaptic potential; GluA, AMPA receptor subunit; IgG (H + L), immunoglobulin (Heavy and Light chain); IHC, inner hair cell; NGS, normal goat serum; oC, organ of Corti; PBS, phosphate-buffered saline; SGN, spiral ganglion neuron.

http://dx.doi.org/10.1016/j.neuroscience.2016.03.043
0306-4522/

following the onset of hearing, synaptic transmission between the IHC and type I SGN matures resulting in increased excitatory postsynaptic potential (EPSP) amplitude at individual type I afferent terminals, and reduced EPSP time to peak and decay time constants (Grant et al., 2010). These data suggest that synaptic transmission strengthens after the onset of hearing; however, it is unclear if the onset of sound stimulation directs these synaptic changes.

Sensory deprivation during development is known to have profound effects on most auditory nuclei in the brain, including the cochlear nucleus, the superior olivary complex, the inferior colliculus and the auditory cortex (for a review see Sanes and Bao, 2009; Sanes and Kotak, 2011), however, its effect on the development of neural circuitry in the cochlea has not been examined. Determining how changes in sensory stimulation alter cochlear circuitry is important because synaptic transmission between IHC and SGN is the first step in transmitting auditory information to the brain, and type I SGN must accurately encode the acoustic signal. Eliminating sensory input to the afferent fibers is difficult to establish without affecting the health of the IHC and consequently the IHC-type I SGN synapse (Forge and Brown, 1982; Platzer et al., 2000). Therefore, conductive deafness has been used to assess how sound input affects the auditory circuitry. In addition to reducing activity along the auditory pathways (Tucci et al., 1999), it also appears to reduce spontaneous drive in these circuits (Tucci et al., 2001). Unilateral manipulations have included ear plugs, ear canal atresia and dislocation of the auditory ossicles. These manipulations have resulted in reduced dendritic branching in the nucleus laminaris (Smith et al., 1983), reduced binaural unmasking (Moore et al., 1999), reduced soma size in the spiral ganglion and cochlear nucleus (Webster, 1983), changes in the expression of AMPA and glycine receptors, increased structural complexity at Calyx of Held synapses in the auditory brainstem nuclei (Wang et al., 2011; Grande et al., 2014), and disruption of inhibitory plasticity in the auditory cortex (for a review see Sanes and Kotak, 2011). While these interventions do not eliminate sound stimulation, they provide a consistent 40–50-dB reduction in sensitivity, and when the firing characteristics of inhibitory neurons in the auditory cortex were compared between gerbils with a conductive hearing loss and those where the cochlea had been removed entirely, equivalent changes were observed (Xu et al., 2007; Takesian et al., 2010). Thus, even moderate reductions in auditory acuity can have a dramatic effect on auditory processing in the central nervous system (CNS), particularly if this occurs during development. While it has been postulated that these deficits arise in the brainstem nuclei (Sanes and Bao, 2009), few studies have examined how this attenuation affects neural innervation in the cochlea. In this study we have sought to unravel the role of sound stimulation on IHC-type I SGN synapse maturation by examining how a conductive hearing loss that was induced prior to the onset of hearing affected the number and structure of IHC-type I SGN synapses in the 10 days following hearing onset.

EXPERIMENTAL PROCEDURES

Animal procedures

All procedures in the study were approved by the University of Auckland Animal Ethics Committee. C57/Bl6 pups were anesthetized at P11 with isofluorane (5% for induction, 2.5% for maintenance of anesthesia) and an incision was made beneath the left ear to expose the ear canal and the tympanic membrane. A conductive hearing loss was induced by tympanic membrane rupture and malleus removal (Xu et al., 2007, 2010; Takesian et al., 2010; Takesian et al., 2012) and the incision was subsequently closed using Histoacryl® (Tissue Seal, LLC, USA). The right ear was left intact and served as the control.

Auditory brainstem responses (ABRs)

Mice with a unilateral conductive hearing loss were anesthetized with urethane (1.6 g/kg) at P10, P15 and P21 and placed on a heated pad to maintain body temperature at 38 °C in a polystyrene box. ABRs were recorded from right and left brainstem respectively in response to stimulation of right (control) and left (dislocated) ears using our standard procedures (Wong et al., 2010; Vlajkovic et al., 2011). Ear plugs were cut from E.A.R classic human foam earplugs, compressed and inserted into the ear canal of the unstimulated ear to avoid ‘‘cross” stimulation, and the animal was placed with this ear down. Three platinum electrodes were placed subdermally; at the mastoid region of the ear of interest, at the scalp vertex and in the left leg, to record the auditory-evoked responses. ES-1 electrostatic speakers (Tucker–Davis Technologies, Alachua, FL, USA) were placed 10 cm away from the ear and 5-ms tone pips between 4 and 40 kHz (0.5-ms rise-fall time) were presented, starting at 90 dB and progressively reducing in intensity by 5 dB. Stimuli were presented and the responses recorded with a Tucker–Davis Technologies auditory physiology workstation (USA) and Biosig digital signal processing software (USA). Responses were averaged from 512 repeats at each sound level (alternating polarity) and the ABR threshold was defined as the lowest intensity at which a wave I and II were visually detected above the noise floor.

Fixed tissue preparation

Following ABR measurements, P15 and P21 mice were euthanized by transcardial injection of urethane. Additional P15 and P21 mice that did not undergo ABR measurements as well as P9 pre-hearing controls were euthanized by intraperitoneal injection of sodium pentobarbitol (90 mg/kg, Nembutal, Vibrac Laboratories, New Zealand). Temporal bones were immediately immersed in Shandon Glyofixx (Thermo-Fisher, Waltham, MA, USA), cochleae were removed and perfused via the oval window and then post fixed overnight (Huang et al., 2012). Cochleae were subsequently decalcified in TBD-1™ Decalcifier (Thermo-Fisher, USA) for 5 min and the bony capsule, lateral wall,