Supplemental Data 4: Modeling sound driven bushy cell spiking

In order to determine whether the change in $P_r$ seen at the endbulb of Held in vitro could account for the changes in first spike latency observed in vivo, we turned to a model of these initial stages of auditory processing. The model included processes beginning with generation of spontaneous and sound-evoked action potentials in the SGN fibers through synaptically-evoked action potentials in the bushy cells. Sound-evoked spike patterns in SGN fibers were modeled using a non-stationary renewal process. The rate of the renewal process is chosen such that modeled action potential rates match the rates observed in auditory nerve fibres of C57Bl/6 mice producing appropriate values for peak rate, adapted rate and spontaneous rate of SGN firing. Action potentials in SGN fibers were then converted to EPSC amplitudes using a model of short-term plasticity (Yang & Xu-Friedman, 2008), with parameters adjusted to replicate the average behavior of endbulbs in control mice. The absolute amplitude of the EPSCs was set by the parameter EPSC$_{amp}$, which was the maximum possible EPSC amplitude (i.e. a release of the entire readily releasable pool of vesicles). The sum of EPSCs from a number of fibers converging onto the same bushy cell was used to drive a model of bushy cell action potential generation. This was done with a single compartment model having Na+, K+ and leak conductances. Whenever available, parameters were chosen according to in vitro experiments from bushy cells. EPSC kinetics and amplitude, as well as bushy cell capacitance and leak resistance were based on values measured during voltage- and current-clamp experiments (Fig. 6). The time of the first action potential of the single compartment model was used as the first spike latency.

To determine whether the effect of CPX I knockout on $P_r$ accounted for the in vivo data, we fixed all other model features. We used identical statistics of action potential firing in SGN axons and identical intrinsic properties of bushy cells. Only the EPSC amplitude and short-term synaptic plasticity changed as a result of the decrease in $P_r$.

The following parameters were varied to obtain different FSL distributions:
1. The number of auditory fibers converging onto a given bushy cell. And for each of those fibers:
2. the spontaneous, peak and adapted rates of action potential firing and
3. the initial EPS conductance relative to the firing threshold of the bushy cell.

Database:

- **in-vivo** auditory nerve fiber (ANF) extracellular recordings from wild-type mice (Fig. 4F)
- **in-vitro** recordings from bushy cells:
  - spike threshold in dynamic clamp experiments (Xu-Friedman and Regehr, 2005)
  - EPSC amplitudes in patch clamp experiments with minimal presynaptic stimulation of ANFs in CPX I⁺/⁺ and CPX I⁻/⁻ mice (this paper)
- **in-vivo** extracellular recordings from AVCN cells in CPX I⁺/⁺ and CPX I⁻/⁻ mice (this paper)

**Short description:**

**Step 1: SGN action potential generation**

*In-vivo* firing patterns of SGNs are mimicked by an inhomogeneous Poisson process. The key point of this first step is, to closely match experimentally observed spike rates (PSTH, see Fig 4F). While sound intensity increases with a \( \sin^2 \) time course for the duration \( \tau_{\text{onset}} \) the release rate increases only during a fraction of this time because the dynamic range of the fibres comprises only a fraction of the stimulus intensity. A good match of the experimental data could be obtained setting this fraction to 0.5. After the rate increased from the spontaneous rate \( (SR) \) to the peak rate \( (PR) \) during \( \tau_{\text{onset}}/2 \) it decays exponentially to the adapted rate \( (AR) \) with a time constant \( \tau_{\text{adapt}} \) of 5 ms. The amplitude of the instantaneous rate is

\[
\text{rate} = \begin{cases} 
SR & t < t_{\text{sound on}} \\
SR + (PR - SR) \cdot \sin^2 \left( \frac{\pi}{\tau_{\text{onset}}} \frac{0.93626}{t_{\text{onset}}} \right) & t_{\text{sound on}} \leq t < t_{\text{sound on}} + \frac{\tau_{\text{onset}}}{2} \\
AR + (PR - AR) \cdot 0.98957 \cdot e^{-\frac{t - (t_{\text{sound on}} + 0.5 \tau_{\text{onset}})}{\tau_{\text{adapt}}}} & t \geq t_{\text{sound on}} + \frac{\tau_{\text{onset}}}{2}
\end{cases}
\]

For an optimal match, \( t_{\text{sound on}} \) had to include a 1.4 ms delay relative to the experimental time of sound onset. To implement the refractory period, we used a renewal function
including an absolute refractory period, $t_{abs}$, and time constant of the relative refractory period, $\tau_{rel}$ (Miller et al., 2001):

$$f_{\text{renewal}}(\Delta t) = \begin{cases} 0 & \Delta t < t_{abs} \\ 1 - \exp\left(\frac{t - t_{abs}}{\tau_{rel}}\right) & \Delta t \geq t_{abs} \end{cases}$$

(2)

where $\Delta t$ is the time since the last AP. Generation of the next AP time point $t_{n+1} = t_n + \Delta t$ was implemented as a inhomogeneous point process with the rate $R_{ves}(t) \times f_{\text{renewal}}$ following the algorithm described by (Berry and Meister, 1998).

Using $SR = 10 \text{ s}^{-1}$, $PR = 575 \text{ s}^{-1}$ and $AR = 261 \text{ s}^{-1}$ this part of the model produced trains of APs with poststimulus time histograms (PSTH) closely resembling the average of in vivo recordings in control and CPX1$^-/-$ mice (Figure 4 main manuscript and Supplemental Figure below).

Supplemental Figure: Validation of the first modeling step: poststimulus spike time histogram of modeled spike times closely matches the experimental data. Furthermore, the instantaneous rate that follows equation 1 is show as a dashed line. For the parameters of the instantaneous rate see text.

SRs and ARs in C57BL/6 SGNs show considerable variability over the population of SGNs (see error bars in Figure 4F of the main manuscript). By adjusting
these parameters, it was possible to construct SGNs that resembled experimental samples. The PR of SGNs recorded in vivo appears to be correlated to the AR. We approximated this correlation as a linear dependence between the logarithms of the two rates. This allowed us to construct random samples of SGNs matching experimental samples in the sense that plots of randomly drawn ARs and matched PRs resembled plots of experimentally determined rates.

Parameters used in step 1:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{\text{sound_on}}$</td>
<td>time at which sound starts</td>
<td>Fig. 8E: 100 ms</td>
</tr>
<tr>
<td>$\tau_{\text{onset}}$</td>
<td>duration of the ramp in</td>
<td>Fig. 8E: 5 ms</td>
</tr>
<tr>
<td>$\tau_{\text{adapt}}$</td>
<td>time constant of SGN rate adaptation</td>
<td>5 ms</td>
</tr>
<tr>
<td>$t_{\text{abs}}$</td>
<td>absolute refractory period</td>
<td>330 µs</td>
</tr>
<tr>
<td>$\tau_{\text{rel}}$</td>
<td>time constant of relative refractory period</td>
<td>410 µs</td>
</tr>
<tr>
<td>SR</td>
<td>spontaneous firing rate for SGN axons</td>
<td>Fig. 8E, bottom left: 2 Hz</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fig. 8E, bottom right: 0.1–1 Hz</td>
</tr>
<tr>
<td>AR</td>
<td>adapted firing rate for SGN axons</td>
<td>Fig. 8E, bottom left: 100 Hz</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fig. 8E, bottom right: 20 randomly drawn values (see text) average 216 Hz</td>
</tr>
<tr>
<td>PR</td>
<td>peak firing rate for SGN axons</td>
<td>Fig. 8E, bottom left: 300 Hz</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fig. 8E, bottom right: 20 randomly drawn values (see text) average 526 Hz</td>
</tr>
</tbody>
</table>

**Step 2: Endbulb synaptic plasticity**

The SGN spike trains were next translated into EPSC amplitudes. In the paper we characterized plasticity at the endbulb of Held synapse made by SGNs onto bushy cells. We used this information to generate a model of endbulb plasticity based on the approach and the nomenclature of Yang and Xu-Friedman, 2008). For a train of activity, the $i^{th}$ EPSC amplitude is given by:
EPSCᵢ = EPSCamp × Fᵢ × Dᵢ ,

where Dᵢ is the fraction of the releasable pool of vesicles that is available, and Fᵢ is the probability of release at that moment, and EPSCamp was the maximum possible EPSC amplitude (i.e. resulting from simultaneous release of the entire readily releasable pool of vesicles). Initially, D₁ is equal to 1, and F₁ is the initial probability of release (Pᵣ). The value of Fᵢ depends on the concentration of a hypothetical calcium-bound form of a sensor (CaF), which had arbitrary units. CaF instantaneously increased by 1 during each AP (occurring at time tᵢ) and decayed exponentially between APs:

\[
\frac{dCa_F}{dt} = -\frac{Ca_F}{\tau_F} + \delta(t - tᵢ)
\]

(4)

Fᵢ depends on the value of CaF immediately before the AP (i.e. at time tᵢ₋ε) according to a simple bimolecular binding reaction characterized by the binding constant Kᵢ:

\[
Fᵢ = F₁ + \frac{(1 - F₁)}{1 + \frac{CaF(tᵢ - ε)}{Kᵢ}}
\]

(5)

EPSCamp was assigned to each SGN individually. Although it was more convenient to write “EPSC”, this is properly thought of as a conductance. The first EPSC (EPSC₁ = EPSCamp × F₁) was set relative to the threshold conductance (Gthresh) that is able to elicit a postsynaptic AP in the bushy cell, Gthresh. A SGN with EPSC₁ < Gthresh is considered sub-threshold, while a SGN with EPSC₁ > Gthresh is considered supra-threshold.

In addition, after release, the fraction of vesicles available decreased. The pool of vesicles recovered at an initial slow rate k₀. This recovery rate was activity dependent, and was modelled as resulting from a calcium-dependent process, using the approach of Dittman and Regehr (1998), which could increase the recovery rate up to a maximal rate kmax. This calcium-dependent process decayed exponentially with a time constant τD and had an affinity for the recovery of KD.

One difference between this model and that of Yang and Xu-Friedman (2008) was that receptor desensitization could be neglected in these mouse strains (originally:
C57BL/6-129SV, back-crossed into C57BL/6 for 5 generations). This was suggested by the paired-pulse kinetics in Fig. 6C.

Once the amplitude of each EPSC in the stimulus train was determined, these EPSC amplitudes were convolved with the EPSC shape measured in voltage-clamp recordings. Finally the EPSC trains from all fibers were summed to yield the synaptic conductance $G_{syn}(t)$.

### Parameters used in step 2:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
</table>
| $F_1$     | Initial release probability | CPX I$^{+/+}$: 0.36  
                        CPX I$^{-/-}$: 0.09 |
| $\tau_F$  | Decay time constant for the facilitation sensor | 30 ms |
| $K_F$     | Affinity of the facilitation sensor for release | 11 |
| $k_0$     | Slow pool recovery rate | 1 s$^{-1}$ |
| $K_D$     | Affinity of the calcium sensor for recovery | 1 |
| $\tau_D$  | Decay time constant for calcium-dependent recovery | 30 ms |
| $k_{max}$ | Maximum pool recovery rate | 20 s$^{-1}$ |
| $EPSC_{amp}$ | Maximum EPSC amplitude | Fig. 8E, bottom left: 4.4$\times G_{thresh}$  
                        Fig. 8E, bottom right: 0.67$\times G_{thresh}$ |

We emphasize that the value of $F_1$ was the only parameter that appears changed between the wildtype and mutant conditions.

### Step 3: Bushy cell spike generation

We next modelled how synaptic input drives action potential generation in the bushy cell. For the integration of few EPSCs in rapid succession this process has successfully been approximated by a perfect integrator (Xu-Friedman and Regehr, 2005). In the present situation, EPSCs might occur with long intervals, so a more complex, conductance based model was devised. The bushy cell membrane potential follows:
\[
\frac{dV_{\text{mem}}}{dt} = -C_{\text{mem}} \sum_{x=K,Na,\text{leak}} (V_{\text{mem}} - V_x^{\text{reversal}}) \cdot G_x(V_{\text{mem}}) + V_{\text{mem}} \cdot G_{\text{syn}}(t)
\]  \hspace{1cm} (6)

where \(V_{\text{mem}}\) is the membrane potential, \(C_{\text{mem}}\) was membrane capacitance, \(G_x\) represents the conductance for K, Na, or leak channels, \(V_x^{\text{reversal}}\) was the reversal potential for conductance X, and \(G_{\text{syn}}\) was the summed synaptic conductance determined in step 2 for multiple SGN inputs \((N_{\text{fiber}})\). Under resting conditions, only small voltage-independent persistent potassium and leak conductances are open.

The model also included conductances for AP generation: rapidly-activating and -inactivating voltage-dependent Na+- and K+-conductances \((G_{\text{Na}}\) and \(G_{\text{K}})\). Those conductances were modeled with a set of extended Hodgkin-Huxley equations (Borg-Graham, 1987). The goal was to mimic AP generation during trains of EPSC. In particular, we adjusted parameters to match the changes in \(G_{\text{thresh}}\) as a result of spiking activity. Xu-Friedman and Regehr (2005) found an increase in \(G_{\text{thresh}}\) to 127%, 168% and 190% of the resting threshold for EPSC frequencies of 100, 200 and 333 Hz respectively. In comparison the conductance-based model with the parameters stated in the following tables reaches 128%, 158% and 200%, which is highly similar.

The implementation of the sodium and potassium conductances used the structure of the I_{Na} and I_{A} currents in (Prinz et al., 2003). Parameters were adjusted to yield the activity dependent AP threshold as explained above.

**K⁺:**

\[
m_\infty = \frac{1}{1 + \exp\left(\frac{V_{\text{mem}} - V_1/2_m}{\text{slope}_m}\right)} \hspace{1cm} (7)
\]

\[
h_\infty = \frac{1}{1 + \exp\left(\frac{V_{\text{mem}} - V_1/2_h}{\text{slope}_h}\right)} \hspace{1cm} (8)
\]

\[
\tau_m = \tau_0 m - \frac{\tau_{\text{ampl}}_m}{1 + \exp\left(\frac{V_{\text{mem}} - V_1/2_m}{\tau_{-\text{slope}}_m}\right)} \hspace{1cm} (9)
\]

\[
\tau_h = \tau_0 h - \frac{\tau_{\text{ampl}}_h}{1 + \exp\left(\frac{V_{\text{mem}} - V_1/2_h}{\tau_{-\text{slope}}_h}\right)} \hspace{1cm} (10)
\]

**Na⁺:**

\(m_\infty, h_\infty, \tau_m\) are as given for K⁺,
\[
\tau_h = \frac{\tau_{\text{fac}_h}}{1 + \exp \left( \frac{V_{\text{mem}} - \tau_{\text{fac}} V_1/2_h}{\tau_{\text{fac}_h}} \right)}
\]

\[
\tau_0_h = \frac{\tau_{\text{ampl}_h}}{1 + \exp \left( \frac{V_{\text{mem}} - \tau_{\text{V1/2}_h}}{\tau_{\text{slope}_h}} \right)}
\]

(11)

Parameters used in Eq. 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>(C_{\text{mem}})</td>
<td>25 pF</td>
</tr>
<tr>
<td>(G_{\text{leak}})</td>
<td>0.65 nS</td>
</tr>
<tr>
<td>(G_{K_{\text{persist}}})</td>
<td>2.165 nS</td>
</tr>
<tr>
<td>(G_K)</td>
<td>2000 nS</td>
</tr>
<tr>
<td>(G_{Na})</td>
<td>4000 nS</td>
</tr>
<tr>
<td>(V_{\text{leak,rev}})</td>
<td>0 mV</td>
</tr>
<tr>
<td>(V_{K_{\text{rev}}})</td>
<td>-78 mV</td>
</tr>
<tr>
<td>(V_{Na_{\text{rev}}})</td>
<td>60 mV</td>
</tr>
<tr>
<td>(N_{\text{fiber}})</td>
<td>Fig. 8E, bottom left: 2, Fig. 8E, bottom right: 20</td>
</tr>
</tbody>
</table>

Parameters used in Eqs. 7–11. Columns represent values for voltage-dependent K and Na conductances.

<table>
<thead>
<tr>
<th>K</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>exponent_m</td>
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<tr>
<td>(V_{1/2_m})</td>
<td>-44.1 mV</td>
</tr>
<tr>
<td>slope_m</td>
<td>-0.37 mV</td>
</tr>
<tr>
<td>(\tau_{0_m})</td>
<td>1.6 ms</td>
</tr>
<tr>
<td>(\tau_{\text{ampl}_m})</td>
<td>1.3 ms</td>
</tr>
<tr>
<td>(\tau_{V1/2_m})</td>
<td>-46.9 mV</td>
</tr>
<tr>
<td>(\tau_{\text{slope}_m})</td>
<td>-10.2 mV</td>
</tr>
<tr>
<td>(V_{1/2_h})</td>
<td>-52.9 mV</td>
</tr>
<tr>
<td>slope_h</td>
<td>4.9 mV</td>
</tr>
<tr>
<td>(\tau_{0_h})</td>
<td>77.2 ms</td>
</tr>
<tr>
<td>(\tau_{\text{ampl}_h})</td>
<td>58.4 ms</td>
</tr>
<tr>
<td>(\tau_{V1/2_h})</td>
<td>-46.9 mV</td>
</tr>
<tr>
<td>(\tau_{\text{slope}_h})</td>
<td>-26.5 mV</td>
</tr>
<tr>
<td>(\tau_{\text{fac}_h})</td>
<td>1</td>
</tr>
</tbody>
</table>
\[ \tau_{\text{fac}_V1/2h} = -47.9 \text{ mV} \]
\[ \tau_{\text{fac}_{\text{slope}}} = -10 \text{ mV} \]

All parameters of the 3rd step were held constant for the two conditions being tested, as experimental data suggested there were no significant differences in intrinsic properties between wt and CPX I\textsuperscript{−/−}.

**Integration of the modules and simulation procedure:**

Figure 9 shows a flow chart of the simulation procedure. We simulated the activity of each considered configuration by conducting steps 1 and 2 for each fiber separately. The sum of the \( n_{\text{fiber}} \) EPSC trains was then used as \( G_{\text{syn}} \) to drive the conductance based model. Each simulation is characterized by
- the number of SGNs: \( N_{\text{fibers}} \)
- the rates SR, PR and AR as well as the synaptic strength (EPSC\(_1\)) of the endbulb for each of these fibers

The model’s output consist in the times at which the bushy cell fires APs. CPX I\textsuperscript{−/−} and CPX I\textsuperscript{+/+} bushy cells (see Supplemental Data 3).

**Two stage convergence: an alternative way to achieve highly reliable timing of first spikes:**

First spike latency distributions with standard deviations below 300 µs can be obtained if 20 to 40 SGNs converge onto a single bushy cell via sub-threshold synapses (Fig. 8E). However, this circuitry is rather robust against lowering the initial release probability and only in the case of small synaptic conductances (\( N_{\text{SGNs}} \times \text{EPSC}_1 < 5 \times G_{\text{thresh}} \)) can a lower initial release probability explain the robust broadening of FSL distributions observed in the CPX I\textsuperscript{−/−} mice *in-vivo*. But reliable first spike timing could also be obtained by a two-step convergence in which few subthreshold fibers converge onto a bushy cell and, at the second stage, several of such bushy cells are coupled by gap junctions between axon collaterals. Such an axonal coupling has been found between mossy fiber axons and between axons of neocortical pyramidal cells (Hamzei-Sichani et al., 2007). A similar
coupling might also be possible in the cochlear nucleus as gap junctions, although rare, have been described between axons, and between dendrites of neurons in the cochlear nucleus by Sotelo and co-workers (1976) and recently by Gomez-Nieto and Rubio (2008).

If such an electrical coupling is in place, the first AP in any of the axons of the network triggers retrograde APs travelling to the somata of the bushy cells that have not fired before. By this a “first triggers all” mechanism is employed that will sharpen the first spike latency distribution in all connected bushy cells. Initial simulations showed that a network of 8 bushy cells, each receiving 5 SGNs, produces first spike times as reliably as a bushy cell receiving 20 SGNs with the same EPSC_{amp}. Due to the lower redundancy of the 5 inputs compared to the 20 inputs, a reduction of initial release probability has very strong impact on the network of bushy cells, leading to sparse firing with large variability in first spike timing (data not shown). Such a network of bushy cells is an interesting functional module: because the conductance of the gap junctions and thereby the size of the bushy cell cluster can be regulated, each cell may either behave as an low convergence cell (Fig.8 E left panel) or collectively with the connected bushy cells as a high convergence cell (Fig.8 E right panel). This might explain why cells of the same PSTH class (PL notch) can show extremely narrow or extremely broad first spike latency distributions.

References:


Rothman, J.S., Young, E.D., Manis, P.B. Convergence of auditory nerve fibers onto bushy cells in the ventral cochlear nucleus: implications of a computational model. J Neuroscience 70, 2562-2583

