Noise-induced hearing adaptation kinetics of the 'cochlear amplifier' maps to $P2X_2$ receptor-dependent auditory brainstem response temporary threshold shift

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ATP-gated ion channels assembled from $P2X_2$ receptor ($P2X_2R$) subunits are expressed in the cochlea and activated by elevated sound levels. $P2X_2R$ contribute significantly to the development of auditory brainstem response (ABR) temporary threshold shift in wildtype mice exposed to sustained sound around safe workplace limits (85 dB SPL). In contrast, mice null for the *P2rx2* gene (*P2rx2*$^{(-/-)}$) that encodes the $P2X_2R$ failed to develop this reversible noise-induced hearing loss. Moreover, when these knockout mice were exposed to louder noise levels, they exhibited disproportionately greater permanent hearing loss than the wildtype controls; hence purinergic hearing adaptation is otoprotective (Housley, *et al.*, 2013). Loss of function mutations in the human $P2X_2R$ gene cause vulnerability to noise-induced and age-related hearing loss (Yan *et al.*, 2013; Faletra *et al.*, 2014; Moteki *et al.*, 2015), supporting a protective role for $P2X_2R$. The time constant for development of the reversible purinergic hearing adaptation in wildtype mice was previously estimated at ˜ 20 minutes based on shifts in ABR thresholds with cumulative noise exposure from 10 minutes to two hours (Housley, *et al.*, 2013).

Here we sought to improve resolution of the kinetics of this purinergic hearing adaptation and to investigate the contribution of the outer hair cell 'cochlear amplifier' to this mechanism. To achieve this, rates of adaptation of hearing sensitivity with noise exposure were compared between wildtype and *P2rx2*$^{(-/-)}$ mice (C57Bl/6J background; anaesthetized (*i.p.*) with a cocktail of ketamine (40 mg/kg), xylazine (8 mg/kg), acepromazine (0.5 mg/kg) or isoflurane (4% induction, 1-1.5% maintenance with O_2)) using ABR and cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) measurements. DPOAEs report changes in the gain of the cochlear amplifier that stem from alterations in cochlear outer hair cell electromechanical transduction and associated organ of Corti micromechanics. We found that both the ABR and DPOAE threshold shifts were largely complete within the first 7.5 minutes of moderate noise exposure (85 dB SPL; 8 – 32 kHz) of wild-type mice. ABR threshold shift after 7.5 minutes noise was 0.77 ± 0.07 of the 7.92 ± 1.06 dB threshold shift measured at 17.5 minutes (means ± s.e.m.; n = 12). Similarly, for the DPOAE, threshold shift at 7.5 minutes noise was 0.76 ± 0.08 of the 14.4 ± 1.6 dB shift at 17.5 minutes (n = 8). As previously noted, the noise exposure failed to produce significant changes in either ABR or DPOAE thresholds in the *P2rx2*$^{(-/-)}$ mice.

These findings document a considerably faster purinergic hearing adaptation to noise than previously reported. Moreover, the similarity in kinetics of ABR and DPOAE measurements implicate the 'cochlear amplifier' as the site of action of adaptation, as ABR reflects downstream neural activity.

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