Frog Saccular Hair Cells Lab

BIE 2011

Goal. To record electrical resonance (tuning) in vertebrate hair cells and measure the underlying voltage-gated currents. In the course of this exercise you will learn how to visualize, record from, and electrically stimulate hair cells that have been isolated from the sensory epithelium.

Background. Hair cells express numerous voltage-gated and Ca^{2+}-gated ion channels in their basolateral membranes, principally K channels (both inward and outward rectifiers of diverse types), but also Ca channels, sodium channels (in some cases, especially immature hair cells) and HCN channels (which pass both K^+ and Na^+ ions). These channels are modulated when voltage is changed by transduction current; in turn, currents through the basolateral channels strongly shape the receptor potential, affecting its timing and sensitivity.

A well-known example is the sharp electrical resonance of frog saccular, turtle cochlear and chick cochlear hair cells, which can be seen in the oscillating response to simple current steps injected through a recording electrode. In papain-dissociated frog saccular hair cells, the oscillations arise from the interplay of two main ion channel types: voltage-gated Ca channels and Ca^{2+}-gated K channels (Hudspeth and Lewis 1988a,b).

Need:

Pulled recording pipettes

Solutions : Dissection, dissociation (with enzymes), bath (external) recording solution, pipette (internal) recording solution

Suck tubing

Chlorided ground electrode and chlorided wire for recording electrode

Access to software - password = mblpass

Equipment

Recording. Axopatch 700B, Digidata 1440 and computer loaded with Clampex and amplifier interface software

Visualizing. Zeiss Axiovert (inverted microscope for isolated cells)

Sutter Instruments micromanipulators for controlling electrode positions

Suggested experiments

1. Understanding the impact of membrane resistance and capacitance on voltage signals in cells (receptor potentials in hair cells)

In current clamp mode with a model cell, deliver small current steps and notice the temporal aspects and steady-state value of the voltage response.

2. Investigate the additional impact of voltage-gated conductances.

In current clamp mode with a hair cell, deliver small current steps and notice the temporal aspects and steady-state value of the voltage response. Use standard solutions. Contrast with the model cell.

3. In voltage clamp mode, characterize the currents evoked by various steps away from resting potential. These currents shape the voltages produced in (2) in response to current steps.

4. Use Cs^+ internal and external solutions to block K channels and reveal the remaining current (usually obscured by the large K^+ currents). This current is critical for both electrical resonance and synaptic transmission.

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Preparation of hair cells (Holt and Eatock 1995, based on Lewish and Hudspeth 1983)

1. Dissect out frog saccule in Standard Frog Solution (High Ca^{2+})
2. Put tissue in protease XXIV (50 µg/ml in low-Ca^{2+} solution) for 10 minutes
3. Remove Otolithic Membrane
4. Switch into papain (500 µg/ml papain and 300 µg/ml L-cysteine in low Ca^{2+} solution) for 20 minutes
5. Switch into bovine serum albumin (500 µg/ml in low Ca^{2+}) for 20 minutes
6. Fill recording chamber with low Ca^{2+} and mechanically dissociate the hair cells using an eyelash
7. Let settle for 10 minutes, then look to see what you've got
8. Switch back to high Ca^{2+} solution for recording.