Differential cochlear afferent neuron response to BDNF in postnatal mouse lacking canonical transient receptor potential channel subtype 3 (TRPC3) expression

A.C.Y. Wong,[1] A. Ng,[1] L. Birnbaumer[2] and G.D. Housley,[1] [1]Translational Neuroscience Facility & Department of Physiology, School of Medical Sciences, The University of New South Wales, NSW 2052, Australia and [2]Laboratory of Neurobiology, National Institute of Environmental Health Science NIH, Research Triangle Park, NC27709, USA.

Canonical transient receptor potential (TRPC) channels belong to the superfamily of non-selective cation-permeable transient receptor potential (TRP) channels, which are widely expressed in the mammalian central and peripheral nervous system. TRPC channels regulate cochlear hair cell Ca^{2+} homeostasis and shape sound transduction and auditory neurotransmission (Raybould *et al.*, 2007; Wong *et al.*, 2013). The cochlear afferent neurons, or spiral ganglion neurons (SGN), innervate the sensory hair cells and SGN neuritogenesis is supported by neurotrophic signaling, particularly through brain-derived neurotrophic factor (BDNF)-TrkB receptor-phospholipaseC/diacylglycerol (DAG) signaling pathway. Here we investigated whether TRPC3 channels known to be expressed by SGN are an effector for this BDNF–TrkB signalling (*via* DAG) in neonatal cultured mouse SGN explants.

TRPC3 channels have been shown to support neural growth cone development, chemotaxis and neuritogenesis in the developing cerebellum (Jia *et al.*, 2007). SGN were prepared from cochleae from P5 wildtype (C129/SvEv background) and TRPC3 null (TRPC3KO) mice. The spiral ganglion was dissected from the cochlear tissue, divided into apical, mid and basal cochlear turns, and were cultured for 48 hours in culture medium containing BDNF (10–100 ng/ml). To identify neurite outgrowth, the explants were immunolabelled with a rabbit polyclonal 200 kDa anti-neurofilament primary antibody, followed by Alexa-594 conjugated secondary antibody. Micrographs were obtained by epifluorescence microscopy. The number of neurites emanating from the explants, the cumulative neurite length and the number of secondary branches, were analysed and quantified with Neuron J/Image J (NIH) software.

Results: Neuritogenesis of the C129/SvEv SGN explants was significantly enhanced by the BDNF treatments. The average cumulative neurite lengths of 100 ng/ml BDNF-treated explants exhibited a significant gradient where Base > Mid > Apex ($p = 0.017$, ANOVA), which was lacking in the corresponding controls (no BDNF; $p = 0.121$, ANOVA). In support of our hypothesis that TRPC3 channels contribute to this BDNF-TrkB signalling mechanism, neuritogenesis was significantly less (4.5× shorter cumulative neurite length) in 100 ng/ml BDNF-treated TRPC3KO SGN basal turn explants compared with the wildtype controls ($p < 0.001$, Holm-Sidak comparisons, two-way-ANOVA).

Conclusion: Our results support TRPC3 channels as an effector of BDNF-mediated neuritogenesis during the establishment of the afferent innervation of the cochlea.

Jia Y, Zhou J, Tai Y & Wang Y. (2007) *Nature Neuroscience* **10**, 559-67.

Raybould NP, Jagger DJ, Kanjhan R, Greenwood D, Laslo P, Hoya N, Soeller C, Cannell MB & Housley GD. (2007) *Journal of Physiology* **579**, 101-13.

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