Supplementary for: *Tonotopic relationships reveal the charge density varies along the lateral wall of outer hair cells*

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S.1. Specific capacitance of lateral wall c_{LW} of OHCs is constant across the cochlea

We write the experimentally derived logarithmic relationships between total voltage-dependent charge, Q_T and lateral wall capacitance C_{LW} and between Q_T and area of the lateral wall, A_{LW} where α_1, α_2, β_1 and β_2 are constants determined from fits of the data (see figure legend of Fig. 3) to

$$Q_T = \alpha_1 ln(C_{LW}) + \beta_1 \tag{S.1}$$

$$Q_T = \alpha_2 ln(A_{LW}) + \beta_2. \tag{S.2}$$

If C_{LW} is a linear function of A_{LW}, then the constants of Eq. (S.1) and Eq. (S.2) are related and we can write

$$\exp(\frac{-\beta_1}{\alpha_1}) = \exp(\frac{-\beta_2}{\alpha_2})c_{LW}. \tag{S.3}$$

Expressing in terms of β_1

$$\beta_1 = \alpha_1\frac{\beta_2}{\alpha_2} - \alpha_1 ln(c_{LW}). \tag{S.4}$$

Substituting Eq. (S.4) into Eq. (S.1) and expanding then

$$Q_T = \alpha_1 ln(c_{LW}) + \alpha_1 ln(A_{LW}) + \alpha_1\frac{\beta_2}{\alpha_2} - \alpha_1 ln(c_{LW}), \tag{S.5}$$

and simplifying

$$Q_T = \alpha_1 ln(A_{LW}) + \alpha_1\frac{\beta_2}{\alpha_2}. \tag{S.6}$$

We find α_1=1.296 pC and α_2=1.3 pC (see legend of Figure 3) therefore $\alpha_1 = \alpha_2$ and Eq. (S.6) is equivalent to Eq. (S.2). We find β_1 and β_2 are -0.7010 and -6.689 pC from the relationships shown in Figure 3. We calculate β_1 with Eq. (S.4) and c_{LW} =0.01 pFμm^{-2} and find it is -0.70012 pC which is the same as -0.7010 pC.

S.2. Specific capacitance of basolateral region c_B of OHCs is constant across the cochlea

We now show that c_b is $\approx$ 0.006 pFμm^{-2} and constant across the cochlea. We write Eq. (S.1) and Eq. (S.2) in a non-dimensional form

$$\frac{Q_T}{e} = \alpha_3 ln(\frac{C_{LW}}{C_B}) + \beta_3 \tag{S2.1}$$

$$\frac{Q_T}{e} = \alpha_4 ln(\frac{A_{LW}}{A_B}) + \beta_4 \tag{S2.2}$$

where e is the charge on an electron at 1.609$\times$ 10^{-19} C; C_B and A_B the capacitance and area of the basolateral region and the constants α_3, α_4, β_3 and β_4 are now expressed in number of electrons. Once again the constants of Eq. (S2.1) and Eq.(S2.2) are related if c_{LW} and c_B are constant across the cochlea then β_3 is written

$$\beta_3 = \alpha_3 \frac{\beta_4}{\alpha_4} + \alpha_3 ln(\frac{c_B}{c_{LW}}). \tag{S2.3}$$

Substituting Eq. (S2.3) into Eq. (S2.1), expanding and simplyfying then

$$\frac{Q_T}{e} = \alpha_3 ln(\frac{A_{LW}}{A_B}) + \alpha_3 \frac{\beta_4}{\alpha_4}. \tag{S2.4}$$

We find α_3=8.09$\times$10^6 electrons and α_4=8.12$\times$10^6 electrons therefore $\alpha_3 \approx \alpha_4$ and Eq. (S2.4) becomes Eq. (S2.2). We find β_3 and β_4 are 3.924$\times$ 10^6 electrons and 7.591$\times$ 10^6 electrons from fits of the data to Eq. (S2.1) and Eq. (S2.2). We calculate β_3 with (S2.3) with c_{LW} and c_B=0.01 and 0.0062 pFμm^{-2} and find it is 3.7$\times$ 10^6 pC which is close to 3.924$\times$ 10^6 where an exact match is obtained if c_B= 0.00635 pFμm^{-2}.

We now show that the unit of the constant α is pC in relationships Eq. 8, Eq 12, Eq. (S.1) and Eq. (S.2). We write the non-dimensional expression Eq. (S2.2) as

$$\gamma = \alpha_4 ln(a) + \beta_4 \tag{S2.5}$$

where $\gamma = Q_T/e$ and a= A_{LW}/A_B. We determine the derivative of γ with respect to a

$$\frac{\partial\gamma}{\partial a} = \frac{\alpha_4}{a}. \tag{S2.6}$$

Expand

$$\frac{\partial Q_T}{\partial A_{LW}} \frac{A_B}{e} = \alpha_4 \frac{A_B}{A_{LW}} \tag{S2.7}$$

Simplify

$$\frac{\partial Q_T}{\partial A_{LW}} = \alpha_4 \frac{e}{A_{LW}} \tag{S2.8}$$

We now do the same for Eq. (S.2) and take the derivative of Q_T with respect to A_{LW} then

$$\frac{\partial Q_T}{\partial A_{LW}} = \frac{\alpha_2}{A_{LW}} \tag{S2.9}$$

This implies RHS of Eq. (S2.8) and Eq. (S2.9) are equivalent and $\alpha_2 = \alpha_4 e$. We find α_2=1.3 and α_4= 8116883 electrons =1.306 pC where we note that we experimentally determine α_2 to be 1.302 pC. Therefore α_2 and α_1 represent the number of electrons and in Eq. 8, Eq. 12, Eq. (S.1) and Eq. (S.2) exhibit units of pC.

S.3. Determination of $\nu = \frac{ze\delta}{\kappa_B T}$ from the tonotopic relationships

The voltage-dependent capacitance when expressed as a function of the voltage is commonly described with the derivative of the two-state Boltzmann function

$$C_{NL} = \frac{\nu Q_T \exp(-\nu(V - Vpeak))}{(1 + exp(-\nu(V - Vpeak)))^2} \tag{S3.1}$$

where ν is

$$\nu = \frac{ze\delta}{\kappa_B T} \tag{S3.2}$$

and z is valence of the charge, e is charge of the electron, δ is the fraction of the distance travelled across the dielectric, κ_B is Boltzmann's constant and T is temperature. The peak capacitance, C_{peak} is the capacitance when $V = V_{peak}$ and is calculated to be

$$C_{peak} = \frac{\nu Q_T}{(1+1)^2} = \frac{\nu}{4} Q_T. \tag{S3.3}$$

We found the experimentally determined A_{LW} increased exponentially with C_{peak} specifically

$$C_{peak} = \alpha_5 ln(A_{LW}) + \beta_5 \tag{S3.4}$$

where α_5 and β_5 are constants determined from the fits to the experimental data (see Figure 3). Equating Eq. (S3.3) into Eq. (S3.4) and rearranging

$$Q_T \approx \frac{4}{\nu} \alpha_5 ln(A_{LW}) + \frac{4}{\nu} \beta_5 \tag{S3.5}$$

The first term of the right hand side of (S3.5) should equate to first term on right-hand side of (S.2)

$$\alpha_2 = \frac{4}{\nu} \alpha_5 \tag{S3.6}$$

substituting values

$$\nu = \frac{4\alpha_5}{\alpha_2} = \frac{9.04 \times 4}{1.3} \approx 27V^{-1}. \tag{S3.7}$$

S.4. Prior experimental evidence that C_{peak} is a non-linear function of the measured membrane area of OHC

There is evidence (see reference [10]) that the maximum non-linear capacitance varies non-linearly across the guinea pig cochlea. In Figure 2a of reference [10] the relationship between $\frac{C_{peak}}{C_L}$ and the length of the OHC, l was described by a linear function although the constants of the relationship were not provided. We write the relationship with slope and intercept of α and β

$$\frac{C_{peak}}{C_L} = \alpha l + \beta \tag{S4.1}$$

where the linear capacitance, C_L was described in terms of l with

$$l = 2.9 \times C_L - 0.24. \tag{S4.2}$$

Substituting Eq. (S4.2) into Eq. (S4.1)

$$\frac{C_{peak}}{\frac{l+0.24}{2.9}} = \alpha l + \beta \tag{S4.3}$$

and expressing in terms of C_{peak} then

$$C_{peak} = \frac{l + 0.24}{2.9} \times (\alpha l + \beta). \tag{S4.4}$$

Expanding

$$C_{peak} = \frac{\alpha}{2.9}\frac{l^2}{1} + \frac{0.24}{2.9}[\alpha l + \frac{\beta l}{0.24}] + \frac{0.24}{2.9}\frac{\beta}{1}. \tag{S4.5}$$

If we write Eq. (S4.5) in terms of C_L where the constants $\alpha_1 = \frac{1}{2.9}$ and $\beta_1 = \frac{0.24}{2.9}$ then Eq. (S4.5) becomes

$$C_{peak} = \alpha\alpha_1 \times l^2 + [\beta_1\alpha + \beta\alpha_1] \times l + \beta\beta_1. \tag{S4.5}$$

In terms of the measured area, A_m (Eq. 4 of main text) Eq. (S4.5) becomes

$$C_{peak} = \alpha\alpha_1 \times \frac{(A_m)^2}{(2\pi r)^2} + [\beta_1\alpha + \beta\alpha_1] \times \frac{A_m}{2\pi r} + \beta\beta_1 \tag{S4.6}$$

and C_{peak} is a quadratic function of the OHC length and area, which is clearly non-linear.

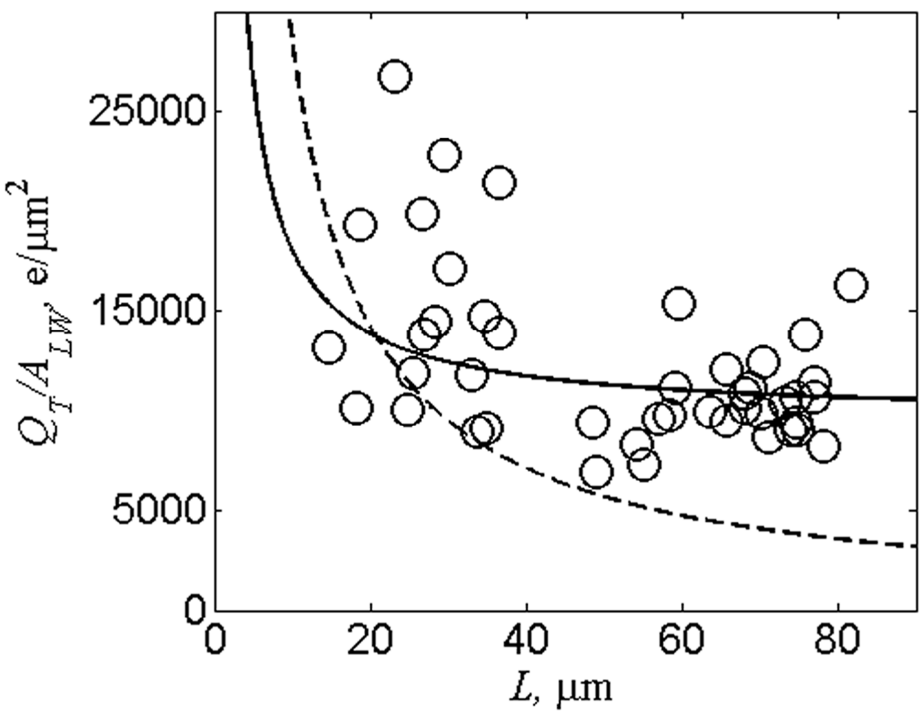

Figure S.1. Charge density calculated on a cell-by-cell basis assuming uniform distribution of charge in the lateral wall. The lines represent the reciprocal relationship for unconstrained (solid line, α = 84,055 *e/μm* β= 9,625 *e/μm²*, R^2 = 0.203) and constrained fits (dashed line, α = 284,305 *e/μm*). Similar weak fits are found for linear, log and exponential functions.

Table S.1

Parameter	Abbreviation
Constants of the relationships	α, β
Area: Membrane, Lateral wall, Non-lateral wall region, Basolateral region, Cuticular plate, Stereocilia bundle	$A_{M}, A_{LW}, A_{NL}, A_{B}, A_{CP}, A_{SB}$
Capacitance: Total, Peak, Linear, Voltage-dependent, Non-lateral wall region, Basolateral region, Cuticular plate, Stereocilia bundle, Non-bell	$C_{T}, C_{peak}, C_{L}, C_{NL}, C_{NLW}, C_{B}, C_{CP}, C_{SB}, C_{NB}$
Specific capacitance: Lateral wall, Basolateral region, Cuticular plate, Stereocilia bundle	$c_{LW}, c_{B}, c_{CP}, c_{SB}$
Fraction of distance traveled across the dielectric	δ
Charge of the electron	e
Frequency	f
Force	F
Boltzmann's constant	κ_B
Length: Total cell, Lateral wall	l,L
Pressure	P
Charge	q
Maximum voltage-dependent charge	Q_T
Resistance: Series, Membrane	R_s, R_m
Cell radius	r
Peak density of lateral wall	ρ_{LW}
Charge density of lateral wall	σ_{LW}
Temperature	T
Voltage	V
Slope factor from fit to Boltzmann	v
Valence of the charge	z

Table S.2: Comparison of fitting different tonotopic relationships to the lateral wall data.

Function	α	*p*-value	β	*p*-value	R^2
1. $Q_T = (\alpha)\ln(A_{LW}) + \beta$	1.300 (0.145)	<0.0001	-6.689 (1.040)	<0.0001	0.658
$Q_T = (\alpha)(A_{LW}) + \beta$	0.001 (0.0001)	<0.0001	1.017 (0.220)	<0.0001	0.603
$Q_T = (\alpha)(A_{LW})$	0.0017 (0.00007)	<0.0001			0.401
2. $Q_T = (\alpha)\ln(C_{LW}) + \beta$	1.296 (0.117)	<0.0001	-0.701(0.310)	0.029	0.744
$Q_T = (\alpha)(C_{LW}) + \beta$	0.112 (0.011)	<0.0001	0.949 (0.189)	<0.0001	0.693
$Q_T = (\alpha)(C_{LW})$	0.1646 (0.006)	<0.0001			0.509
3. $C_{peak} = (\alpha)\ln(A_{LW}) + \beta$	9.04 (1.09)	<0.0001	-46.262 (7.867)	<0.0001	0.619
$C_{peak} = (\alpha)(A_{LW}) + \beta$	0.008 (0.001)	<0.0001	7.270 (1.632)	<0.0001	0.575
$C_{peak} = (\alpha)(A_{LW})$	0.0118 (0.0005)	<0.0001			0.375
4. $C_{peak} = (\alpha)\ln(C_{LW}) + \beta$	8.918 (0.934)	<0.0001	-4.352 (2.466)	0.085	0.684
$C_{peak} = (\alpha)(C_{LW}) + \beta$	0.768 (0.090)	<0.0001	7.022 (1.475)	<0.0001	0.636
$C_{peak} = (\alpha)(C_{LW})$	1.158 (0.04)	<0.0001			0.440

Table S.3. OHC parameters for adult male‡

Parameter	Values
c_{LW}, (pF/μm^2)	0.010 (0.0007)
c_{LW} per cell, (pF/μm^2)	0.010 (0.0017)
σ_{LW}, (pC/A_{LW})	1.30 (0.15)
ρ_{LW}, (pF/A_{LW})	9.04 (1.09)
A_B , (μm^2) *	437 (70)
C_{NLW} , (pF)	5.17 (0.64)
Weight (grams)	580 (45)
Diameter of OHC (μm)	10.93 (1.77)

*Values calculated from Q_T versus A_M (Table 1, function 4)
‡The numbers in parenthesis are the standard deviation of the estimates