Cholinergic efferent synaptic transmission regulates the maturation of auditory hair cell ribbon synapses

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This document includes four Supplementary Figures with legends.
Supplementary Figure 1. Potassium currents in immature IHCs from α9nAChs KO mice.

(a,b), Potassium currents recorded from a control and α9nAChR KO immature IHC (P4-P5), respectively. Membrane currents were elicited in response to depolarizing voltage steps in 10 mV increments from –143 mV (holding potential –84 mV) to the various test potentials shown by some of the traces. All IHCs expressed K⁺ currents characteristic of immature IHCs with similar amplitudes [1]. (c), Current-voltage curves from seven P4 control and nine P4-P5 KO α9nAChs mice.
Supplementary Figure 2. Exocytotic Ca\textsuperscript{2+} dependence is normal in α9nAChR KO immature IHCs

Data are from apical coil control (black) and α9nAChR KO (red) immature IHCs (P5-P7). (a), $I_{C_a}$ and corresponding $\Delta C_m$ recordings as described in figure 2 (main manuscript). (b), Average $I_{Ca}$-voltage (bottom) and $\Delta C_m$-voltage (top) curves in control and α9nAChR KO IHCs. (c), Synaptic transfer curves obtained as described in figure 2 (main manuscript). Fits in C are according to eqn. 1. Power values were: control 3.6 ± 0.4, $n = 7$; KO 3.3 ± 0.4, $n = 6$. 
Supplementary Figure 3. Current and voltage responses in mature IHCs from control and KO α9nAChR and Syt 2 mice.

(a,b), Potassium currents recorded from a control and a KO IHC of adult α9nAChR and Syt 2 mice, respectively. Membrane currents were elicited in response to depolarizing voltage steps in 10 mV increments from −144 mV (holding potential −64 mV) to the various test potentials shown by some of the traces. All IHCs expressed K⁺ currents characteristic of mature IHCs (Iₖ, Iₖ,f and Iₖ,n) with similar amplitudes (see Table 1 in main manuscript and also Ref. 1). (c,d), Voltage responses under whole-cell current clamp in a control and a KO IHC from mature α9nAChR and Syt 2 mice, respectively. Responses were elicited by applying depolarizing current injections in 100 pA increments from the IHC resting membrane potential. For clarity, only a few voltage responses are shown.
Supplementary Figure 4. Diagram illustrating how the efferent system controls the maturation of the immature cochlea.

Schematic representation of an immature (left) and adult (right) IHC with afferent fibres (blue), and cholinergic axosomatic (green) and axodendritic (orange) efferent terminals. Note that the number of axosomatic efferents in adult IHCs (shown faded with “?”) is extremely small [2,3] and it is currently unknown whether they have physiologically relevant role, or whether they are “leftovers” from an incomplete synaptic restructuring. The release of ACh from the efferent fibres contacting immature IHCs involves synaptotagmins 1 and 2 (A; indicated by colour-coded vesicles). When ACh binds to α9α10AChRs on IHCs it causes Ca\(^{2+}\) influx that activates SK2 channels and IHC hyperpolarization. This hyperpolarization prevents spontaneous action potential activity, thus influences its frequency and pattern (B). Normal action potentials during the second postnatal week (~P7-P12) are required for the linearization of the exocytotic Ca\(^{2+}\) dependence (C) in adult IHCs [4]. Here we found that
when α9AChRs or synaptotagmin 2 are absent the linearization did not occur, providing the first indication for a functional role of the efferent system in the developing cochlea. The altered action potential activity in early postnatal IHCs from α9AChRs or synaptotagmin 2 knockout mice would also affect the patterning of spike activity in the immature afferent fibres (D), which in turn could disrupt the sharpening of tonotopic maps in the auditory brainstem nuclei known to mainly occur during the first postnatal week [5]. Note that the round and ellipsoid black structures in IHCs define ribbons tethering glutamate-containing synaptic vesicles, the fusion of which to the presynaptic site is regulated by otoferlin and synaptotagmin 1 in immature IHCs and otoferlin and synaptotagmin 4 in adult cells [6]. Glutamate release will activate the postsynaptic afferents. With development, the spontaneous discharge patterns of afferent fibres becomes more regular.
References


