Transcriptional responses to noise stress in mice

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Exposure to excessive noise and the associated hearing loss is an increasing global problem in modern society. Following acoustic overstimulation a shift in hearing sensitivity is observed and depending on the intensity of the noise, a temporary (TTS), or permanent, threshold shift (PTS) will occur. In contrast to PTS, changes in hearing sensitivity after TTS-inducing noise are reversible. Many cochlear structures affected by noise overstimulation have been identified (*e.g.* Chen, 2006; Hirose & Liberman, 2003; Liberman & Dodds, 1984), and investigations into related changes in gene expression have been carried out. These studies focused on loud, PTS-inducing noise, in rats (*e.g.* Cho *et al*., 2004; Kirkegaard *et al*., 2006) and mice (*e.g.* Gratton *et al*., 2011; Tornabene *et al*., 2006). It is, however, also important to understand the possible transcriptional responses underlying TTS. Here we report the regulation of cochlear gene expression in C57Bl/6J mice in response to TTS-inducing noise.

Evoked auditory brainstem responses (ABR) were measured to broadband clicks, or 16 kHz tonepips, before and after 30 min noise exposure (86 dB or 95 dB, 4 – 32 kHz). Control mice underwent the same procedure, but were not exposed to noise. Experiments were conducted on mice anaesthetized with a ketamine/xylazine/acepromazine cocktail as previously described (Cederholm *et al*., 2012), and in accordance with University of New South Wales' Animal Care and Ethics Committee approval. That noise regime produced on average 12 ± 1.1 dB TTS for 86 dB noise, and on average 41 ± 3.0 dB for 96 dB noise. Separate experiments showed that these levels of TTS fully recovered within two weeks. Cochlea RNA extraction (RNeasy Plus Mini kit, Qiagen) was performed on tissue collected 1, 2, 4, 8 and 24 h after the noise exposure (pentobarbital overdose prior to tissue collection). cDNA template was hybridized to the Affymetrix® mouse gene array 1.1ST. Gene expression analysis was performed 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.

We identified a number of genes that were up-regulated across all five times with TTS. The two most highly regulated genes after 86 dB noise exposure showed almost 20-fold up-regulation at 4 h. Up-regulation typically commenced two h after the noise exposure. We have shown, to our knowledge, for the first time transcriptional responses to TTS-inducing noise. The majority of the genes previously reported to be regulated after PTS-inducing noise was not observed to be responsive to TTS-level noise in our study. 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.

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