SOP Test 17 - Electroretinogram (ERG) Measurements in Larval Zebrafish

1 Purpose

Record whole field potential of the eye in response to light.

2 Scope

This electrophysiological technique is used to record the field response of the eye in response to light. It is a convenient tool to locate the defect in the visual pathway of a blind mutant. A flat ERG implies absence of light perception by photoreceptors, absence of the b-wave, lack of photoreceptor synapse function, and reduced b-waves are mostly indicative of reduced light perception.

3 Safety requirement

4 Associates documents

5 Notes

There are numerous variations depending on the particular assay. The described method is suitable for a general assessment of outer retina function. Many variations of the light used (light of different spectral composition, monochromatic light) or of background light conditions are possible. Additionally pharmacological treatment can be used to isolate the subcomponents, for instance to isolate the direct photoreceptor contribution (a-wave).
Recordings can also be done in larvae without paralysis or with MESAB instead of Esmeron, although Esmeron is preferable, because it targets muscles and not nerve cells.

6 Quality control

7 Equipment

- Amplifier (any commercially available electrophysiology amplifier will do)
- Binocular microscope containing light source equipped with infrared filter (e.g. Zeiss Stemi SV8, Oberkochen, Germany)
- DAQ Board NI PCI 6035E connected to a NI BNC-2090 BNC terminal Faraday cage
- General purpose stimulator (Master-8, A.M.P:I., Jerusalem, Israel)
- Light guide
- Light source (e.g. 24V, 250W projection lamp Liesegang Diafant 250, Düsseldorf, Germany)
- Mechanical shutter (custom built; commercial ones work as well)
- Micromanipulator with holder (e.g. M3301, WPI, Stevenage, UK)
- Micropipette puller (e.g. P-87, Sutter Instruments CO, USA)
- Neutral density filter wheels
- Oscilloscope (e.g., HM408 Hameg GmbH, Frankfurt am Main, Germany)
- Pre-Amplifier (e.g. P55 A.C. GRASS technologies, Astro-Med Inc., West Warwick, USA)
- Recording electrode (e.g. GC100-10, Harvard Aparatus Ltd, Kent, UK) with a tip diameter between 20-30 µm
- Reference electrode (e.g. an Ag/AgCl pellet, HLA-003, Axon Instruments, Union City CA, USA)
- Recording software (e.g. LabVIEW VI)

8 Supplies

E3 Medium
Esmeron® (Organon Teknika, Eppelheim, Germany)
Syringe (attach e.g. Microleader tip, Eppendorf, Hamburg, Germany)
Sponge (35mm diameter to fit the petridish)

Brush
Petridish (35mm diameter)

9 Procedure

1. Recordings should be performed inside a Faraday cage to reduce electrical noise.
2. Connect the light source to the light guide, the neutral density filter wheels and the mechanical shutter.
3. Connect both the recording electrode and the reference electrode to the amplifier via the pre-amplifier.
4. Connect the amplifier to the oscilloscope and the DAQ Board.
5. Use a binocular microscope for positioning of the larvae.

Preparation of Electrodes

6. Prepare a micropipette with the desired tip diameter (15-30µm) using a micropipette puller, fill it with E3 Medium using a syringe and attach it to the micropipette holder.
7. Attach the micropipette holder to the micromanipulator.
8. Fix the reference electrode on the petridish containing the sponge.
9. Place a piece of paper tissue onto the reference electrode.
10. Moisten the sponge and the paper with E3 Medium.

Placement of the Zebrafish Larva

11. Larvae need to be dark adapted for minimum 30min.
12. In order to minimize bleaching of the visual pigment, always work under red illumination.
13. Place a single larva onto the pellet of the reference electrode using the brush, and orient it in such way that one eye is directed to the light beam.
14. Paralyze the larva with a drop of Esmeron®.
15. Position the recording electrode on the centre of the cornea using the micromanipulator.
    The recording electrode should just slightly touch the eye, do not insert the electrode into the eye.
16. Ensure that the light guide is positioned properly, so that the light beam is directed at the larval head.

Standard Measurements

17. Turn on all components of the setup equipment.
18. Adjust desired light intensity using the neutral density filter wheels.
19. For standard measurements use a stimulus duration of 100 - 1000ms and an acquisition time of 1000-2000ms, depending on the stimulus duration.
    A 100ms stimulus is sufficient for eliciting a- and b-waves; for recording a d-wave a longer stimulus duration (of 1000ms) is required.
    A scan rate of 1000Hz, and a bandpass of 1-100Hz is used for all our recordings.
20. Observe recordings on the oscilloscope, or alternatively use a computer and a customized software (e.g. LabView Software).
21. In order to get an averaged curve, repeat measurements for 5 times with a defined interstimulus interval of 5000-7000ms.

10 Supporting Information

Fleisch, VC, Jametti T, Neuhauss, SCF* (2008). Electroretinogram (ERG) Measurements in Larval Zebrafish. *Cold Spring Harbor Protocols* 10.1101 prot4973

Makhankov, YV, Rinner, O, Neuhauss*, SCF (2004). An inexpensive device for non-invasive electroretinography in small aquatic vertebrates. *Journal of Neuroscience Methods* 135, 205-210