Abstract: 57O

Novel primary afferent auditory and vestibular neuron phenotypes in the mouse inner ear resolved using peripherin promoter-driven transgene reporters and subtype-selective immunofluorescence

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Background: Resolving the expression profiles of the sub-populations of auditory and vestibular afferents spanning development and aging informs the physiology of hearing and balance. The cochlear primary afferents (spiral ganglion neurons – SGN) innervating the inner hair cells (IHC) have recently been classified as Type Ia, Ib and Ic via scRNAseq analysis (Sun, *et. al.*, 2018). Outer hair cells (OHC) are innervated by Type-II SGNs, whose function contributes to the sensory drive of the medial olivocochlear efferent feedback loop that controls the electromotility of OHC, underlying amplification and filtering of sound transduction. Type-II SGNs can be delineated by immunolabelling for the type III intermediate filament peripherin (Cederholm, *et. al.*, 2022). In the vestibular system, Type-I hair cells are innervated by calyx afferents and dimorphic afferents, while Type-II hair cells synapse with dimorphic afferents and peripherin immunopostive neurites terminating at bouton synapses (Leonard, *et. al.* 2002). To advance understanding of inner ear primary afferents with regard to neuron population, distribution and synaptic architecture, we established a reporter mouse line (TNF-43) which utilises elements of the peripherin promoter.

Methods: The TNF-43 transgenic mouse line was generated through microinjection of the $Prph_p$-hDTR-IRES-mCherry plasmid into the pronucleus of a C57Bl/6J mouse. Transgene positive neurons express the human diphtheria toxin (hDTR) and mCherry fluorescent reporter. With approval of the UNSW Animal Care & Ethics Committee, the distribution of the TNF-43 mCherry-positive afferent innervation of the cochlea and vestibular systems was mapped in inner ear tissue obtained from mice from birth to 4 months of age. The tissue was fixed in paraformaldehyde, decalcified and then either cryosectioned at 40 µm and immunolabelled floating; or were kept whole and processed through a method we optimised for visualising immunolabelled neurons intact with CUBIC/PEGASOS clearing and Light sheet Z1 imaging. Antibodies used included: mCherry (transgene reporter), TUBB3 (pan-neuronal), PRPH (Type-II SGN/bouton afferents), CALB1 (Calbindin - Type-Ia SGN marker), CALB2 (Calretinin - Type-Ib marker/calyx and dimorphic afferents) and POU4F1 (Brn3a - Type-Ic SGN marker).

Results: The TNF-43 transgenic mouse model identified a discrete sub-population of mCherry-positive auditory neurons. This subpopulation shows overlap with immunofluorescence markers across SGN subtypes. Most distinctly, the transgenic subpopulation shows significantly increased expression in the most basal (high frequency – encoding) region of the spiral ganglion, which was greatest in the first post-natal week and declined with age. Expression is also observed in small/medium diameter neurons of the vestibular system, consistent with innervation of non-calyx (type II) vestibular hair cells. The expression patterns of TNF-43 mCherry positive neurons have been assessed in comparison to their overlap with known type and subtype markers of auditory and vestibular ganglion neurons. This work contributes to the molecular differentiation of inner ear afferents with regard to gene regulation and proteomic plasticity. The TNF-43 transgenic mouse line provides the opportunity for hDTR-based selective-ablation of the resolved neuron subpopulations, to achieve new insights into inner ear physiology.

References: Cederholm JME, Parley KE, Perera CJ, von Jonquieres G, Pinyon JL, Julien J-P, Ryugo DK, Ryan AF, Housley GD. 2022 *Front. Neurol – Neuro-Otol*. Doi: 10.3389/fneur.2022.962227 / Leonard RB and Kevetter GA. *Brain Res* 2002 **928**:8-17 / Sun S, Babola T, Pregernig G, So K, Nguyen M, Su M, Palermo A, Bergles DE,1 Joseph C. Burns JC, Müller U. 2018 *Cell.* **174**(5):1247-1263

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