Variations in OHC-generated Voltage and DPOAEs with low EP

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Abstract. Endocochlear potential (EP) is essential for cochlear amplification, by providing the voltage drop needed to drive outer hair cell (OHC) transducer current, which leads to OHC electromechanical force. An early study using furosemide to reversibly reduce EP showed that distortion product oto-acoustic emissions (DPOAEs) recovered before EP. This indicated that cochlear amplification may be able to adjust to a new, lower EP. To investigate the mechanism of this adjustment, EP and locally-measured OHC-generated voltage were measured simultaneously in gerbil, with intraperitoneal injection of furosemide to reduce EP.

INTRODUCTION

Cochlear amplification is a frequency- and sound-level-dependent feedback of outer hair cells (OHCs) that sharpens frequency tuning and enhances sensitivity, especially to low and moderate level sounds. The mechanism of cochlear amplification is thought to be achieved by OHC electromechanical force [1]. Endocochlear potential (EP), which provides the voltage drive needed for OHC transducer current, is essential for cochlear amplification. Reduced EP leads to a decrease and linearization of input-output function of basilar membrane velocity [2], threshold elevations and alterations in auditory-nerve fiber tuning [3], and elevations in compound action potential (CAP) thresholds [4]. Furosemide is a loop diuretic that has ototoxic effects, primarily on the stria vascularis, and reversibly decreases EP. A study by Mills and colleagues [5] using furosemide to reversibly reduce EP, showed that distortion product oto-acoustic emissions (DPOAEs) recovered before EP. This suggested that cochlear amplification may be able to adjust to a lower EP. In this paper, locally-measured OHC-generated voltage and DPOAEs were studied during furosemide-induced EP reduction, to investigate this possible adjustment process.

METHODS

Animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Columbia University. Adult female gerbils with CAP response were used in the experiments. Following anesthesia, the animal was placed in a supine position with the pinna removed. A horizontal incision was made to expose the bulla, which was opened with forceps to expose the cochlea. 120 mg/kg furosemide was injected intraperitoneal (i.p.) to reduce EP. 0.5 ml of warm saline was administered every 2 hrs.

To investigate the variation of cochlear mechanics under low EP, EP and OHC-generated voltage were measured simultaneously. EP was measured via a ~10 um diameter hole in the scala media (SM) of the second basal turn of the cochlea. The reference electrode was placed on the muscle of right leg, and the animal body was grounded. For both working and reference electrodes, microelectrode holders (World Precision Instruments) with an Ag-Ag/Cl pellet were used. OHC-generated voltage was measured using a tungsten microelectrode with ~ 1-micron tip diameter (FHC Inc.

Bowdoin, ME). The electrode was advanced to be close to the basilar membrane via a ~100 µm diameter hole, in the scala tympani (ST) of the basal turn of the cochlea where the best frequency (BF) is 15-20 kHz. A reference electrode was placed in the muscle at the neck. CAP thresholds were measured before and after the ST cochleostomy to ensure a healthy starting cochlea.

Sound stimulation was generated by a Tucker-Davis Technologies (TDT) System driving a Radio Shack dynamic speaker, connected in a closed-field configuration to the ear canal (EC). The calibration of sound was performed within the EC using a Sokolich ultrasonic probe microphone. Responses were measured for ~1 s and averaged. Two types of stimuli were applied: single-tone and two-tone. Single-tone stimuli were swept in steps of 1 kHz between 1 kHz and 40 kHz. For the two-tone experiments, two pure tones (f_1 and f_2) with equal SPLs and fixed frequency ratio $f_2/f_1 = 1.25$ were applied. f_2 frequencies were swept in steps of 1 kHz between 1 kHz and 40 kHz.

RESULTS

Similar to the finding of Mills et al [5], after i.p. furosemide was administered, EP decreased quickly, reached its minimum within 20 ~ 40 min, and slowly recovered over ~ 140 min (see Fig.1a for an example). The time course varied somewhat between animals. The EP has not fully recovered in our preparations to date, and lack of full EP recovery is common in the literature, for example [5].

Two-stages Recovery of OHC-generated Voltage

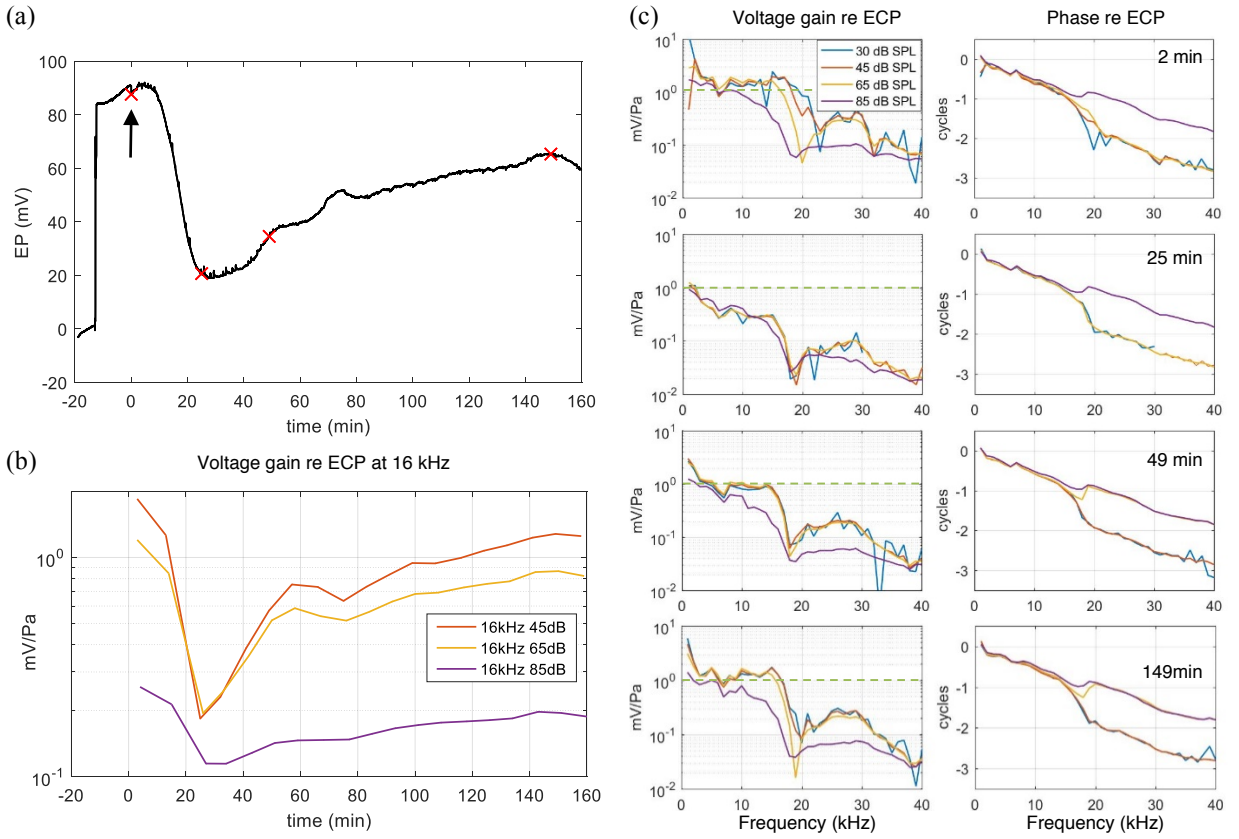


FIGURE 1. (a) EP variation versus time. The arrow shows the time of 120 mg/kg furosemide i.p. injection (0 min). Red crosses indicate the corresponding times for the frequency responses in Fig.1c. (b) Voltage variation at 16 kHz for three stimulus levels. Voltage gain and phase were normalized by ear canal pressure (ECP). (c) Frequency responses at 2 min, 25 min, 49 min, and 149 min after furosemide injection. The green dashed line at 10^0 is a guide for the eye, to compare the magnitude at the different times. The microphone output goes positive for negative pressure and this 180° offset was not subtracted in the presented data. (Gerbil #644)

EP and OHC-generated voltage were simultaneously measured, following furosemide injection. The voltage responses were measured for stimulus SPLs of 30, 45, 65 and 85 dB. Fig.1a shows the EP variation, and Fig.1c shows the frequency response of voltage measured 2, 25, 49 and 149 min after furosemide injection. As the top figure of Fig.1c shows, the voltage response showed cochlear amplification. The rapid phase accumulation observed at 15-20 kHz indicates that we were detecting traveling wave responses, which shows that our voltage measurement is dominated by the responses of local OHCs. The voltage was tuned to a BF of ~ 19 kHz, which was determined by the frequency for which phase accumulated 1.5 cycles. To avoid the notches near BF (likely due to the cancellation of voltage responses) the 16 kHz response was considered in more detail in Fig. 1b. Normalized to the EC pressure, the voltage started with a significant nonlinear compression, comparing the 16 kHz responses at 45 and 65 dB SPL. After furosemide injection, EP dropped and reached its minimum (~ 20 mV) at ~ 25 min. Along with the EP drop, a decrease of the voltage amplitude was observed (middle panels of Fig.1c). As an example, the 16 kHz 45 dB SPL response dropped from 1.2 mV/Pa to 0.2 mV/Pa. At the same time, the nonlinear compression was greatly altered. Following furosemide, the normalized response for 30 dB, 45 dB and 65 dB SPL were similar at all the frequencies, indicating a linear response and absence of cochlear amplification. The 85 dB SPL response retained nonlinearity following furosemide, likely because that OHC transduction mechanism was ~ saturated – this can be considered "passive nonlinearity". After that, EP and voltage slowly recovered, and stabilized at ~ 150 min. The bottom panel of Fig.1c shows the frequency response when the EP had stabilized. It indicates some but not full recovery of nonlinear compression. The 16 kHz 45 dB SPL response had substantially but not fully recovered.

Fig.1b shows the time course of normalized voltage with the 16 kHz stimulus at 45, 65 and 85 dB SPL. Compared to the time course of EP (Fig.1a), at ~ 30 min, the voltage amplitudes started to recover when EP was still low (~ 20 mV). In the EP recovery phase, a two-stages recovery of voltage response was observed. At the first stage (25 min ~ 50 min), the amplitude of 45 dB and 65 dB SPL responses were significantly increased, but the nonlinear compression was still small. At the second stage, their amplitude stayed almost unchanged, but the nonlinear compression started to recover significantly at 50 ~ 60 min, then stayed unchanged. This can also be seen in Fig.1c.

DPOAE Recovers Nearly Fully

In parallel to the single tone data acquisition, DPOAEs were tracked in time after the furosemide injection. Two equal amplitude tones with frequency ratio of $f_2/f_1 = 1.25$ were delivered at stimulus SPLs of 55, 65 and 75 dB. Fig.2a shows the time course of the amplitudes of the $2f_1$-f_2 DPOAE with f_1=12.8 kHz and f_2 = 16 kHz. Fig.2b and Fig.4e are the spectral graphs of the two-tone and their DPOAEs for the three SPLs. DPOAE amplitudes of $2f_1 - f_2$ started to recover before EP (see Fig 2a and Fig.1a). This increase in the DPOAE supports the finding by Mills et al [5], who hypothesized that the OHCs were adapting to lower EP. Another observation was that DPOAE amplitudes with stimulus levels of 55 and 65 dB SPLs recovered completely although the EP remained subnormal.

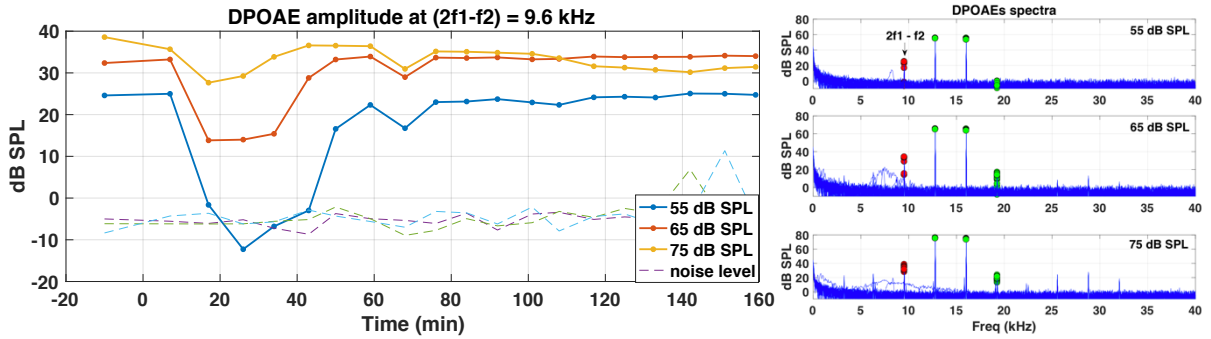
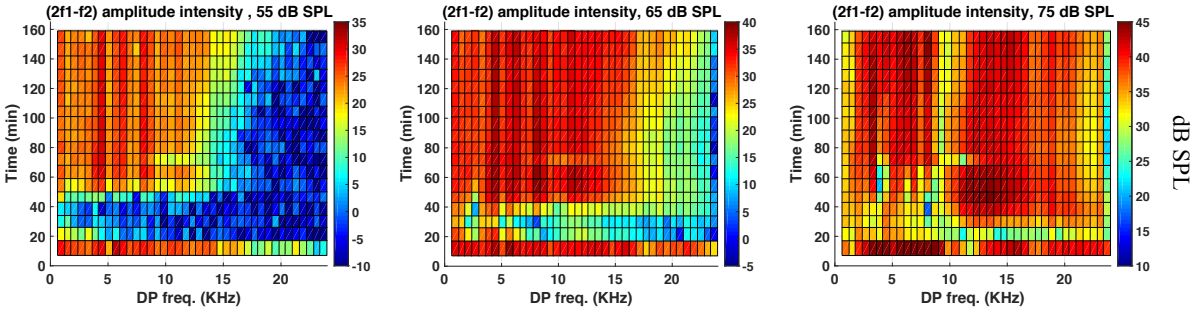


FIGURE 2. (a) DPOAE ($2f_1$-f_2) amplitudes change in time after the furosemide injection at stimulus frequency of f_1 = 12.8 kHz and f_2 = 16 kHz, for three sound stimuli of 55, 65 and 75 dB SPLs. (b) Frequency spectra of three sound stimuli and their DPOAEs. ($2f_1$–f_2) amplitudes plotted in red balls. (Gerbil #644)

The mesh plots in Fig.3 show how the $2f_1$-f_2 DPOAE changed in time following furosemide, through the full frequency range, with sound stimuli of 55, 65 and 75 dB SPL. Each horizontal row represents DPOAE amplitudes at a specific time after the furosemide injection. For the sound stimuli of 55 and 65 dB SPL, with the gradual recovery

of EP, DPOAE amplitudes almost fully recovered for $2f_1 - f_2 \leq 17$ kHz, which is equivalent to $f_1 \leq 23$ kHz and $f_2 \leq 28$ kHz. However, for the 75 dB SPL sound stimulus, higher frequencies recovered more quickly, but not to their initial levels.



(a) (b) (c)

FIGURE 3. Time course of the ($2f_1$-f_2) emission amplitudes at through a whole frequency range of 1 kHz ≤ ($2f_1$–f_2) ≤ 25 kHz with sound stimulus of (a) 55 dB SPL, (b) 65 dB SPL and (c) 75 dB SPL. (Gerbil #644)

Evidence of OHC Adjustment During Furosemide Injection

The aforementioned results were able to be repeated. Moreover, in another preparation (#652), there were some other interesting findings as shown in Fig.4. With pure tone stimulation, the second harmonics in the voltage responses (Fig.4b) recovered over ~ 50 – 140 min, and the amplitudes recovered to a level even greater than before furosemide injection. Also, for the DPOAE 65 dB SPL response (Fig.4c), we observed recovery to a level greater than before furosemide injection at ~ 30 – 50 min. These observations indicate that a change of OHC operating point occurred as the system recovers.

DISCUSSION

Recalling the Davis model [6] (Fig.5a), the current *i(t)* that flows through OHCs during small ciliary deflections ($\Delta G << G_{ap}$), is approximately

$$i(t) = I_0 + (\Delta G I_0 R_{ap}^2 / R_{tot}) \times sin\omega t$$

Where R_{ap} is the resistance of the OHC's apical membrane, ΔG is the amplitude conductance change due to ciliary deflections at radian frequency ω, R_{tot} is the summation of basolateral resistance, the SM resistance and the ST resistance. I_0 is the steady OHC current

$$I_0 = \frac{(E_{EP} - E_{OHC})}{R_{tot}}$$

E_{OHC} is the intracellular OHC potential, which is taken as -60 mV. Then we can write the measured OHC-generated voltage in this study as

$$v(t) = R_{body} \Delta G (E_{EP} - E_{OHC}) R_{ap}^2 / R_{tot}^2 \times sin\omega t$$

Where R_{body} is the internal body resistance of the gerbil.

To get an idea for how the cochlear amplification changed along with the EP, we can use this model to "predict" the voltage that would have been measured if cochlear amplification *did not change* with EP change. Because we actually expect amplification to change with EP [2], we do not expect this prediction to be born out and to emphasize this we put "predict" in parentheses. Define a factor $F = R_{body} \Delta G R_{ap}^2 / R_{tot}^2$, so that $v(t) = F(E_{EP}\text{-}E_{OHC})$. With the assumption that cochlear amplification stays the same, F would be a constant, and can be calculated using the measured voltage and EP value before it begins its furosemide-induced reduction. Using this constant F, any difference between the measured and "predicted" voltage amplitude could reflect a change in cochlear amplification. However, a difference could also be due to a change in conductance set point. Either of them would cause a change in ΔG.

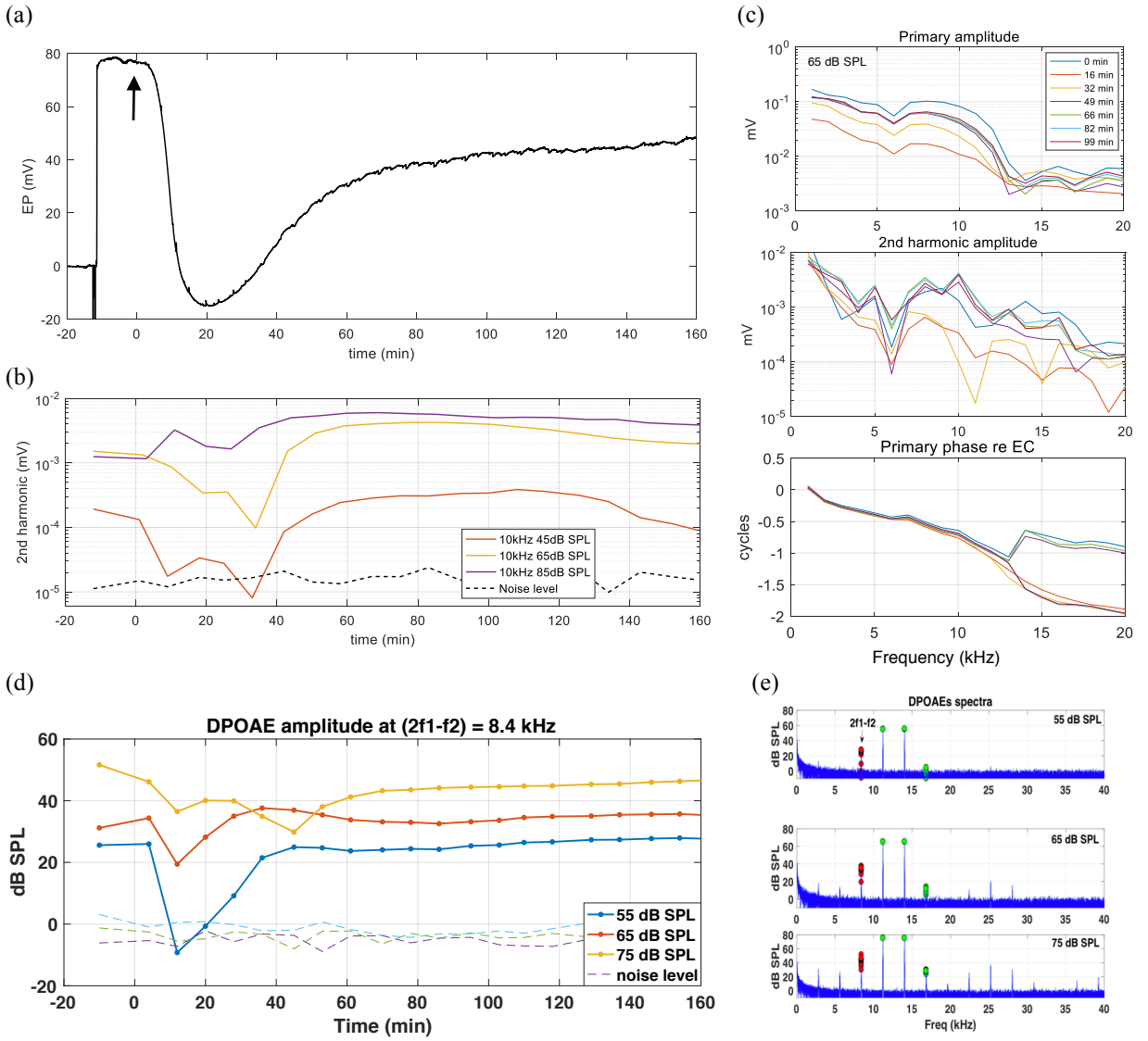


FIGURE 4. (a) Time course of EP. The arrow shows the time of 120 mg/kg furosemide i.p. injection (0 min). (b) Time course of the 2nd harmonics of the voltage at 10 kHz stimulus. (c) Frequency responses of fundamental and 2nd harmonics for a stimulus level of 65 dB SPL. BF ~ 14 kHz. Voltage phase was normalized by ECP. (d) DPOAE ($2f_1$-f_2) amplitudes change in time after the furosemide injection at stimulus frequency of f_1 = 11.2 kHz and f_2 = 14 kHz, for three sound stimuli of 55, 65 and 75 dB SPLs. (e) Frequency spectra of three sound stimuli and their DPOAEs. ($2f_1 - f_2$) amplitudes plotted in red balls. (Gerbil #652)

Fig.5b shows the comparison of measured and "predicted" voltages in the 16 kHz responses (preparation as in Figs.1-3.) For the 30, 45 and 65 dB SPL responses, significant differences can be seen between the measured and "predicted" voltages. At 0 – 25 min, while EP was decreasing, the differences were increasing, most likely due to the increasing loss of cochlear amplification. The biggest differences were at ~ 25 min, when EP was at its lowest level. When EP started to recover, the difference started to decrease, which means the cochlear amplifier started to recover. However, the differences between "predictions" and measurements did not recover to zero at the end: the cochlear amplifier did not recover as much as EP recovered. Moreover, the difference seemed to grow at ~ 60 min. The reason for this time-shift in recovery of cochlear amplification is not known.

For the responses at 85 dB SPL stimulus, the measurements match the "predictions" relatively well, most likely because at this high sound pressure level cochlear amplification was small, so our "prediction" does pretty well. In this case the difference between predictions and measurement, and lack of predicted degree of recovery suggests that in addition to reduction in cochlear amplification, the OHC operating point has changed, diminishing ΔG.

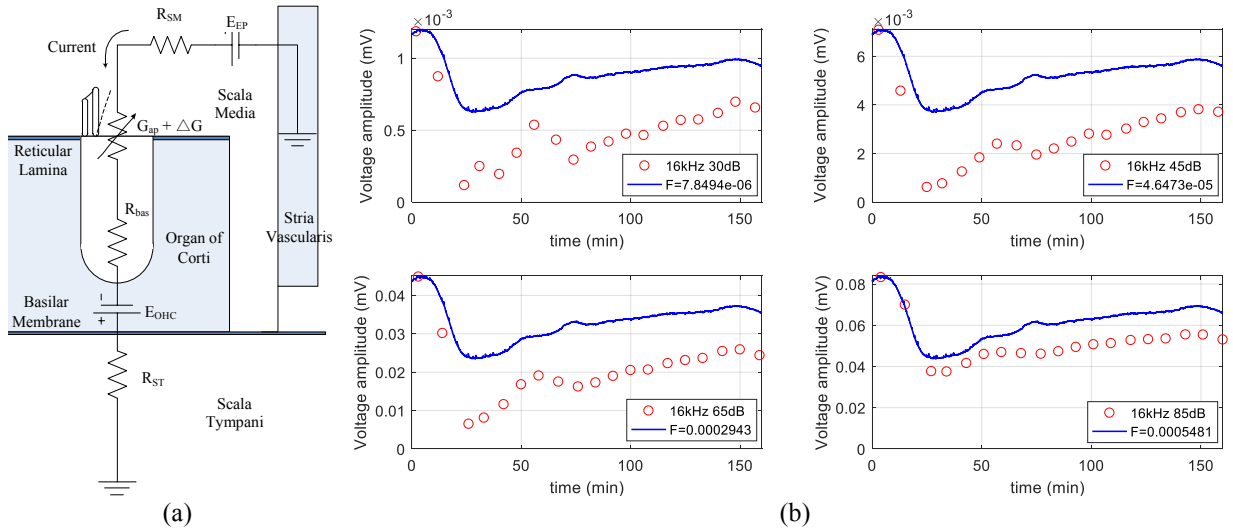


FIGURE 5. (a) Diagram of the Davis Model [6,7]. (b) Comparison of measured OHC-generated voltage (red circles) and "predicted" voltage if cochlear amplification is unchanging (blue curve). F is the factor $F = R_{body}\Delta G R_{ap}^2 / R_{tot}^2$, which estimated by the first points of voltage and EP measured at ~ 2 min after furosemide injection. See Fig.1a for the EP. (Gerbil #644)

In conclusion, low EP caused an expected loss in OHC receptor current, and thus in the OHC-based voltage. The recovery from low EP followed a multi-stage time course: Initially the OHC-based voltage recovery slightly preceded EP recovery, and then EP recovery caught up. Voltage harmonics and DPOAEs could recover fully and even overshoot as EP recovered. The OHC response to low EP thus seems to include an operating point shift that boosts nonlinear distortion and reduces amplification.

ACKNOWLEDGEMENTS

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