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Abstract

The electroretinogram (ERG) is an electrophysiological tool used to measure electrical activity originating in the outer retina in response to a light stimulus. Defects occurring at various levels of the retina can easily be detected by ERG measurements. Furthermore, the shape of the ERG response points toward the likely retinal cell type responsible for the deficit. Thus, this method is particularly useful for a rapid assessment of retinal function in genetically or pharmacologically manipulated animals. A typical ERG curve can be subdivided into three components: a small initial a-wave originating in photoreceptor activity, a large positive b-wave reflecting mainly ON bipolar cell depolarization, and a d-wave occurring at light offset. Here we present a noninvasive protocol for taking ERG measurements in larval zebrafish (4-7 days post-fertilization [dpf]). We use an extracellular recording electrode which is placed onto the surface of the cornea of the larva, and a light flash of a defined intensity and duration which is applied to evoke a response. In a typical larval ERG trace, we are able to record ERG a-, b-, and d-waves.

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Protocol

Electroretinogram (ERG) Measurements in Larval Zebrafish

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INTRODUCTION

The electroretinogram (ERG) is an electrophysiological tool used to measure electrical activity originating in the outer retina in response to a light stimulus. Defects occurring at various levels of the retina can easily be detected by ERG measurements. Furthermore, the shape of the ERG response points toward the likely retinal cell type responsible for the deficit. Thus, this method is particularly useful for a rapid assessment of retinal function in genetically or pharmacologically manipulated animals. A typical ERG curve can be subdivided into three components: a small initial a-wave originating in photoreceptor activity, a large positive b-wave reflecting mainly ON bipolar cell depolarization, and a d-wave occurring at light offset. Here we present a noninvasive protocol for taking ERG measurements in larval zebrafish (4–7 days post-fertilization [dpf]). We use an extracellular recording electrode which is placed onto the surface of the cornea of the larva, and a light flash of a defined intensity and duration which is applied to evoke a response. In a typical larval ERG trace, we are able to record ERG a-, b-, and d-waves.

MATERIALS

Reagents

 E3 medium

Esmeron/Zemuron (Organon/Schering Plough) (0.8 mg/mL in E3 medium)

Zebrafish larvae (*Danio rerio*)

Equipment

Amplifier (electrophysiology)

We use a custom-made amplifier; however, any commercially available electrophysiology amplifier is acceptable.

Brush

Data acquisition (DAQ) board (NI PCI 6035E) connected to a BNC terminal block (NI BNC-2090; National Instruments)

Faraday cage

Light guide (i.e., halogen bulb covering the visible spectrum)

Light source (24-V, 250-W projection lamp) (e.g., Liesegang Diafant 250)

Mechanical shutter (custom built or commercial)

Micromanipulator (e.g., M3301; WPI Inc.)

Micropipette holder (WPI Inc.)

Micropipette puller (e.g., P-87; Sutter Instruments)

Microscope (binocular, containing light source equipped with infrared filter) (e.g., Zeiss Stemi SV8)

Neutral density filter wheels (two filter wheels covering five log units)

Unattenuated light intensity over the larval head is 3100 lux (optical density [OD] is equal to 0 log unit), as measured by a light meter (Tekronix J17).

Oscilloscope (e.g., HM408; HAMEG) or computer and customized recording software (e.g., LabVIEW Virtual Instrument [VI])

Petri dish (35-mm diameter)

Preamplifier (general purpose AC) (e.g., P55; GRASS Technologies)

Recording electrode (borosilicate glass micropipette) (e.g., 300016; Harvard Apparatus Ltd.), with a tip diameter of 15-30 μm

Reference electrode (Ag/AgCl pellet) (e.g., HLA-003; Molecular Devices)

Sponge or other fluid-absorbent material (35-mm diameter, to fit Petri dish)

Stimulator (general purpose) (Master-8; A.M.P.I.)

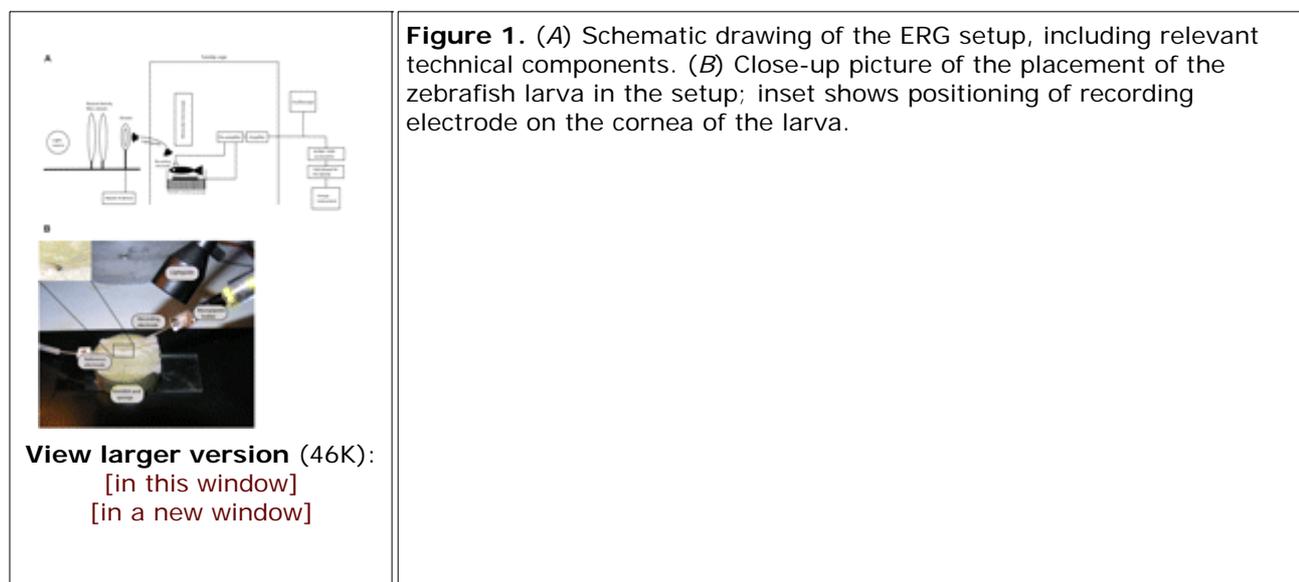
Syringe

Tissue

METHOD

Recording Setup Assembly

An assembled recording setup is shown in [Figure 1](#) . Recordings should be performed inside a Faraday cage to reduce electrical noise.



1. Connect the light source to the light guide, the neutral density filter wheels and the mechanical shutter. *Neutral density filter wheels are used to adjust the desired light intensity. The shutter is controlled by computer-based software (Master-8 software; A.M.P.I.).*
2. Connect both the recording electrode and the reference electrode to the amplifier via the preamplifier. *Preparation of the recording electrode is described in Step 5.*
3. Connect the amplifier to the oscilloscope and the DAQ Board.

4. Set up a binocular microscope, to be used for positioning of the larvae.

Preparation of Electrodes

5. Prepare a recording electrode (micropipette) with the desired tip diameter (15-30 μm) using a micropipette puller.

Using the P-87 puller, we employ the following parameters: heat = 367, pull = / , velocity = 16, time = 250, pressure = 500. However, these parameters will vary extensively depending on the type of puller (and filament) used.

Make sure that the tip diameter is correct and that the tip end is smooth and not damaged. Tips do not need to be flame-polished.

6. Fill the micropipette with E3 medium using a syringe, and attach it to the micropipette holder.
7. Attach the micropipette holder to the micromanipulator.
8. Place a sponge or other fluid-absorbent material (35 mm in diameter) in a 35-mm Petri dish. Fix the reference electrode to the Petri dish containing the sponge. Place the pellet of the reference electrode in the center of the binocular microscope's visual field.
9. Place a piece of tissue onto the reference electrode.
10. Moisten the sponge and the tissue with E3 medium.

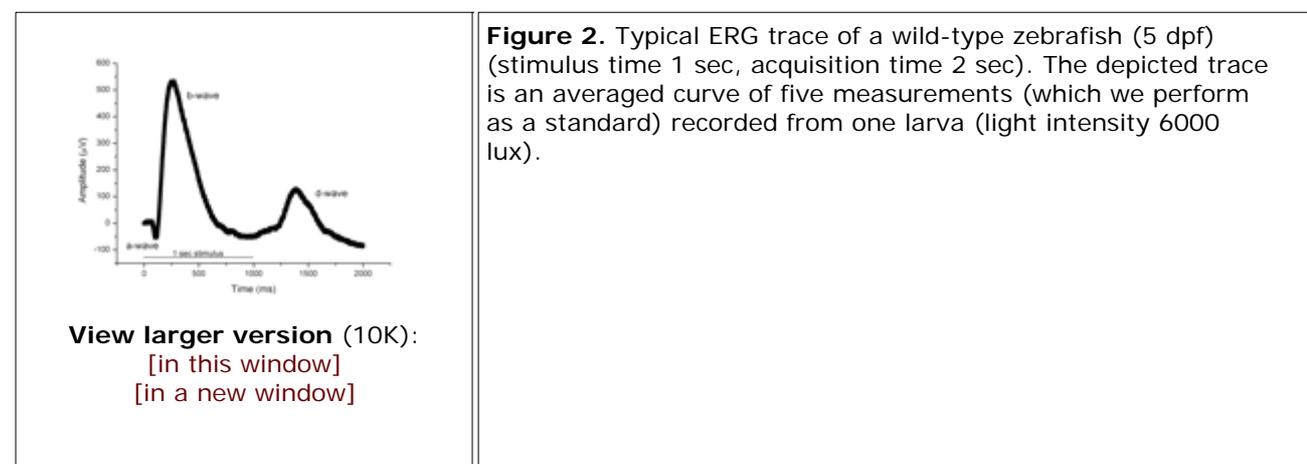
Placement of Larva

In order to minimize bleaching of the visual pigment, always work under red illumination.

11. Adapt larvae to the dark for a minimum of 30 min. Place a single larva onto the pellet of the reference electrode using a brush. Orient the larva such that one eye is directed to the light beam.
12. Paralyze the larva with a drop of Esmeron.
13. Position the recording electrode on the center of the cornea using the micromanipulator. *The recording electrode should just slightly touch the eye; do not insert the electrode into the eye.*
14. Position the light guide such that the light beam is directed at the larval head.

Standard Measurement Recording

An example of an ERG trace is shown in Figure 2 .



15. Turn on all components of the setup equipment.

16. Adjust the desired light intensity using the neutral density filter wheels.

During standard recordings, shut off all external light sources (including the infrared light). If desired, recordings can also be done under different background illumination levels.

17. To take standard measurements, use a stimulus duration of 100-1000 msec and an acquisition time of 1000-2000 msec, depending on the stimulus duration.

A 100-msec stimulus is sufficient for eliciting a- and b-waves; a longer stimulus duration (of 1000 msec) is required for recording a d-wave.

We use a scan rate of 1000 Hz and a bandpass of 1-100 Hz for all recordings.

The total signal amplification of the amplifier and preamplifier equals 1000X.

18. Observe recordings on the oscilloscope, or alternatively use a computer and customized recording software (e.g., LabVIEW VI).

See Troubleshooting.

19. To produce an averaged curve, repeat measurements five times with a defined interstimulus interval of 5000-7000 msec (Fig. 2).

The interstimulus interval can be adjusted as desired; if recordings are done using different light intensities, intervals can be longer to gain time for turning the filter wheels.

TROUBLESHOOTING

Problem: A measurable electrical response occurs without a sample.

[Step 10]

Solution: This is due to the photovoltaic effect of an oxidized reference electrode. Confirm that the reference electrode shines brightly metallic. If not, clean it carefully with fine grinding paper. This should abolish the photoelectric response.

Problem: Irregular waves (e.g., several peaks) are observed.

[Step 18]

Solution: There may be a defect in the recording electrode, such as an end that is not smooth or a narrow tip. Change the recording electrode.

Problem: Small b-wave amplitude (<150 μ V) is observed, even with bright light.

[Step 18]

Solution: Confirm that the recording electrode is well positioned on the center of the cornea and remove any surplus E3 medium on the sponge. Alternatively, change the recording electrode.

Problem: High background noise affects measurement.

[Step 18]

Solution: Consider the following:

- Make sure that all components of the setup are properly grounded. This may require adjustment during the initial setup.
- Place a metal grid in front of the whole assembly to reduce noise (Fig. 1).
- Use a 50-Hz filter on the amplifier.
- A recording electrode with a small opening diameter also produces electrical noise; use an electrode with a tip diameter of at least 15 μ m.

DISCUSSION

The ERG setup described here can be used to measure ERG responses of larval zebrafish, as well as *Medaka* larvae and tadpoles of *Xenopus laevis* (Makhankov et al. 2004). Moreover, it can be easily adapted for

measurements of adult zebrafish (with use of an oxygenation chamber) and other aquatic species. Larval zebrafish begin to show reliable ERG responses at 4 dpf. It is important to keep in mind that all responses measured in these larvae are cone-driven responses, since rods do not contribute to the ERG response until 15 dpf. Therefore the larval retina during this time span is considered to be functionally cone-dominant (Branchek 1984; Bilotta et al. 2001).

The use of specialized recording software (e.g. LabVIEW VI) allows creation of different paradigms (e.g., bleaching, double flash paradigm, etc.), which can be enormously useful in addressing specific biological questions of interest.

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Recipe

E3 medium

5 mM NaCl

0.17 mM KCl

0.33 mM CaCl₂

0.33 mM MgSO₄

0.1% methylene blue (Sigma)

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