Analysis of the gene expression response to a temporary reduction in hearing sensitivity

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Acoustic overstimulation leads to a shift in hearing sensitivity, and related hearing loss, and is a global problem in modern society. The intensity of the noise determines if the shift in hearing sensitivity is temporary (TTS) or permanent (PTS). While many studies have investigated the effect of noise on cochlear structures (*e.g.* Chen, 2006; Hirose & Liberman, 2003; Liberman & Dodds, 1984) and gene expression after PTS-inducing noise (*e.g.* Cho *et al.*, 2004; Kirkegaard *et al.*, 2006; Gratton *et al.*, 2011; Tornabene *et al.*, 2006), it is also imperative to examine the possible transcriptional response to TTS-inducing noise. We used a gene array approach to determine cochlear gene regulation in response to TTS-inducing noise in C57Bl/6J mice.

Evoked auditory brainstem responses (ABR) were measured to broadband clicks, or 16 kHz tone pips, before and after 30 min noise exposure (86 dB or 95 dB, 4 – 32 kHz), or before and after 30 min of no-noise in control mice. Separate experiments measuring ABR to broadband clicks, or 4, 16, 24 and 32 kHz tone pips to these noise regimes were also performed in order to elucidate the time of recovery and verify the TTS. All mice were anaesthetised with a ketamine/xylazine/acepromazine cocktail as previously described (Cederholm *et al.*, 2012), and in accordance with University of New South Wales (UNSW) Animal Care and Ethics Committee approval. Cochlear RNA extraction was performed on tissue collected 1, 2, 4, 8 and 24 h after the noise exposure. cDNA templates were hybridised to the Affymetrix® mouse gene array 1.1ST, with gene expression analysis carried out using GenePattern software (http://www.broadinstitute.org/ cancer/software/genepattern). Statistically significant changes in gene expression were identified as having a p-value of <0.001 and a minimum of a 2-fold up- or down-regulation. The noise regimes produced on average a 12 ± 1.1 dB shift for 86 dB noise, and on average a 41 ± 3.0 dB shift for 95 dB noise. These shifts were confirmed to be TTS and fully recovered within 1 and 3 weeks, respectively.

We found 63 genes that were significantly regulated compared to no-noise controls, with the largest number of responsive genes at 4 h. 18 of the genes found in the 86 dB study, were also found at 95 dB. Identified genes were mainly involved in transcriptional regulation, tight junction, morphology, cell migration, inflammatory and oxidative stress responses. A number of the identified genes were verified using real-time qPCR. We have shown, to our knowledge, for the first time transcriptional responses to TTS-inducing noise. The TTS-regulated gene set we have identified here likely reflects cellular responses to noise stress that contribute to hearing adaptation and protection from noise-induced hearing loss (Housley *et al.*, 2013).

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