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Methods in Molecular Biology (MiMB): Mouse Retinal Phenotyping - Methods and Protocols *Ex vivo* functional evaluation of synaptic transmission from rods to rod bipolar cells in mice.

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Abstract

Mice have been widely used as a model organism to study mechanisms of phototransduction and synaptic transmission in the retina. Genetic manipulations and electrophysiological techniques for analysis of photoreceptor and rod bipolar cell function in mice are uniquely advanced. Here, we describe a set of biochemical and electrophysiological techniques for evaluation of synaptic transmission at the rod-rod bipolar cell synapse, which represents the first and key step in the processing of dim-light visual information.

Keywords

Retina; rod photoreceptor; rod bipolar cell; immunoblot; immunofluorescence; suction electrode; patch clamp

1 Introduction

Vision is initiated when the visual pigments of the photoreceptors in the retina absorb photons of light. The activated visual pigment initiates a phototransduction cascade, which reduces the circulating dark-current flowing in through the outer segment, followed by a hyperpolarization of the photoreceptor inner segment membrane potential [1,2]. This change reduces neurotransmitter release to second order neurons, which then allows the neural circuitry of the retina to process and optimize the visual signal transmitted to the brain [3]. Comprehensive evaluation of synaptic transmission from rods to rod bipolar cells (RBC) requires both biochemical and electrophysiological approaches. This combination of approaches enables better understanding of how biochemical or morphological changes in photoreceptors and at the synapse alter the signal transfer to the rod bipolar cell. Standard biochemical assessment includes analysis of retinal morphology, subcellular localization of

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major phototransduction and synaptic components by immunofluorescence, quantification of the key signaling proteins by Western blotting and assays of their activity. ERG recording represents a simple means for initial electrophysiological characterization of rod to RBC signaling. In-depth electrophysiological analysis of rod-RBC signaling is typically performed with the suction electrode technique and patch clamp recordings from retinal slices (Fig 1) [4–6]. Because of the polarity of the rod photoreceptors, the suction electrode method offers robust measurements of photocurrents without the need to penetrate the cell membrane. Patch clamp recordings from retinal slices, made with a vibrating microtome and embedded in agar, offer the possibility to record high-quality light responses from the intact retinal circuitry with minimal cell damage. Here we describe general biochemical methods and electrophysiological measurements of membrane currents and voltages at the first visual synapse between rods and RBC.

2 Materials

2.1 Eyecup Preparation and Isolation of Retina

1. 1X PBS
2. 21-gauge needles
3. Dissecting tools: Curved iris scissors, straight forceps, spring microdissection scissors (see Note 1)

2.2 Immunoblot Analysis

1. 10% SDS
2. DC Protein Assay kit
3. 4X NuPage LDS sample buffer
4. NuPage 4–12% Bis-Tris precast gels
5. 20X NuPage MES SDS running buffer
6. Protein ladder
7. iBLOT gel transfer stacks
8. PBST: 1X PBS containing 0.1% Tween-20
9. Blocking buffer: 5% milk in PBST
10. ECL Plus western blotting detection reagents

2.3 Retinal Morphology

1. 4% PFA in 1X PBS. For 100 ml, take 80 ml of 1X PBS and heat it to approximately 60°C in a ventilated hood, taking care to not let it boil. Add 4g of paraformaldehyde powder to the heated PBS solution. Slowly raise the pH to 6.9 by adding 1 N NaOH dropwise from a pipette until the solution clears. Make up the volume to 100 ml, cool and filter the solution. Store either at 4°C for 1–2 weeks or make aliquots and freeze at –20°C.

2. Paraffin processing: Ethanol, xylene, paraffin
3. Superfrost Plus charged slides
4. Harris Hematoxylin solution
5. 1% acid alcohol
6. 0.2% ammonia water
7. Eosin-phloxine staining solution
8. Permount

2.4 Immunofluorescence

1. Tissue Freezing medium
2. 0.1% Triton X-100 in PBS
3. 3% BSA in PBS
4. Vectashield antifade mounting medium

2.5 Measurement of Rhodopsin Concentration

1. 3% Lauryldimethylamine N-oxide (LDAO) in PBS

2.6. PDE6 activity assays

1. Buffer A: 20 mM Tris-HCl (pH 7.5), 120 mM NaCl, 1 mM MgSO₄, and 1 mM β -mercaptoethanol
2. [³H]cGMP
3. AG1-X2 resin: (a) Use AG1-X2 (Bio-Rad, catalog #143–1255) anion exchange resin, hydroxide form, 200–400mesh, 100g. (b) To begin, resuspend the resin in 500mL of distilled/deionized H₂O and vacuum filter on Büchner funnel. (c) Wash the resin 3 times with 500mL of 0.5M NaOH each time, vacuuming each addition of 500mL. (d) After washing with NaOH, wash with 500mL of distilled/deionized H₂O. (e) Next, wash the resin 3 times with 500mL of 0.5M HCl each time, vacuuming each addition of 500mL. (f) Add 500mL of distilled/deionized H₂O to the resin, gently stir, and gently remove funnel from the vacuum flask to check the pH of the funnel's flow with an indicator strip. If pH is <5, then go ahead and filter through. Repeat these steps until the pH reaches 5. (g) When pH reaches 5, stir resin to resuspend and transfer to the glass bottle. Store the resin at +4°C.
4. Bacterial alkaline phosphatase
5. Trypsin
6. cGMP

2.7 Agar

1. Prepare a backstop for slice experiments (see *Methods 3.2.4*) by melting 5% by weight agar (Sigma-Aldrich # 9002–18-0, 7.5 g in 150 mL purified water) (see Note 2). Pour into 150 mm Petri dish, and store at 4°C. On the day of experiment, cut out a small square and glue to microtome specimen plate.
2. For the ground electrode bridge (see *Materials 2.13*) melt 3.5% by weight (2.5 g in 50 mL 1 mM NaCl) agar until clear and free of bubbles. Using a 10 mL syringe, slowly fill polyethylene tubing (Intramedic, PE205) and let cool. Cut to suitable length for ground electrode (1.5–2 inches) and store in a Ziplock bag at 4°C.
3. On the day of experiment, prepare agar for slice embedding (see *Methods 3.2.4*) by melting 3.5% by weight (0.75 g in 50 mL Ames' HEPES) low gelling temperature agar (Sigma-Aldrich # 39346–81-1) until clear and free of bubbles. Pour into 50 mL Falcon tube and store at 42°C.

2.8 External Bath Solution: Ames' medium

1. Add two vials of Ames' medium powder (Sigma-Aldrich # A1420), 3.8 g NaHCO₃, and ~14 mL of Penicillin/Streptomycin (to prevent contamination) to 2000 mL purified water (final volume) (see Note 3).
2. Check osmolarity and adjust to 280 mOsm. Store at 4°C until needed (see Note 4).

2.9 Slicing solution

1. Add one vial of Ames' medium powder (Sigma-Aldrich # A1420), 0.887 g of NaCl, 2.38 g of HEPES (10 mM), and 7 mL of Penicillin-Streptomycin to 1000 mL purified water (final volume).
2. Adjust pH to ~7.4 using 1 N NaOH or 1 M HCl.
3. Check osmolarity and adjust to 280 mOsm. Store at 4°C until needed.

2.10 Suction electrode internal pipette solution

1. For suction electrode internal solution use normal HEPES-buffered (10mM) Ames' medium (see *Methods 3.2.3*).

2.11 Patch-clamp Potassium Aspartate (K-Asp) internal pipette solution

1. To purified water add: 125 mM K-Aspartate, 10 mM KCl, 10 mM HEPES, 5 mM N-methyl glucamine-N- (2-hydroxyethyl) ethylenediamine-N,N,N-triacetic acid, 0.5 mM CaCl₂, 1 mM ATP-Mg, and 0.2 mM GTP-Mg.
2. Adjust pH to 7.3 with NMG-OH.
3. Check osmolarity and adjust to 280 mOsm. Store in 1.5 mL Eppendorf tubes at –18°C, until needed.

2.12 Patch-clamp Cesium internal pipette solution for Ca²⁺ current measurements

1. To purified water add: 110 mM Cs-Methanesulfonate, 12 mM Tetramethylammonium-Cl, 10 mM HEPES, 10 mM EGTA, 2 mM QX-314, 11 mM ATP-Mg, 0.5 mM GTP-Tris, and 0.5 mM MgCl₂.
2. Adjust pH to 7.3 with N-methyl-glucamine hydroxide.
3. Check osmolarity and adjust to 280 mOsm. Store in 1.5 mL Eppendorf tubes at –18°C, until needed.

2.13 Preparing electrodes

1. Hold the ground and recording electrode wires (Ag/AgCl) in 1 M NaCl and run a 15 V 1 Hz square pulse between them for ~ 10 s.
2. For suction electrode pipettes, use borosilicate glass with an outer diameter of 1.5 mm and inner diameter of 0.86 mm (Sutter Instruments, # B150–86-10).
3. For patch clamp pipettes, use borosilicate glass with an outer diameter of 1.2 mm and inner diameter of 0.69 mm (Sutter Instruments, # B120–69-10).
4. Pull pipettes of desired width using a micropipette puller (Sutter Instruments P-97).

3 Methods

3.1 Biochemical protocols

3.1.1 Eyecup preparation and Isolation of Retina

1. These procedures are usually carried out under ambient light conditions. Red light is used only if the mouse needs to be in a dark-adapted state.
2. Place curved iris scissors under the eyeball and press down gently to make the eyeball pop out a bit. Enucleate the eyeball by severing the optic nerve (See Note 5).
3. Immerse the enucleated eye immediately in 1 ml chilled 1X PBS for 2 min to wash away extra tissues from around the eyeball.
4. Using straight forceps, transfer eye to the lid of a 35mm dish that contains chilled 1X PBS. This helps maintain the shape of the eye as you work with it during dissection.
5. Use straight forceps to anchor the eyeball with the cornea facing up and then use a 21-gauge needle to make a single puncture wound in the front of the eye by inserting the needle 1–2 mm into the eyeball.
6. While holding the eyes still with your forceps, insert the lower blade of the spring microdissection scissors into the incision and start cutting along the cornea-scleral divide. Try to keep your lower blade under the iris to cut both cornea and iris. Using your straight forceps, slowly rotate the eye 360°, making

small incisions all the way around. Lift and remove the iris and cornea away from the rest of the eye to make the eyecup (See Note 6).

7. Place the eye on its side and press down gently on the back of the eye, forcing the lens out. The inside of the eyecup is lined by the retina.
8. Keep the eye lying on its side and make a cut with the spring microdissection scissors on the top side of the eye. Gently grasp the retina with one pair of forceps while holding down the rest of the eye with another pair of forceps. Pull the retina gently away from the retinal pigment epithelium (RPE) and the rest of the eye to obtain free retinas.

3.1.2 Immunoblot Analysis

1. Immerse two isolated retinas in 100 μ l of 10% SDS (See Note 7).
2. Solubilize the retinas by brief sonication (2 pulses, 3 secs each), boil at 95°C for 5 min and then centrifuge at 13,000 rpm at room temperature for 5 min.
3. Determine concentration of the protein supernatant using the DC Protein Assay with known concentrations of bovine serum albumin dissolved in 10% SDS serving as standards.
4. Add 4X NuPage LDS sample buffer to the protein samples and heat at 95°C for 5 min. Centrifuge the heated samples at 7,000 rpm for 30 secs to bring down the condensate.
5. Separate the retinal lysates according to their molecular weights by denaturing polyacrylamide gel electrophoresis (PAGE) along with protein standards. For this, use the NuPage 4–12% Bis-Tris precast gels and the NuPage MES SDS running buffer. Electrophorese at 100V until the dye front reaches the bottom of the gel.
6. Pry the gel plates open with a spatula. Remove the stacking gel and carefully immerse the rest of the gel in distilled water.
7. Use the iBLOT gel transfer device and the iBLOT gel transfer stacks to transfer the separated proteins from the polyacrylamide gel onto nitrocellulose membranes (20V, 7 min).
8. Disassemble the transfer stack. Incubate the nitrocellulose membrane on an orbital shaker with blocking solution for 1h at room temperature, followed by appropriate dilution of the primary antibody overnight at 4 °C and then appropriate HRP-coupled secondary antibody for 1h at room temperature. Primary and secondary antibody dilutions are made in blocking buffer. The membrane was washed with PBST (3 times, 5 min each) after every incubation step.
9. Proceed to develop the blots using chemiluminescent detection method. We use the ECL Plus Western Blotting Detection Reagents from GE Healthcare.

3.1.3 Retinal Morphology

1. Euthanize mice and enucleate eyeballs as described in 3.1. Transfer the eyeballs to a 35mm dish containing 4% paraformaldehyde solution, poke through the cornea with a 21-gauge needle, and immerse in 1.5 ml of 4% paraformaldehyde solution for 1h at room temperature to allow the fixative to reach the interior parts of the eye.
2. Remove the cornea and lens to make eyecups as described in 3.1. Return the eyecups to 4% paraformaldehyde for overnight fixation at 4 °C. This step allows the fixative to now directly act on the retina.
3. Next day, during paraffin processing, dehydrate the fixed eyecups by passing through an ethanol gradient (80% to 100%), immerse them in xylene and finally infiltrate them with paraffin.
4. Embed the processed eyecups in paraffin and cut 5 µm sections (using Leica RM2135). Place the sections on Superfrost Plus microscopic slides and store at room temperature until use.
5. For visualization of retinal morphology, stain the paraffin sections with hematoxylin and eosin using standard protocol. Deparaffinize and rehydrate the sections by sequential immersion in xylene, ethanol gradient (from 100% to 80%) and water. Then stain the sections with Harris hematoxylin solution, differentiate in 1% acid alcohol, and blue in 0.2% ammonia water. Wash the slides in water in between the different steps.
6. Counterstain the hematoxylin stained sections in eosin-phloxine solution and dehydrate by sequential immersion in ethanol gradient (from 80% to 100%) and xylene. Mount the stained slides with xylene-based Permount medium and visualize under Olympus BX51 microscope.

3.1.4 Immunofluorescence

1. Enucleate, poke, and cut the mouse eyes into eyecups as described in 3.3. Immerse the eyecups in 4% paraformaldehyde solution for 3 hours at room temperature. Infiltrate the fixed eyecups with 30% sucrose solution overnight at 4°C.
2. The next day, embed the eyecups in tissue freezing medium (TBS) and freeze into cryo blocks on dry ice. Cut 10 µm sections (using Microm HM 505E), place on Superfrost Plus slides and store at -80 until use.
3. For visualizing localization of different proteins in the retina, immunofluorescence assays were performed on the cryosections. Before staining, warm the slides up to room temperature. First incubate the sections with 0.1% Triton X-100 in PBS for 30 min, followed by a 1h incubation with the appropriate dilution of the primary antibody in 3% BSA in 1X PBS. Then stain the sections with goat anti-rabbit AlexaFluor 546 or goat anti-mouse AlexaFluor 488 secondary antibodies (Molecular Probes) in 3% BSA in 1X PBS for 1h. Wash the slides in 1X PBS (3 times, 5 min each) after every incubation (See

Note 8). Counterstain sections with TO-PRO3 nuclear stain for 10 min at room temperature, briefly rinse in 1X PBS and mounted using Vectashield Antifade Mounting Medium. Visualize and image the stained sections on a Zeiss LSM 510 confocal microscope.

3.1.5 Flat mount of retina

1. Whole retinal flat mounts were done to determine the spatial expression of proteins across the retina. For this, enucleate the eyes as described above in 3.1, leaving a section of the optic nerve attached to the eye. Poke, fix and cut the eyes to make eyecups as described in 3.3. Carefully separate the retina from the RPE as described in 3.1 and fix the free-floating retinas in 4% paraformaldehyde for 6h at room temperature.
2. Wash the whole retinas with 1X PBS, permeabilize by immersion in 0.1% Triton X-100 in PBS for 30 min at room temperature and incubate with appropriate primary antibody in 3% BSA in 1X PBS overnight at 4°C.
3. Add appropriate AlexaFluor 488 or 546 conjugated secondary antibody and TO-PRO3 in 3% BSA in 1X PBS to the retinas and incubate for an hour. Wash the retinas in 1X PBS (3 times, 5 min each) after every incubation.
4. Place the retinas on a Superfrost Plus slide with outer segments of the photoreceptor cells facing up. Make 4–5 radial cuts to flatten the retina (should look like petals of a flower) and then mount gently with antifade mounting medium (See Note 9).

3.1.6 Measurement of Total Rhodopsin Content in the Retina (See Note 10)

1. All steps in this procedure are conducted under dim red light except where mentioned.
2. Euthanize mouse and remove eyes as described in 3.1. Immediately place each eyeball in a 1.5 ml polyethylene tube and freeze by immersing in a liquid nitrogen bath.
3. To each eye, add 400 µl of cold 1X PBS. Sonicate 2–3 times, 5 secs each. Place the tube in ice for 10 sec in between pulses to prevent overheating of the sample. Then, to each eye, add 630 µl of cold 3% LDAO in PBS. Repeat sonication as before (After sonication, sample will turn black/brown)
4. Centrifuge at 13,000 rpm for 4 min at RT. Collect supernatant into fresh microcentrifuge tubes. Wrap in Aluminum foil and store on ice till spectra is measured.
5. Measure absorption spectra before and after bleaching the sample by exposure to bright light for 2 min. Calculate rhodopsin concentration based on the decrease in absorbance at 500 nm before and after bleaching (Molar extinction of rhodopsin $\epsilon = 42,000 \text{ M}^{-1}\text{cm}^{-1}$).

3.1.7 PDE6 activity assays

1. Isolate 2–4 mouse retinas as per the procedure described in section 3.1.
2. Homogenize the retinas by sonication (2–3, 5-sec pulses each, 10 sec gaps in between pulses) in Buffer A (See Materials 2.). Briefly centrifuge the homogenate (3000g, 2 min) to remove cell debris and store at –80°C until use.
3. To measure basal PDE6 activity (See Note 11), we use an activity assay with [³H]cGMP. Incubate the retinal homogenates in 40 µl of Buffer A containing 0.1 U bacterial alkaline phosphatase and 10 µM [³H]cGMP (equivalent to 100,000 cpm) for 10 min at room temperature. Stop the reaction by addition of 0.5 ml of 20% suspension of cold AG1-X2 cation exchange resin. Incubate the samples for 6 min at room temperature, vortexing every 2 mins. Spin down the samples at 10,000g for 3 min. Remove 0.25 ml of the supernatant and add to 5 ml of scintillation fluid. Mix well and measure in a scintillation counter.

3.2 Physiological Recording from Rods and Rod Bipolar Cells.

3.2.1 Experimental preparations

1. Before the experiment, dark-adapt the mouse for at least 12h.
2. On the day of experiment, heat and maintain the external bath solution at ~35–37°C and equilibrate with 5% CO₂/95% O₂.
3. Fill a light-tight retinal tissue storage container (see Methods 3.2.2) with 100 mL heated Ames' medium, equilibrate with 5% CO₂/95% O₂ and maintain at 32°C.
4. For suction electrode experiments, heat 5 mL of Ames' HEPES media to RT for suction internal solution (see Methods 3.2.3).
5. For patch clamp experiments, thaw ~ 1.5 mL of desired patch-clamp internal solution. Put 100 mL of cold Ames' HEPES slicing solution on ice bath and oxygenate with 100% O₂ (see Methods 3.2.4).
6. To ensure the retina is in its basal dark-adapted state, all subsequent experimental procedures are performed in total darkness under infrared light illumination using infrared converters and goggles.

3.2.2 Retina dissection

1. Euthanize the mouse by cervical dislocation.
2. Using curved scissors, remove both eyes and place on filter paper.
3. Using a sharp razor blade (Wilkinson), cut a slit through the cornea.
4. Move the eyes into a small Petri dish filled with 32°C equilibrated Ames' medium.
5. Using the pre-made slit as a starting point, cut out and remove the cornea.
6. Carefully remove the lens and the vitreous from the eyecups (see Note 12).

7. Using a glass Pasteur pipette, move the eyecups into the light tight storage container with Ames' medium equilibrated with 5% CO₂/95% O₂, and maintain at 32°C until use (see Note 13).

3.2.3 Suction electrode recordings

1. Coat the ground electrode with an agar bridge and mount the recording chamber. Fill recording electrode with Ames' HEPES (see Note 14). Make sure it contains no bubbles.
2. Using a curved scalpel blade (Bard-Parker #10), hemisect one eyecup and carefully detach the retina from the retinal pigment epithelium (RPE).
3. Using a sharp blade, chop the retina into small slices.
4. With a transfer pipette, transfer retina pieces to the empty recording chamber.
5. Let the retina slices and photoreceptors settle to the bottom (~2 min), then turn on perfusion (2 mL/min, 35–37°C).
6. Under infrared light find a suitable retinal slice with rod outer segments sticking out. Lower the recording electrode and draw a rod outer segment into the pipette using gentle suction.
7. Stimulate with flashes of light and record photocurrent responses.

3.2.4 Patch Clamp recordings

1. Coat ground electrode with an agar bridge and mount to recording setup.
2. Using a curved scalpel blade (Bard-Parker #10), hemisect one eyecup, trim the edges and carefully detach the retina from the RPE.
3. To a small 35 mm Petri dish, add a small amount of low gelling agar to cover the bottom of the dish. Drag forceps through agar, and when it starts to solidify, add previously prepared piece of retina with a transfer pipette.
4. Carefully remove excess Ames' media with coiled Kimwipe. Make sure the retina piece lays flat on the agar bed photoreceptor side down, and avoid touching the retina.
5. Gently add two drops of agar on top of the retina. Cover with plastic tube, and gently fill the plastic tube with agar to embed the retina (see Note 15). Move the Petri dish to an ice bath to cool.
6. After ~45 s, carefully remove the plastic tube containing the retina in agar and gently push out the agar block.
7. Cut out a small block containing the retina piece and move the agar block to microtome specimen holder with pre-glued agar backstop.
8. Superglue the agar block to the backstop photoreceptors facing forward, move the specimen holder into the slicing chamber and add 100 mL ice cold oxygenated Ames' HEPES.

9. Using a microtome (Leica VT100 S) vibrating at maximum speed, cut slices with a thickness of 200 μm (see Note 16).
10. Remove the slices from the vibratome blade with a sharp scalpel (Bard-Parker # 11) and transfer to your recording chamber with a transfer pipette. Place a slice anchor on top of the slice to prevent moving.
11. Mount the recording chamber, turn on perfusion (8 mL/min, 35–37°C) and insert ground electrode.
12. Fill the recording electrode with desired internal solution. Make sure the tip contains no bubbles (see Note 17).
13. Apply positive pressure to the electrode and insert into bath. Run a seal test to check the pipette is of desired size.
14. Using infrared light find a healthy rod or rod bipolar cell (see Note 18).
15. Gently lower the electrode with micromanipulators, and when the tip barely touches the cell membrane, remove negative pressure to make a high resistance seal.
16. Apply short pulses of gentle negative pressure to rupture the cell membrane and achieve whole cell mode. Run a seal test to check for membrane ($\sim 1\text{G}\Omega$) and input resistance ($\sim 20\text{M}\Omega$).

4 Notes

1. Materials in 2.1–2.6 and 2.7–2.13 are for the biochemical and electrophysiological analyses, respectively.
2. Use purified deionized MilliQ ultrapure ‘Type 1’ water (18 $\text{M}\Omega\text{-cm}$ at 25°C) for all solutions.
3. Always use Ames’ medium, created specifically to support retinal tissue in short-term cell cultures [7], for external bath solution.
4. Osmolarity is checked with a standard osmometer and pH is adjusted using a standard pH meter, both calibrated before each experiment.
5. If needed, cut the skin in the corners and tissue around the eyeball to make enucleation easier.
6. Effort should be made to squeeze the eyeball as little as possible and not to distort its shape while making eyecups.
7. SDS may precipitate at 4°C. Therefore, the lysis buffer needs to be warmed prior to use.
8. PBS with 0.05% Tween 20 can be used for the washing steps to reduce background if high background is a problem.
9. Care should be taken not to damage the structure of the outer retina by excessive pressing during mounting of flattened retinas.

10. Total rhodopsin content in dark-adapted mouse retinas is an indirect measure of the overall volume of photoreceptor outer segments, which is useful in comparison phototransduction and synaptic signaling in experimental and control mouse strains.
11. Basal (spontaneous) activity of PDE6 sets the kinetics and sensitivity of rod responses [8,9]
12. During the dissection, make sure the retina stays attached to the RPE.
13. For eyecup transfer use a glass Pasteur pipette with a rubber head on the tip instead of the back.
14. For suction electrode recordings pull pipettes with an initial width of ~ 5 μm and fire polish the pipette tip to a final width of ~ 1 μm , or a tip resistance of ~ 900 k Ω .
15. For plastic tube for making agar slice, use 10 mL syringe cut to a length of ~ 0.5 inches.
16. Make the microtome cutting-blade by cutting a sharp (Wilkinson) blade in half and coat with Sigmacoat[®] (Sigma-Aldrich #SL2) to reduce friction.
17. For rod patch clamp use pipettes with a tip resistance of ~ 16–20 M Ω . For RBC patch clamp use pipettes with a tip resistance of ~ 10–12 M Ω .
18. If cell damage has occurred on the retinal slice, dead cells can be removed by gently sucking away a layer of dead cells using a pipette with a broken tip.

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Fig 1.

Electrophysiological recordings from rods and rod bipolar cells. Suction electrode recordings can be made to measure the photocurrent from the rod outer segment. Patch clamp recordings can be done from rods and rod bipolar cells to measure the photocurrent or photovoltage. Modified from Pahlberg et al, 2011 [3]. Used with permission.

Fig 2.

Current and voltage responses in the retina. **(a)** Measurements of rod photocurrent recorded with a suction electrode. The figure shows rod photocurrent responses to flashes of light of increasing strength. Modified from Pahlberg et al, 2017 [10]. Used with permission. **(b)** Measurements of rod photovoltage with K-Asp internal solution. The rod is held in current-clamp-mode ($I=0$) to enable measurements of photovoltage. **(c)** Measurements of inner segment conductances with K-Asp internal solution. The figure shows a dark-adapted voltage step-family from a mouse rod. The cell was held at $V_m = -40$ mV and stepped for 200 ms to hyperpolarizing potentials in 5-mV increments up to $V_m = -80$ mV. Modified from Majumder et al, 2013[11]. Copyright (2013) National Academy of Sciences. Used with permission. **(d)** Rod bipolar cell responses. The rod bipolar cell was held at $V_m = -60$ mV and stimulated with brief flashes of light of increasing strength.