Submission ID #: 61491
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18754803

Title: Direct-Coupled Electroretinogram (DC-ERG) for Recording the Light-Evoked Electrical Responses of the Mouse Retinal Pigment Epithelium

Authors and Affiliations: Kiyoharu J. Miyagishima[1], Congxiao Zhang[2,*], Volha V. Malechka[3,*], Kapil Bharti[2], and Wei Li[1]
*These authors contributed equally

[1]Retinal Neurophysiology Section, National Eye Institute, National Institutes of Health
[2]Ocular and Stem Cell Translational Research Section, National Eye Institute, National Institutes of Health
[3]Human Visual Function Core, National Eye Institute, National Institutes of Health

Co-Corresponding Authors:
Kiyoharu Miyagishima
kiyoharu.miyagishima@nih.gov

Wei Li
liwei2@nei.nih.gov

Co-authors:
zhangc@nei.nih.gov
volha.malechka@nih.gov
kapilbharti@nei.nih.gov

Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **51**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Josh Miyagishima**: The DC-ERG technique can be used for the non-invasive evaluation of retinal pigment epithelium function to monitor age-related changes or disease progression and to assess pharmacological intervention effects **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Connie Zhang**: This technique improves upon DC-ERG reproducibility and ease of use by simplifying the capillary electrode preparation. It can also be supplemented with a standalone software application to facilitate analysis **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the National Institutes of Health (NIH).

Protocol

2. Capillary Electrode Filling

2.1. Begin by carefully sliding a 25-gauge syringe needle through the silicone rubber gasket to the back wall of the electrode holder **[1]**.

2.1.1. WIDE: Talent inserting needle into gasket *Videographer: Important/difficult step*

2.2. Fill the base of the electrode holder with degassed HBSS (H-B-S-S) while slowly retracting the needle, taking care not to introduce bubbles **[1-TXT]**, and replace the threaded cap without tightening **[2]**.

2.2.1. Base being filled *Videographer: Important/difficult step* **TEXT: See text for HBSS degassing details**
2.2.2. Cap being placed

2.3. Reinsert the syringe needle to fill the empty space within the cap with HBSS **[1]** and hold the capillary horizontally while filling to prevent solution escaping from the other end **[2]**.

2.3.1. Cap being filled *Videographer: Important/difficult step*
2.3.2. Capillary being filled *Videographer: Important/difficult step*

2.4. Grasping the filled capillary at the bent end, slowly insert the other end through the loosened cap **[1]** and tighten the screw cap into place **[2]**.

2.4.1. End being inserted into cap NOTE: 2.4.1 and 2.4.2 in one shot *Videographer: Important/difficult step*
2.4.2. Cap being tightened

2.5. Tilt the electrode holders with the capillary electrodes facing up to allow any bubbles to flow out **[1]** and degas the capillaries in a vacuum chamber for 5-10 minutes **[2-TXT]**.

2.5.1. Holders being positioned
2.5.2. Talent starting vacuum **TEXT: Escaping gas will push HBSS from holders**

2.6. Then slowly release the vacuum **[1]** and refill the electrode holders and glass capillaries as demonstrated **[2]**.

2.6.1. Vacuum being released

2.6.2. Holders and/or capillaries being filled

3. Microelectrode Holder Preparation

3.1. To make a custom stand for the microelectrode holder, remove the black polyacetal clips from one side of a number-8 T-clip **[1]** and use a cylinder base with magnetic ball joints machined in half to adjust the height **[2]**.

3.1.1. WIDE: Talent removing clip(s)
3.1.2. Stand

3.2. Secure the modified T-clips to the magnetic ball mounting screws with M3-sized nuts **[1]** and slide an approximately a 1-inch tapered wooden handle modified from a cotton tipped cleaning stick into the stand at an angle to secure the microelectrode holder into the custom-made T-clip-magnetic ball joint stand **[2]**.

3.2.1. Talent securing clips to screws NOTE: 3.2.1 – 3.3.1 in one shot and audio slated
3.2.2. Holder being secured to stand

3.3. Then use the rare earth magnet cylinder base to securely position the customized electrode holder stand onto the metal plate of the stage **[1]**, enabling a 360-degree rotation on a 180-degree axis **[2]**.

3.3.1. Talent positioning base
3.3.2. Holder being rotated

4. Electrode Test

4.1. To perform an electrode test, first gently lower the fully assembled, HBSS-filled capillary microelectrodes into a small container of HBSS **[1]**.

4.1.1. WIDE: Talent lowering electrodes into HBSS

4.2. Place the needle ground electrode **[1]** and the silver-silver chloride-sintered pellet reference electrode in the same HBSS to complete the circuit **[2]** and select or create an appropriate identifier to describe the mouse to be tested **[3]**.

4.2.1. Needle ground electrode being placed into HBSS NOTE: 4.2.1 – 4.2.2 in one shot
4.2.2. Reference electrode being placed into HBSS
4.2.3. Talent selecting identifier, with monitor visible in frame

4.3. To select the direct-coupled electroretinogram protocol to be performed, click **Protocols** and select **DC-ERG** (D-C-E-R-G) **[1]**.

4.3.1. SCREEN: screenshot_1_t2: 00:17-00:26

4.4. Click **Run**. A dialog box will pop-up with the patient information. If the information is correct, click **Yes** and proceed to Step 1 of 6 **[1]**.

4.4.1. SCREEN: screenshot_1_t2: 00:26-00:33

4.5. Close the doors to the faraday cage **[1]** and click **Impedance** to display the impedance mode **[2]**.

4.5.1. Talent closing door(s)
4.5.2. Talent clicking Impedance

4.6. Verify that the values for the mouth reference, tail ground, and recording electrodes are acceptable and click **Step** to proceed to step 4 of 6 to test the baseline stability **[1]**.

4.6.1. SCREEN: screenshot_1_t2: 00:37-00:55 *Video Editor: please speed up*

4.7. To begin viewing the traces, click **Preview**. The traces should be low noise with a peak-to-peak amplitude of less than 200 microvolts **[1-TXT]**.

4.7.1. SCREEN: screenshot_1_t2: 00:56-03:50 *Video Editor: please speed up* **TEXT: Slight <500 microvolt/80 s drift that gradually fades to baseline acceptable**

5. Mouse and Electrode Positioning

5.1. To position the mouse and electrodes for an experiment, anesthetize the cornea of a sedated mouse with a drop of 0.5% proparacaine hydrochloric acid **[1-TXT]** before dilating the cornea with a drop of 2.5% phenylephrine hydrochloric acid and 0.5% tropicamide **[2]**.

5.1.1. WIDE: Talent adding proparacaine HCl onto eye NOTE: all shots except 5.4 and SC in Section 5 are audio slated *Videographer: More Talent than mouse in shot* **TEXT: Anesthesia: ketamine 80 mg/kg + xylazine 8 mg/kp i.p.**
5.1.2. ECU: Drop being applied to eye

5.2. In the ERG system software, verify that the correct patient is selected **[1]**.

5.2.1. SCREEN: screenshot_2_t1: 00:02-00:08

5.3. Click **Protocols**. Under **Protocol Description**, select **DC-ERG** and click **Run**. Click **Yes** to verify that the correct test is being performed **[1]**.

5.3.1. SCREEN: screenshot_2_t1: 00:09-00:15

5.4. Use Step 1 of 6 to turn on a dim red light inside the dome **[1]** and place the mouse on a heated recording table **[2-TXT]**.

5.4.1. Red light being turned on in dome
5.4.2. Talent placing mouse onto table **TEXT: Trim whiskers to prevent glass capillary electrode twitching disturbance during recording** *Videographer: More Talent than mouse in shot*

5.5. Use forceps to carefully tent the skin of the rear leg **[1]** and, using one hand to firmly hold the needle electrode, use the other hand to insert the electrode subcutaneously into the rear leg to secure the electrode into place **[2]**.

5.5.1. Skin being tented NOTE: 5.5.1 – 5.5.2 in one shot
5.5.2. Shot of electrode held firmly, then electrode being inserted

5.6. Place the reference silver-silver-chloride electrode into the animal's mouth **[1-TXT]** so that the sintered pellet rests along the back cheek and is held in place behind the teeth **[2]**.

5.6.1. Electrode being placed into mouth **TEXT: Gold electrodes increase noise** NOTE: 5.6.1 – 5.6.2 in one shot
5.6.2. ECU: Shot of pellet resting on back cheek held in place behind teeth *Videographer/Video Editor: can skip if not clear shot*

5.7. Before placing the capillary electrodes onto the eye, position the electrode holder with the glass capillaries oriented vertically **[1]** and flick the electrode holder with an index finger to remove any bubbles that may have been introduced **[2]**.

5.7.1. Talent picking up holder/orienting holder in vertical position NOTE: 5.7.1 – 5.7.2 in one shot
5.7.2. Holder being flicked *Videographer: Important/difficult step*

5.8. Use a 25-gauge needle to fill the capillary tips with HBSS **[1]** and inspect the capillaries to ensure that there are no air bubbles trapped in the tips **[2]**.

5.8.1. Talent filling tip NOTE: 5.8.1 – 5.8.2 in one shot
5.8.2. Shot of needle without bubbles *Videographer: Important step*

5.9. Position the electrode holder stand so that the open tips of the HBSS-filled capillaries are in gentle contact with the cornea **[1]** and, taking care to avoid introducing bubbles, invert the lubricant eye gel dispenser to discard the initial drops **[2]**.

5.9.1. ECU: Holder being positioned in contact with cornea NOTE: 5.9.1 – 5.9.2 in one shot *Videographer: Important step*
5.9.2. Dispenser being inverted/drops being discarded *Videographer: Important step*

5.10. Then place a drop of lubricant eye gel onto each eye to maintain conductivity and to prevent desiccation during the recording **[1]**.

5.10.1. Gel being placed onto eye *Videographer: Important step*

6. Direct-Coupled Electroretinogram (DC-ERG) Recording

6.1. To record the DC-ERGs, click **Step** to select step 5 of 6 **[1]** and click **Impedance** to use the **Impedance Checking** screen to examine the resistances of the left and right eyes **[2]**.

6.1.1. WIDE: Talent clicking Step, with monitor visible in frame
6.1.2. SCREEN: screenshot_2_t1: 02:46-02:51

6.2. The impedance values for the recording electrodes at each eye should be similar and the impedance values for both the ground and reference electrodes should be less than 10 kiloohms **[1]**.

6.2.1. SCREEN: screenshot_2_t1: 03:09-03:23 *Video Editor: please speed up and please emphasize red values with "recording … similar" and please emphasize the yellow values with "both the ground … kilooohms"*

6.3. Click **Preview** to view the traces for the left and right eyes and wait up to 10 minutes for a stable baseline to be achieved **[1]**.

6.3.1. SCREEN: screenshot_2_t1: 08:52-09:57 *Video Editor: please speed up*

6.4. Then click **Stop** to exit the trace preview **[1]** and click **Run** to start the recording **[2]**.

6.4.1. SCREEN: screenshot_2_t1: 11:00-11:02
6.4.2. SCREEN: screenshot_2_t1: 11:11-13:25 *Video Editor: please speed up*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?

2.1.-2.4., 5.7.-5.9.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
Filling the electrode (2.1-2.4) and removing bubbles are the single most difficult aspects of the procedure (5.7). I can have another set of electrodes ready (bubble-free) prior to the JOVE film crew arriving.

Additionally, the screen capture videos showing the appropriate DC-ERG responses are difficult to perform and record (videography) given the dark lighting conditions and limited space. However, I will record those independently using the screen capture software suggested by JOVE and upload them prior to scheduling the actual shoot with JOVE.

Results

7. Results: Representative DC-ERG Traces and Workflow Analyses

7.1. Here a sample dataset from conditional knockout and wild type mice is shown **[1-TXT]**.

7.1.1. LAB MEDIA: Figure 2 **TEXT: *i.e.*, MiR-204 ko/ko cre/+ mice**

7.2. In this analysis, the trace suffered from minute bubbles in the electrode **[1]** that increased the peak-to-peak noise in the trace **[2]**.

7.2.1. LAB MEDIA: Figure 2A top panel *Video Editor: please add/emphasize arrows and Noise text*
7.2.2. LAB MEDIA: Figure 2A top panel *Video Editor: please emphasize blue line*

7.3. In a separate analysis, the bubbles were eliminated using the vacuum chamber prior to assembling the microelectrodes within the electrode holder stands **[1]**.

7.3.1. LAB MEDIA: Figure 2A bottom panel

7.4. The best fit lines to the initial 25 seconds **[1]** can be calculated **[2]**, allowing the drift corrected responses to be replotted **[3]** and the amplitudes of the DC-ERG components to be identified **[4]**.

7.4.1. LAB MEDIA: Figure 2B *Video Editor: please emphasize green portion of data lines*
7.4.2. LAB MEDIA: Figure 2B *Video Editor: please emphasize blue lines in graphs*
7.4.3. LAB MEDIA: Figure 2C
7.4.4. LAB MEDIA: Figure 2C *Video Editor: please add/emphasize figure keys*

7.5. As expected, reduced expression of K_{ir} (keer) 7.1 potassium channels greatly attenuates the c-wave **[1]** and fast oscillation **[2]**, indicating a significant impairment of the retinal pigment epithelium electrical properties **[3]**.

7.5.1. LAB MEDIA: Figures 2D and 2E *Video Editor: please add/emphasize C-wave text and arrow in Figure 2D and emphasize grey C-wave data bar in Figure 2D*
7.5.2. LAB MEDIA: Figures 2D and 2E *Video Editor: please add/emphasize FO text and arrow in Figure 2D and emphasize grey FO data bar in Figure 2E*
7.5.3. LAB MEDIA: Figures 2D and 2E

7.6. Here the relative amplitudes of the DC-ERG components normalized to wild type and plotted against the relative two largest light-evoked a-wave amplitudes are shown **[1]**.

7.6.1. LAB MEDIA: Figures 2F-2H

7.7. The reduction in the a-wave response to the brightest light intensity **[1]** suggests a delay in the recovery of the sensitivity due to a visual cycle impairment **[2]**.

7.7.1. LAB MEDIA: Figures 2F-2H *Video Editor: please add/emphasize asterisk/data point indicated by asterisk*

7.7.2. LAB MEDIA: Figures 2F-2H

Conclusion

8. Conclusion Interview Statements

8.1. **Josh Miyagishima**: Remember to achieve a stable baseline prior to moving onto the mouse recording and to reinspect the electrodes periodically for bubbles that may have been introduced **[1]**.

8.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (4.2)

8.2. **Connie Zhang**: Dark-adapted ERGs can be recorded *prior* to the DC-ERG to measure the rod-driven retinal function. Light-adapted ERGs can also be performed *after* the DC-ERG to evaluate cone-driven retinal responses **[1]**.

8.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera