Chapter 26

Whole-Cell Patch-Clamp Recording of Mouse and Rat Inner Hair Cells in the Intact Organ of Corti

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Abstract

Whole-cell patch clamping is a widely applied method to record currents across the entire membrane of a cell. This protocol describes application of this method to record currents from the sensory inner hair cells in the intact auditory sensory epithelium, the organ of Corti, isolated from rats or mice. This protocol particularly outlines the basic equipment required, provides instructions for the preparation of solutions and small equipment items, and methodology for recording voltage-activated and evoked synaptic currents from the inner hair cells.

Key words Inner hair cell, Cochlea, Organ of Corti, Whole-cell patch-clamp, Voltage clamp, Voltage-activated current, Evoked synaptic current

1 Introduction

Whole-cell patch-clamp electrophysiology has been used to measure the electrical potential and currents from a variety of cell types including the sensory hair cells from various animals, including (but not limited to) frog [1], turtle [2], chick [3], guinea pig [4], rat [5], and mouse [6]. These recordings have been invaluable to examining the biophysical mechanisms underlying function of these sensory cells, including mechanoelectrical transduction [7], electrical tuning in nonmammalian hair cells [8], membrane properties of mammalian inner and outer hair cells [9], the mechanisms of afferent neurotransmitter release [10], and synaptic transmission at olivocochlear efferent synapses [11]. While whole-cell patch-clamp recordings were originally performed on isolated hair cells, the ability to perform recordings in the intact organ of Corti has greatly expanded the capacity to examine the physiology of the sensory hair cells genetically, tonotopically, and in association with their synaptic contacts and neighboring cells. Recordings from the intact organ of Corti have proven essential to our increasing understanding of the developmental changes underlying the maturation

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of the sensory hair cells, afferent and efferent synaptic transmission, and glutamate uptake by neighboring support cells. This chapter describes the materials and protocols necessary for recording whole-cell currents from inner hair cells in the acutely isolated organ of Corti. Although emphasis is placed on the recording and isolation of voltage-gated currents and evoked synaptic currents, very similar techniques can be used to record currents from the outer hair cells [12], the afferent boutons [13], as well as supporting cells within the organ of Corti [14]. Finally, although this chapter discusses only the whole-cell configuration, the methods outlined can be adapted to investigate other patch-clamp configurations.

2 Materials

2.1 Isolating the Organ of Corti

1. Dissecting stereomicroscope (e.g., Olympus SZ51 or Leica MZ75).
2. Dissection scissors and forceps (Fine Science Tools).
3. 35-mm petri dishes.

2.2 Coverslip Fabrication to Hold the Isolated Organ of Corti

1. Glass coverslips (e.g., 0.8–12 cm diameter round glass coverslips).
2. Stainless steel insect (minutien) pins (e.g., length of 1 cm and diameter of 0.1 mm, Fine Science Tools).
3. Heating platform.
4. Sylgard.

2.3 Electrode Fabrication and Filling

1. Programmable pipet puller (e.g., Narashige PC-10 or Sutter Instrument Company P-97).
2. Thin-walled borosilicate glass tubing (e.g., 1 mm capillaries with filament, World Precision Instruments).
3. Recording electrode filling pipet (e.g., MicroFil needle, World Precision Instruments).

2.4 Whole-Cell Patch-Clamp Recordings

1. Patch-clamp microscope with DIC (e.g., Zeiss AxioExaminer, Olympus BX51).
2. Precision micromanipulators and electrode holder for patch clamping (e.g., Sutter Instrument Company MP-285).
3. Patch-clamp amplifier and digitizer (e.g., Molecular Devices Multiclamp 700A and Digidata 1322A).
4. Acquisition and analysis software (e.g., pClamp and/or WinWCP [Windows Whole Cell Program] developed by John Dempster at the University of Strathclyde Glasgow).

5. Vibration isolation table, perimeter, and Faraday cage (e.g., TMC).
6. Gravity-fed, local perfusion system (*see* **Note 1**).
7. Drug delivery pipet (e.g., MicroFil needle, World Precision Instruments).
8. Coarse micromanipulator (e.g., Sutter Instrument Company MP-85).
9. Isolated constant current stimulator (e.g., Digitimer Ltd. DS3).
10. Stimulating electrode holder (e.g., electrode holder for theta glass, Warner Instruments).
11. Theta-glass tubing (e.g., Warner Instruments).
12. Insulated silver wire (e.g., 0.25 mm, teflon coated silver wire, Warner Instruments).

2.5 Solutions

Prepare all solutions using ultrapure water and analytical grade reagents.

1. Household bleach (e.g., Clorox).
2. External solution (mM): 155 NaCl, 5.8 KCl, 0.9 $MgCl_2$, 1.3 $CaCl_2$, 0.7 NaH_2PO_4, 5.6 D-glucose, and 10 HEPES, pH 7.4, 300 mOsm.
3. Internal solution (mM): 150 KCl, 3.5 $MgCl_2$, 0.1 $CaCl_2$, 5 EGTA, 5 HEPES, and 2.5 Na_2ATP, pH 7.2, 290 mOsm (*see* **Note 2**).

3 Methods

3.1 Experiment Preparation

Solutions, coverslips, and both cleaning and stimulating electrodes can be made well in advance of experiments. Recording electrodes should be made the day of experiments.

3.1.1 Solutions

1. Prepare external solution, usually in 1 L volumes, filter, and store at 4 °C for at most 2 weeks.
2. Prepare internal solution usually in 50 mL or 100 mL volumes on ice, aliquot in 5 mL volumes, and store at −20 °C for at most 2 months. Keep on ice and use immediately once thawed for experiments. Discard any extra solution.

3.1.2 Coverslip Fabrication to Hold the Isolated Organ of Corti

1. Place the coverslip on a very warm hot plate.
2. Place a minutien pin across the diameter of the coverslip.
3. Use a standard pipet tip to place a drop of prepared Sylgard on one end of the pin to secure it to the coverslip. The heat cures the Sylgard immediately. Be careful the Sylgard drop is not too

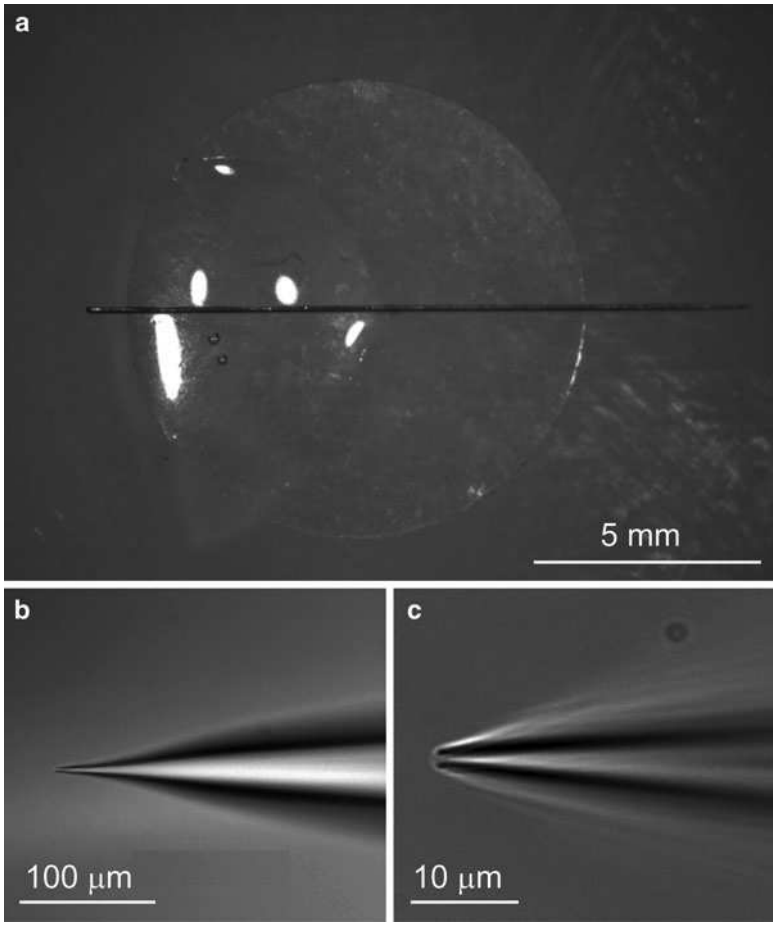

Fig. 1 Preparation of coverslips and recording electrodes. (**a**) A properly prepared coverslip is fabricated from round coverslip glass (with a diameter of approximately 1 cm) and an insect pin (with a length of approximately 1 cm and diameter of 0.1 mm) affixed with a drop of Sylard. (**b**, **c**) An appropriately shaped recoding electrode, at lower magnification (**b**) and also higher magnification (**c**), tapers rapidly and has an opening diameter generally between 1 and 2 μm

high or it can obstruct access to the organ of Corti during patch-clamp recordings. A finished coverslip is shown in Fig. 1a.

4. Coverslips can be rinsed with distilled water after experiments and reused.

3.1.3 Recording Electrode Fabrication

1. Pull recording electrodes from borosilicate glass using a multi-stage puller. A starting protocol is: Heat 1: 65.1, Heat 2: 58.0 (Narishige PC-10). However, adjustments will need to be made. In most cases, the temperature of the final pull needs to be increased/decreased to pull electrodes with smaller/larger tip diameters, respectively.

2. Examine the tip for shape and diameter under a compound microscope. The tip should taper rather steeply, and the final diameter varies depending on the recordings being performed (Fig. 1b, c). Tip diameters of approximately 2 μm have resistances of 1–3 MΩ with the above solutions, whereas smaller tip diameters (1 μm) have resistances of 5–6 MΩ. *See* **Note 3** for more discussion on electrode resistances.

3.1.4 Cleaning Pipet Fabrication

1. Under a stereomicroscope break the tip of a recording electrode with dissecting forceps so that the tip diameter is approximately 10–30 μm.

3.1.5 Stimulating Electrode Fabrication

Electrodes for stimulating olivocochlear efferent terminals can be fabricated from the same glass pipets used for recordings (creating a unipolar electrode) or, alternatively, from theta-glass capillaries (creating a bipolar electrode). Fabrication of a bipolar electrode is described below. Fabrication of a unipolar electrode would be similar except that the second pole is connected to the bath ground.

1. Cut two pieces of insulated silver wire to a length of approximately 5 cm.
2. Remove insulation from one end of each of the silver wires to expose about 0.5–1 cm of uninsulated wire.
3. Immerse the exposed part of the wires in full strength household bleach for 15–30 min until the wire obtains a purple-gray color. Rinse with distilled water and use.
4. Remove insulation from the other end of each of the silver wires to expose about 3–4 mm or just enough to allow for electrical continuity with stimulator.
5. Pull the theta-glass capillary using the following protocol: single step with a heating value of 65.1 (Narishige PC-10).
6. Under a stereomicroscope, break the tip of the stimulating electrode with dissecting forceps, so that the tip diameter is approximately 20–40 μm. *See* Subheading 3.1.5, **step 9** for instructions on measuring the resistance values.
7. Fill each compartment of the stimulating electrode approximately half way with external solution.
8. Insert a silver wire into each compartment of the stimulating electrode and mount the electrode to a holder.
9. With the aid of a fine micromanipulator, place the tip of the stimulating electrode inside the recording bath (filled with extracellular solution). Use a voltmeter to measure the electrode resistance across the cable connectors of the electrode holder. Resistance for bipolar electrodes should not be greater than 1–2 MΩ.
10. Connect the mounted electrode to the stimulator.

3.2 Isolating the Organ of Corti for Patch-Clamp Recordings

Isolating the organ of Corti (for patch-clamp recordings and other techniques) requires practice and is best learned with the help of someone experienced in the dissection. An excellent video tutorial is available [15]. Isolation of the organ of Corti generally becomes more difficult as the animals mature and the structures of the inner ear calcify. Apical (low frequency turns) are generally easier to isolate than basal (high frequency) turns. Organs of Corti are best isolated from rats and mice younger than 1 month of age (although older ages are possible). The age of animals used to obtain tissue, also depends on the intent of the experiments: many voltage-gated currents show developmental changes in expression [16].

1. Remove the temporal bone containing the inner ear from the skull using dissecting scissors and place immediately in a 35 mm petri dish filled with ice-cold external solution.
2. Under a dissecting microscope isolate the inner ear from the temporal bone using forceps and place into a new 35 mm petri dish filled with ice-cold external solution containing the submerged coverslip.
3. Remove the organ of Corti from the inner ear using forceps.
4. Remove the overlying tectorial membrane using forceps.
5. Position the organ of Corti under the insect pin of the coverslip so that the inner hair cells are accessible from one side of the pin (in the *y*-axis). A properly positioned organ of Corti is shown in Fig. 2 (*see* **Note 4**).

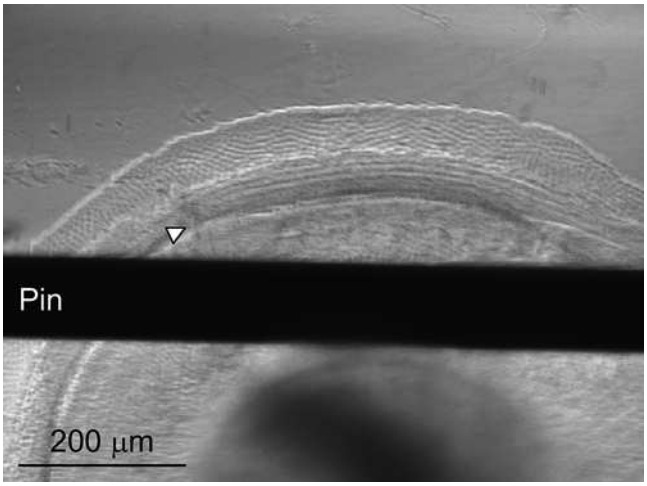

Fig. 2 Low magnification micrograph of an isolated organ of Corti positioned on a coverslip. A properly positioned organ of Corti is firmly and flatly anchored under the insect pin and also allows for ample room to access the row of inner hair cells (*open arrowhead*) by the recording electrode

3.3 Preparing the Isolated Organ of Corti for Patch-Clamp Recordings

Depending on the recordings to be performed, a necessary step described here is the removal of supporting cells that overlie the basal ends of the inner hair cells and/or the terminals of the efferent projections.

1. Place the coverslip with the organ of Corti into the recording chamber. The organ of Corti must be kept under constant perfusion at a rate of approximately 1–3 mL/min.
2. Bring the organ of Corti into view first under low power (10×) and then under higher power (40× or 60×). Gently raise the objective but (ideally) keep the objective in contact with the bath (external) solution. Raising the objective allows room to position the cleaning pipet.
3. Place the cleaning pipet onto the electrode holder. Use the micromanipulators to position the cleaning electrode into the bath and under the microscope objective.
4. Incrementally lower the cleaning pipet and objective until the cleaning pipet is in the same field of view as the organ of Corti.
5. Gently position the cleaning pipet onto the organ of Corti just below the row of inner hair cells (toward the spiral ganglion cells in the *y*-axis). Apply gentle suction while moving the cleaning pipet below the row of inner hair cells. (Suction can also be applied with the aid of a 50 mL syringe connected to the electrode holder.) The goal is to carefully remove the supporting cells and connective tissue that cover the base of the inner hair cells (*see* **Note 5**).
6. Repeat this step until the basal ends of at least a few inner hair cells have been sufficiently exposed. An organ of Corti before and after cleaning is shown in Fig. 3.
7. Raise the objective (while still keeping it in contact with the bath solution) and retract and dispose of the cleaning pipet.

3.4 Achieving the Whole-Cell Patch-Clamp Recording Configuration

The procedure for whole-cell patch clamping inner hair cells is very similar to the procedures used to patch-clamp other cells. In all cases, ideal procedures are somewhat personal and can vary from cell to cell. Excellent reviews outlining the general theory and application of patch clamping are available elsewhere [17].

1. Fill the recording electrode approximately halfway with filtered internal solution (*see* **Note 2**). Be sure there are no air bubbles in the tip. Place the recording electrode onto the electrode holder and apply positive pressure. The electrode should be at approximately a 30° angle with the coverslip and organ of Corti.
2. Use the micromanipulators to position the recording electrode into the bath and under the microscope objective.

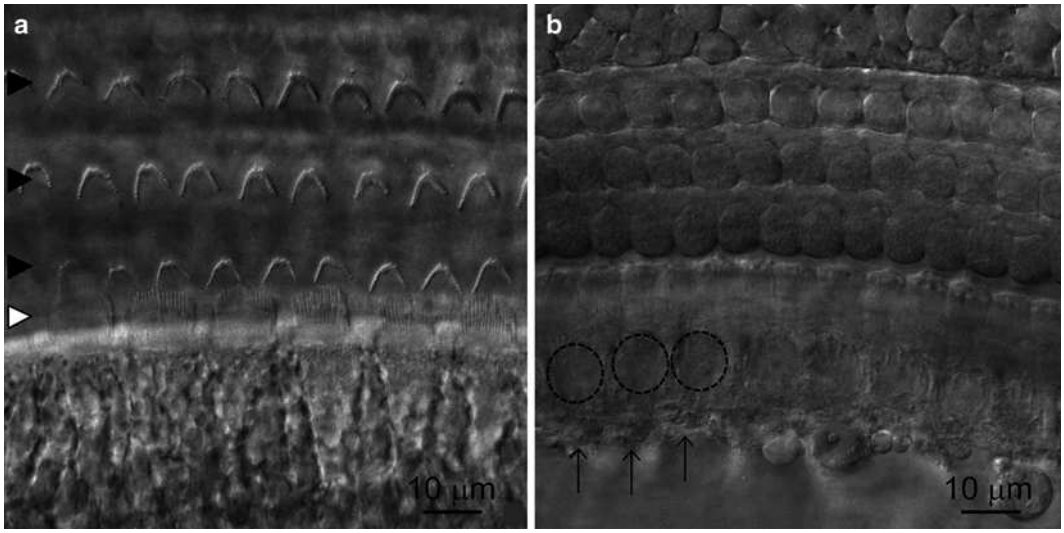

Fig. 3 High magnification of an isolated organ of Corti before and after cleaning. (**a**) Hair cell stereocilia are visible in a preparation before cleaning. The three rows of outer hair cells (*closed arrowheads*) and a single row of inner hair cells (*open arrowhead*) are indicated. (**b**) In the cleaned organ of Corti, the relative positions of the hair cells are similar but the basal ends are now exposed for the first three inner hair cells (*arrows*). Nuclei are also clearly visible (*circled*)

3. Verify the appropriate resistance (size) of the electrode with a test pulse (*see* **Note 3**).
4. Verify the appropriate (negative) holding potential.
5. Incrementally lower the recording electrode and objective until the recording electrode is in the same field of view as the organ of Corti.
6. Verify the appropriate positive pressure on the electrode. There should be a visible stream from the tip of the electrode and yet pressure should not be so high as to cause cells to detach from the organ of Corti.
7. Choose a healthy hair cell for patching. Indicators of poor condition (indicating a hair cell should not be patched) include disrupted or missing stereocilia, cell swelling, particle movement in the nucleus or cytoplasm.
8. Bring the recording electrode alongside the base of the inner hair cell to be patched. The electrode tip and the cell membrane of the hair cell should be in the same plane. Focus up and down to verify that the tip of the electrode is indeed in the same plane as the hair cell and neither above nor below.
9. Gently slide the recording electrode along the lateral wall of the hair cell to verify that the electrode is positioned on the hair cell membrane (and not the membrane of a supporting cell).

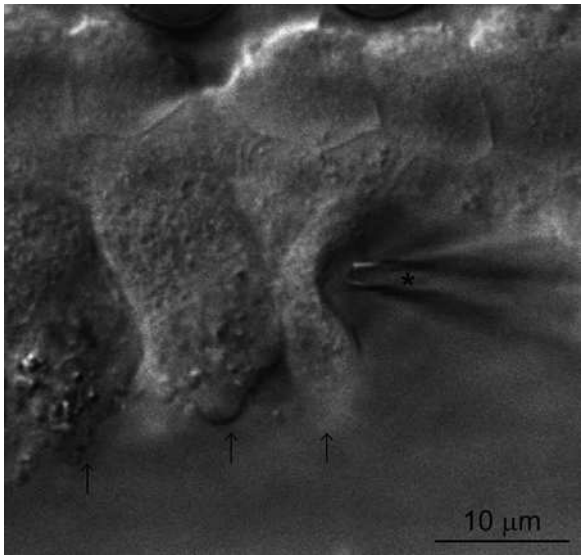

Fig. 4 High magnification of an isolated organ of Corti showing the proper approach to an inner hair cell with a recording electrode. Supporting cells surrounding the inner hair cells have been removed to allow access by the recording electrode (*) to the basal ends of the first three inner hair cells (*arrows*). The membrane of one of the inner hair cells and the tip of the recording electrode are in focus (in the same plane) and the positive pressure of the recording electrode forms a visible indentation on the inner hair cell membrane

10. If necessary readjust positive pressure and/or the position of the recording electrode so that there is a visible indentation of the hair cell membrane by the recording electrode (Fig. 4).
11. Release positive pressure and simultaneously apply slight negative pressure to the recording electrode.
12. Monitor the membrane resistance. (Both software packages referred to above are able to continuously measure and check this parameter). It should increase and achieve a gigaohm seal. For some hair cells, changes in resistance happen quickly, whereas for other hair cells changes happen more gradually. If the membrane resistance stabilizes without reaching a gigaohm seal, increase negative pressure gradually until a gigaohm seal is achieved.
13. Once a gigaohm seal has been achieved, apply gentle, rapid negative suction to achieve the whole-cell configuration.
14. If a gigaohm seal cannot be achieved, raise the objective (while still keeping it in contact with the bath solution), retract and dispose of the recording electrode, and begin with a new recording electrode and hair cell.
15. The approximate membrane properties of a healthy cell in the whole-cell configuration are (in voltage clamp mode) a mem-

brane capacitance of 10 pF, a membrane resistance of 200–400 MΩ, a series resistance of up to 20 MΩ, and (in current clamp mode) a resting membrane potential of –60 mV. These values depend on a variety of factors, especially the size of the cell, the size of the recording electrode, the quality of the whole-cell configuration, and the solutions.

16. In voltage-clamp mode, large outward (potassium) currents are evoked in response to depolarizing voltage steps and are another indication that the whole-cell configuration is established correctly.
17. The isolated organ of Corti, under constant perfusion and at room temperature, can last for up to 1 h. Indications that the preparation should be replaced include loss of integrity of the cell membranes, swollen cells, and particle movement in the nuclei or cytoplasm.

3.5 Recording and Analyzing Whole-Cell Voltage Clamp Currents

The biophysical properties of the currents to be recorded determine the voltage clamp protocols. The most conspicuous voltage-gated currents in inner hair cells from hearing animals are relatively quickly activating BK currents and relatively slowly activating K^+ currents. Recording and isolating these currents is described here to illustrate the general principles required to analyze whole-cell currents.

1. As part of preparing for experiments (*see* Subheading 3.1), make 5–10 mL of 100 nM IBTX in external solution and load into a gravity-fed, local perfusion system (*see* **Note 1**).
2. After preparing the isolated organ of Corti for patch-clamp recordings (*see* Subheading 3.3), use a coarse micromanipulator to position the drug delivery pipet approximately 300 μm from the recording electrode and just above the sensory epithelium.
3. After achieving the whole-cell configuration (*see* Subheading 3.4), apply on-line series resistance correction. With low resistance recording electrodes, series resistances are typically 5 MΩ and can often be compensated by 70 % on-line. Series resistance correction may need to be adjusted during the recordings.
4. From a hyperpolarized membrane potential (–60 mV) depolarize the membrane to approximately 40 mV in 10 mV steps. Depolarization durations should be approximately 20–100 ms to achieve steady-state outward currents. Allow sufficient time between depolarization steps (2–10 s) (*see* **Note 6**). Finally, leak subtraction can be incorporated into these protocols. Previous experiments [18, 19] have utilized a standard P/4 protocol [20]. Finally, because of their fast activation, low pass filter BK currents at 10 kHz and sample (digitize) at 50 kHz.

3.5.1 Pharmacological Isolation

Although less than ideal, BK currents can be separated from the more slowly activating K^+ currents by the time of measurement. Because BK currents activate with a tau of <1 ms, which is much faster than the other slowly activating K^+ currents (tau ≈6 ms), currents measured 1 ms after application of the depolarizing step are mostly BK currents [18]. Pharmacological isolation of currents is often the preferred method of isolation (*see* **Note 6**). BK currents can be isolated from the remaining K^+ currents by off line subtraction after pharmacological blockade. IBTX is a toxin highly specific for BK channels [21].

1. Record whole-cell currents in IBTX using the same voltage protocol used in control (no toxin) conditions (*see* **Note 8**).
2. Offline subtract records obtained in IBTX from those obtained in control conditions (which represent the total K^+ currents) to obtain the isolated BK currents. (Most acquisition and analysis software provides features to subtract records.) Further analyses of these records can be performed as required. For example, activation kinetics can be fit to the original records and/or the isolated BK currents. In addition, current-voltage (*I-V*) or conductance–voltage (*G-V*) relationships can be calculated from the original records and/or the isolated BK currents. Excellent explanations on preparing and analyzing *I-V* and *G-V* curves are available elsewhere [17].

3.6 Recording Evoked Synaptic Currents

Before the onset of hearing, efferent terminals originating from the medial superior olive in the brainstem contact IHCs [22]. This direct efferent innervation of the IHCs peaks functionally during the second postnatal week [23]. Spontaneous efferent synaptic activity can be recorded postsynaptically from the IHC using methodology similar to that described above. Efferent synaptic activity can also be evoked and recorded postsynaptically from the IHC using the protocol described below.

1. After preparing the isolated organ of Corti for patch-clamp recordings (*see* Subheading 3.3), use a precision micromanipulator to position the extracellular stimulating electrode under the row of IHCs and in contact with the preparation.
2. After achieving the whole-cell configuration (*see* Subheading 3.4), deliver stimulating pulses at a rate of 1 Hz while holding the IHC at –90 mV. Begin with pulse amplitudes between 100 and 200 μA and durations of current (or voltage) between 100 and 500 μs (*see* **Note 9**).
3. If no synaptic currents are elicited, reposition the stimulating electrode towards or away from the row of IHCs (in the *y*-axis) and/or into or out of the organ of Corti (in the *z*-axis). Stimulation of the efferent terminals projecting to the IHCs is achieved most efficiently by placing the stimulating electrode

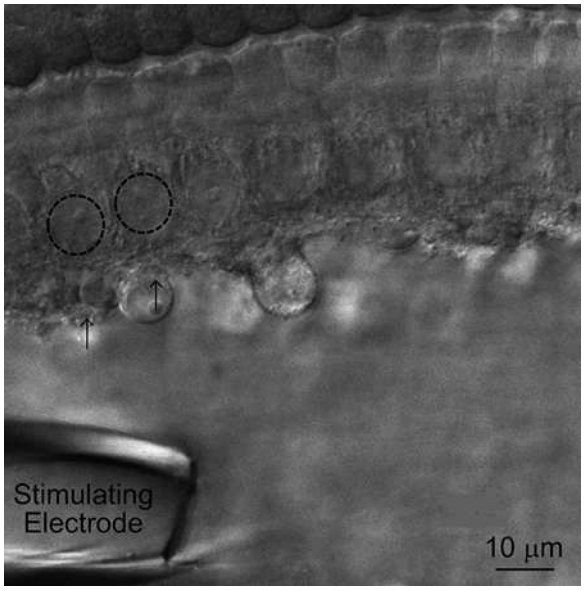

Fig. 5 High magnification of an isolated organ of Corti showing the proper positioning of recording and stimulating electrodes. The basal ends of two inner hair cells (*arrows*) and their nuclei (*circled*) are visible relative to the stimulating electrode

near the row of IHCs, but not necessarily directly below the recorded IHC. When repositioning the stimulating electrode, do so slowly and carefully so as not to compromise the whole-cell patch-clamp configuration. A properly positioned stimulating electrode is shown in Fig. 5.

4. The amplitude and duration of the pulse is another factor that can be modified for successful stimulation. Importantly, the stronger the stimulation, the more fibers are recruited and the larger the synaptic response (also, the lower the failure rate). Changing the polarity of the pulse may also affect the response.
5. From these records, quantal analyses can be performed to determine important parameters of neurotransmitter release at the efferent-IHC synapse and are described excellently elsewhere [24].

4 Notes

1. The gravity-fed, local perfusion system can be very simple and consist of a syringe connected to the delivery pipet by nothing more than tubing and a stopcock. On the other hand, a variety of commercially available and also computer-driven systems (e.g., Warner Instruments VC-8) are available for use.

Application of solutions using a picospritzer (Parker) may be preferable, to precisely control small ejection volumes (pL) and ejection times (ms) [12].

2. Typically the osmolarity of the internal solution is 10–15 mOsm less than that of the external solution to promote formation of a gigaohm and obtain long and stable recordings. K^+-based internal solutions are also suitable for recording synaptic currents. An alternative to K^+-based internal solutions, especially when recording Ca^{2+} currents, is the following solution (mM): 120 $CsMeSO_3$, 13 TEA, 5 HEPES, 0.35 $CaCl_2$, 3.5 $MgCl_2$, 1 EGTA, 2.5 Na_2ATP, pH 7.2, 290 mOsm). $CsMeSO_3$ can be replaced by CsCl, or CsGluconate.
3. In general, more rapidly tapering electrodes are preferable to more slowly tapering electrodes, since the former reduces electrode resistance. The size of the electrode depends on the size of the currents to be recorded. While smaller (higher resistance) electrodes more easily form gigaohm seals, larger lower resistance electrodes reduce series resistance errors associated with large recorded currents. Series resistance errors are deviations of the membrane potential from the clamped potential caused by currents passing across the electrode and cell resistances in series. Series resistance errors make voltage-dependent outward currents appear to activate more slowly and have lower magnitudes at steady state. These effects become more dramatic as the current and associated voltage deviation, $V = IR$, increases.
4. Proper positioning of the organ of Corti under the insect pin is important. Enough of the organ of Corti must be exposed above the insect pin to allow access to the inner hair cells by the recording electrode. At the same time, enough of the epithelium must be securely positioned under and below the pin to firmly anchor the organ of Corti to the coverslip. Finally, the organ of Corti must be flatly positioned under the pin. If the organ of Corti is too loose, cleaning of supporting cells may dislodge the organ of Corti from the coverslip. Approaching cells with the recording electrode becomes more difficult if the epithelium is not positioned flatly under the pin.
5. The basal ends of inner hair cells are more inaccessible in organs of Corti obtained from younger animals (<P12) but become more exposed in older organs of Corti (>P14). Depending on the recording age, cleaning overlying cells may or may not be necessary. If the cleaning of overlying cells is not necessary, a recording electrode, filled with external solution and under positive pressure, can be pushed through the tissue below the row of IHCs, to open space for later access with another recording electrode filled with internal solutions.

6. In general, when recording large, outward K^+ currents, it is best to keep the depolarization duration as short as possible. Short durations avoid the accumulation of extracellular K^+, which shifts the Nernst potential of K^+, thereby reducing the driving force of K^+ efflux and decreasing current magnitudes in a time-dependent manner. Longer durations between depolarizing steps and continuous perfusion with external solution also prevent accumulation of extracellular K^+. Importantly, previous work shows that K^+ accumulation is minimal under these conditions [17].
7. Blocking substances are used in one of two main ways to separate currents. First, it may be possible to block all currents except the current of interest. Depending on the range of channels present, this may involve one or more blockers, sometimes in combination with ionic substitution. Secondly, a blocker that is specific for the channel of interest may be used. Current is recorded in the presence of the blocker and that result is subtracted from current obtained in the absence of the blocker to visualize the current of interest.
8. IBTX is difficult to washout. Ideally, to verify recording stability, whole-cell currents should be measured after washout of the pharmacological agent, using the same voltage protocol used in control and treatment conditions. Control and washout currents should be comparable to verify stability.
9. Repositioning the stimulating electrode is preferable to applying very large or prolonged electrical stimuli. Larger stimuli reduce recording stability. Prolonged stimuli can result in stimulation artifacts that overlap with evoked synaptic currents.

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