Phenotyping the differential innervation of the peripherin knockout mouse cochlea

C. Perera,[1] G. von Jonquieres,[1] J.L. Pinyon,[1] K.W. Parley,[1] J.M.E. Cederholm,[1] J.-P. Julien,[2] A.F. Ryan[3] and G.D. Housley ,[1] [1]Translational Neuroscience Facility & Department of Physiology, School of Medical Sciences, UNSW Australia NSW 2052, Australia, [2]Department of Psychiatry & Neuroscience, Laval University, Quebec, G1J 2G3, Canada and [3]University of California, San Diego and the VA Medical Center, 9500 Gilman Drive, La Jolla, CA 92093, USA.

Peripherin (Prph) is a neuronal type III intermediate filament, that, as it name suggests, is primarily expressed by neurons outside the CNS and contributes to axon outgrowth and synaptic stablization. By way of example, the majority of small and intermediate–sized dorsal root ganglion neurons express Prph. In the mouse cochlea Prph is only expressed by the unipolar type II spiral ganglion neurons, which are the 5% of primary afferents that innervate the outer hair cells. We recently reported that the Prph knockout mouse lacked contralateral suppression, where noise presented to one ear failed to cause rapid medial olivocochlear (MOC) efferent-based suppression of sound transduction in the opposite ear, evident as a reduction in the amplitude of distortion product otoacoustic emissions. This was associated with disruption of the type II spiral ganglion neuron (type II SGN) innervation of the cochlear outer hair cells (Froud *et al.* 2015), assessed using neurofilament 200 immunofluorescence and blockface SEM tomography. These approaches showed that there were few remaining type II SGN fibres in the outer spiral bundles (OSBs) and an absence of afferent boutons at the base of the outer hair cells in the apical of the two turns of the mouse cochlea. In the present study we further resolved the nature of Prph expression throughout the mouse cochlea using double immunofluorescence labelling of Prph alongside NF200 and β-III tubulin (tuj1), and independently determined the impact of loss of expression of peripherin on the synaptic boutons by imaging the terminal swellings of the type II SGN using parvalbumin immunofluorescence. The study was undertaken on cochlear tissue fixed with paraformaldehyde, following a protocol for euthanizing the mice with pentobarbital (Lethabarb) approved by the UNSW Australia Animal Care and Ethics Committee. The experiments revealed that in adult wildtype mice, tuji1 immunolabelling of OSB peripherin positive type II SGN fibres were in greater numbers than that evident with NF200 labelling, and in the knockout mice, more tuj1-positive fibres were resolved in the apical turn region than that previously reported using NF200. However, proportionately more OSB fibres were also evident in the WT mice, so the differential remained. The disruption of the synaptic boutons at the outer hair cells was also confirmed for the apical turn region based on parvalbumin immunofluorescence in cyrosections. In the basal (high frequency encoding) region, parvalbumin labelling resolved OSB fibres and their bouton-like structures at the basal pole of the outer hair cells and potentially along the associated Deiters' supporting cells, but this was still considerably weaker than that seen in the WT cochlear tissue. Overall, these data support the concept that the type III intermediate filament protein peripherin is a key protein for the maturation of the type II spiral ganglion innervation of the cochlea, and that absence of expression of this protein disrupts the afferent innervation of the outer hair cells. This supports the concept that sensory coding from the 'cochlear amplifier', which is derived from the outer hair cell electromotility, drives the MOC efferent feedback suppression. Supported by NHMRC grant APP1052463.

Froud KE, Wong ACY, Cederholm JME, Klugmann M, Sandow SL, Julien J-P, Ryan AF, Housley GD. (2015) Type II spiral ganglion afferent neurons drive medial olivocochlear reflex suppression of the cochlear amplifier. *Nature Comm* **6**:7115.