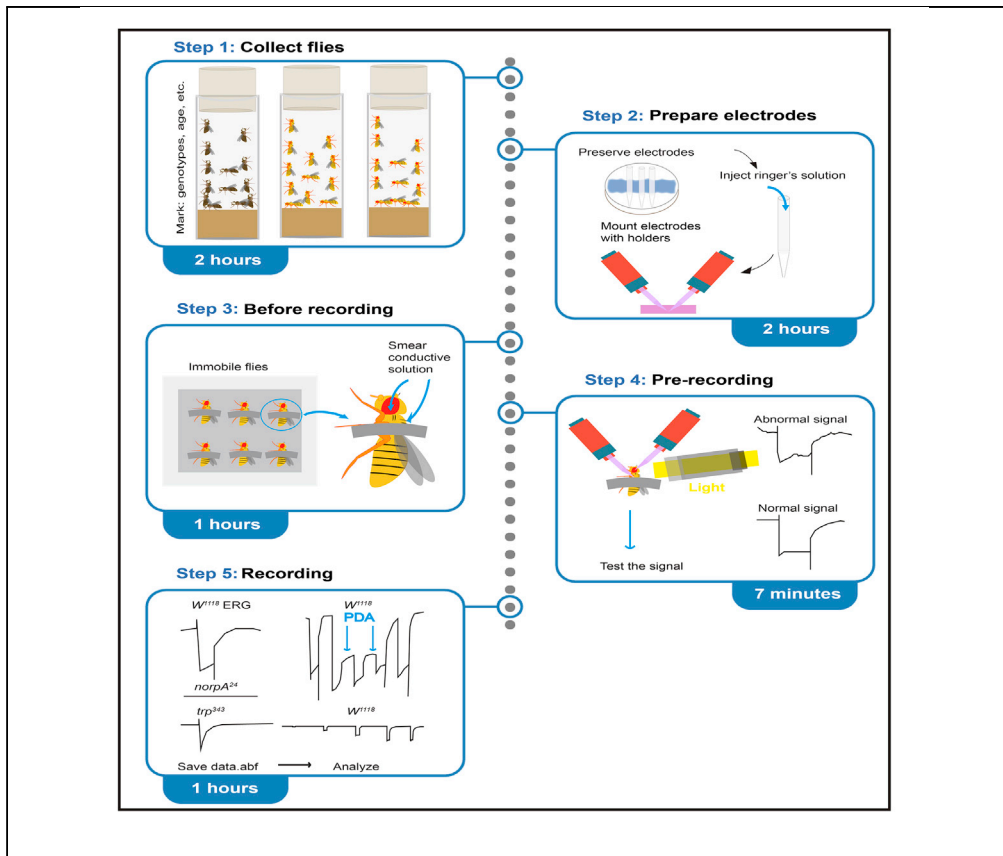


## Protocol

# Protocol for electroretinogram recording of the *Drosophila* compound eye



Jinglin Wu, Yao Tian,  
Wei Dong, Junhai  
Han

dongwei@swmu.edu.cn  
(W.D.)  
junhaihan@seu.edu.cn  
(J.H.)

### Highlights

Optimized protocol to detect the rapid response upon light stimulation of *Drosophila*

Protocol facilitates large-scale genetic screening of *Drosophila*

Detailed procedures for ERG recordings under normal and restrictive conditions

*Drosophila* phototransduction is a well-established model for characterizing biological processes, such as calcium signaling. Here, we present a protocol for electroretinogram (ERG) recording, an *in-vivo* physiological assay extensively used in *Drosophila* phototransduction research to measure light-evoked field potential responses of photoreceptors and neurons in the lamina neuropil. We describe fly preparation and electrode placement, followed by pretest setup, ERG recording, and data analysis. This protocol enables assessment of photoreceptor performance and visual synaptic transmission between photoreceptors and downstream lamina neurons.

Wu et al., STAR Protocols 3,  
101286  
June 17, 2022 © 2022 The  
Author(s).  
[https://doi.org/10.1016/  
j.xpro.2022.101286](https://doi.org/10.1016/j.xpro.2022.101286)



## Protocol

Protocol for electroretinogram recording of the *Drosophila* compound eyeJinglin Wu,<sup>1,3</sup> Yao Tian,<sup>1,3</sup> Wei Dong,<sup>2,\*</sup> and Junhai Han<sup>1,4,5,\*</sup><sup>1</sup>School of Life Science and Technology, Key Laboratory of Developmental Genes and Human Disease, Southeast University, Nanjing 210096, China<sup>2</sup>Key Laboratory of Medical Electrophysiology of Ministry of Education and Medical Electrophysiological Key Laboratory of Sichuan Province, Institute of Cardiovascular Research, Southwest Medical University, Luzhou, Sichuan 646000, China<sup>3</sup>These authors contributed equally<sup>4</sup>Technical contact<sup>5</sup>Lead contact\*Correspondence: [dongwei@swmu.edu.cn](mailto:dongwei@swmu.edu.cn) (W.D.), [junhaihan@seu.edu.cn](mailto:junhaihan@seu.edu.cn) (J.H.)  
<https://doi.org/10.1016/j.xpro.2022.101286>

## SUMMARY

*Drosophila* phototransduction is a well-established model for characterizing biological processes, such as calcium signaling. Here, we present a protocol for electroretinogram (ERG) recording, an *in-vivo* physiological assay extensively used in *Drosophila* phototransduction research to measure light-evoked field potential responses of photoreceptors and neurons in the lamina neuropil. We describe fly preparation and electrode placement, followed by pretest setup, ERG recording, and data analysis. This protocol enables assessment of photoreceptor performance and visual synaptic transmission between photoreceptors and downstream lamina neurons.

For complete details on the use and execution of this protocol, please refer to Han et al. (2006), Hu et al. (2012), and Wu et al. (2021).

## BEFORE YOU BEGIN

Electroretinogram (ERG) can be performed on any live adult fly. Researchers can maintain the flies according to their own experimental needs, but the experimental group and control group flies should be of the same age. It is recommended to adapt the flies to the dark at least 12 h before recording, due to long-term adaptation processes such as light-dependent Gq and transient receptor potential-like (TRPL) channel translocation (Katz and Minke, 2018; Gu et al., 2020). The flies used in the following protocols were chosen to present comparable recording results. We raised the flies on yeast-based food at 25°C under 12 h light/dark cycles and maintained the ambient relative humidity at 60%, unless other specific conditions were required. Temperature was chosen based on the results of temperature gradient tests, which demonstrated that *Drosophila* have a strong behavioral preference to dwell at ambient temperatures between 23°C and 25°C (Sayeed and Benzer, 1996).

Detailed information on the flies is presented in these papers, including genotype and age (Hu et al., 2012; Han et al., 2006; Wu et al., 2021).

## Collect flies

⌚ Timing: ~1 h

1. Collect 1-day-old  $w^{1118}$ , *Lai-Gal4/UAS-TeTxLC*, *Lai-Gal4/+*, and *UAS-TeTxLC/+* flies in new tubes.



**Note:** Carefully label tubes with corresponding information about the flies [genotype, age, sex, eye color (white vs. red, etc.)].

△ **CRITICAL:** Except when comparing the signals of the same genotype at different ages, it is best to choose flies of the same age. Different conditions and ages of *Drosophila* lead to different ERG signals. Therefore, it is necessary to ensure the status of incubation, and age of both the experimental group and control group are consistent, so the results can be compared.

- Put flies in the incubator until recording.

### Prepare electrodes

⌚ **Timing:** ~2 h

- For ERGs, normally place a recording electrode on the eye, and randomly place a reference electrode on the animal (typically in the thorax).
- Fabricate electrodes for ERG recording (Electrode information: length 100 mm, OD 1.0 mm, ID 0.58 mm, without filaments, Cat# 1B100-4) with the P-97 Flaming/Brown type micropipette puller with the program: heat = 625, pull = 85, velocity = 78, time = 98.

**Note:** Under these conditions, the electrodes possess a low resistance of approximately 1–10 MΩ. Apply the software pCLAMP 10.6, and add voltage to measure the pipette resistance.

△ **CRITICAL:** Proper electrode resistance is very important for the successful recording of ERG. Tip length has little correlation with electrode resistance. Most of the resistance of a microelectrode is in the electrode shank behind the tip. Electrodes with higher resistance will likely have a longer shank and a smaller cone angle and opening size at the tip (see P-97 Flaming/brown micropipette puller operation manual for details).

- Put the prepared electrodes on plasticine within a dish to prevent tip fracture (Figure 1).

**Note:** We usually prepare more than ten electrodes at a time and use them in step 7. Provided the electrodes are free of dust, they can be stored and used at any time.

⏸ **Pause point:** Electrodes can be stored in a dish for up to one year.

- Use the prepared electrodes as recording electrodes and reference electrodes.

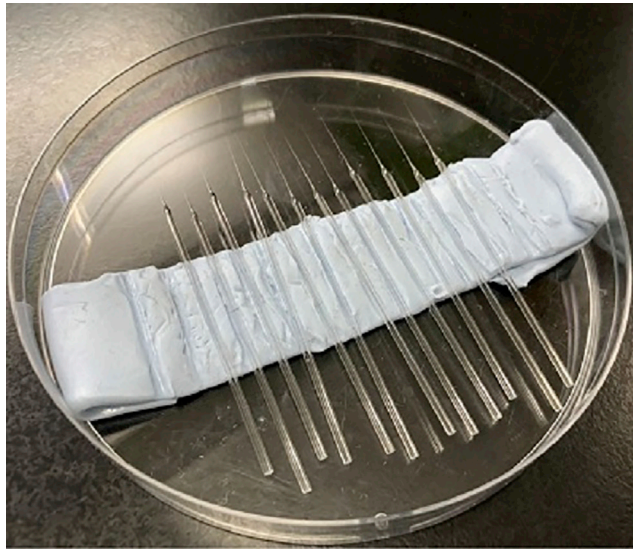
**Note:** Break off the tip of the reference electrodes to avoid piercing the thorax. Unlike the recording electrodes, the resistance of the reference electrodes is not important for successful ERG recording.

- Prepare Ringer's solution, then filter the solution with a combined filter for solvent with a 0.22 μm Millipore filter membrane before filling the electrodes.

⏸ **Pause point:** Ringer's solution can be stored at room temperature (20°C–26°C) for two weeks.

- Fill the electrodes with Ringer's solution. Cut the front 2/3 of a microloader tip and install it on the needle of a 1 mL sterile syringe (Figure 2).

**Note:** With this filling device, the Ringer's solution is drawn and injected into the electrode with as few bubbles as possible.



**Figure 1. The prepared electrodes**

Keep the electrode tip away from the dish edge. Electrodes are placed on plasticine within the dish. It is useful to place a larger diameter tip on the cornea and a smaller diameter tip to on the thorax.

**Alternatives:** The solution can be injected with a micro-sample syringe.

△ **CRITICAL:** Fewer bubbles results in a higher signal-to-noise ratio. Avoid bubbles in the process of electrode filling. 1) Make sure the electrodes are clean and without dust. 2) Ringer's solution must be filtered before filling the electrode. 3) Keep a slow and constant injection speed. 4) Maintain the injecting solution in the electrode when withdrawing the syringe. 5) Tighten the electrode holder of the manipulator with the proper intensity, which will make the solution squeeze out slightly from the tip of the electrode.

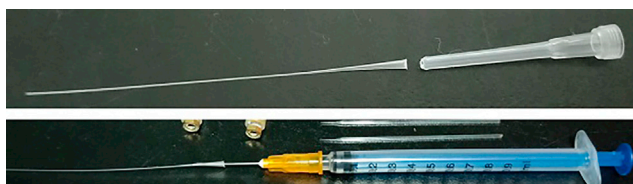
9. Fill the microelectrode with Ringer's solution, then put the microelectrodes on the holders and tighten the holders.

**Note:** Attach the microelectrode to holders on a micromanipulator.

10. In the Faraday cage, fix the recording electrode with the holder on the right micromanipulator and fix the reference electrode with the holder on the left micromanipulator.

**Note:** To prevent concretion of the condensed solution, these electrodes' tips are immersed in Ringer's solution until the experiment.

△ **CRITICAL:** The Faraday cage can protect its interior from external electric fields without interference and enhance the signal-to-noise ratio.



**Figure 2. Microloader and filling device**

Top, the cutting position of micro loader tips; Bottom, the filling device: microloader tips with a sterile syringe.

## KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Chemicals, peptides, and recombinant proteins</b>		
Sodium chloride	Sigma-Aldrich	Cat# 746398
Potassium chloride	Sigma-Aldrich	Cat# P4504
Calcium chloride dihydrate	Sigma-Aldrich	Cat# C0556
Sodium bicarbonate	Sigma-Aldrich	Cat# S5761
Phenol red	Sigma-Aldrich	Cat# P3532
Double-sided tape and Single- sided tape	Deli	N/A
Plastic board 6 cm × 5 cm × 2 cm	Deli	N/A
Borosilicate Glass Capillaries	World Precision Instruments	Cat# 1B100-4
Microloader	Eppendorf	Cat# 5242956003
Sterile syringe with needle 1 mL	Bidi	Cat# 300841
Tubes 1.5 mL	Eppendorf	Cat# 30108051
Dish with 30 mm diameter	Corning	Cat# 430165
Electrode jelly	Parker Laboratories	N/A
Millipore Filter Membrane, Organic-system, 0.22 μm	Sangon Biotech	Cat# F513139
Combined Filter for Solvent	Sangon Biotech	Cat# F502000
Micro-Sample Syringe, 100 μL	Sangon Biotech	Cat# F519163
Soft clay	Faber Castell	N/A
High precision straight end tweezers	Electron Microscopy China	Cat# EP5622
Illuminometer (measuring range:0.01–20000lux)	Tai Shi	Cat# 1330A
<b>Experimental models: Organisms/strains</b>		
<i>Drosophila: w<sup>1118</sup></i> (adult)	Bloomington Drosophila Stock Center	RRID: BDSC3605
<i>Drosophila: w*; P[UAS-TeTxLC]</i> (adult)	Bloomington Drosophila Stock Center	RRID: BDSC28837
<i>R92A10AD attP40; R17D06DBD attp2</i> (adult)	Aljoscha Nern	N/A
<b>Software and algorithms</b>		
Clampfit 10.6	Molecular Devices	N/A
Clampex 10.6	Molecular Devices	N/A
AxoScope 10.6	Molecular Devices	N/A
pCLAMP 10.6	Molecular Devices	RRID: SCR_011323 <a href="https://www.moleculardevices.com/products/axon-patch-clamp-system/acquisition-and-analysis-software/pclamp-software-suite">https://www.moleculardevices.com/products/axon-patch-clamp-system/acquisition-and-analysis-software/pclamp-software-suite</a>
GraphPad Prism 7	GraphPad	RRID: SCR_002798 <a href="https://www.graphpad.com/scientific-software/prism/%20RRID:%20SCR_002798/">https://www.graphpad.com/scientific-software/prism/%20RRID:%20SCR_002798/</a>
<b>Other</b>		
Electronic shutter controller	Newport	N/A
Electronic shutter	Newport	76992
Digitizer	Molecular Devices	1550B
Intracellular electrometer	Warner Instruments	IE-210
Constant current lamp power supply	Newport	68938
Low voltage halogen lamp	Philips	409850
Condensing lens assembly	Newport	60008
Optical fiber	Newport	40230
Collimating beam probe	Newport	77644
Computer	Lenovo	L195wD
Stereo microscope	Nikon	SMZ800
Tilting stand with magnetic base	Narishige	TM-1
Micromanipulator	Sarasota	KITER
Microelectrode holders	Warner Instruments	MEH8

(Continued on next page)

**Continued**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bandpass filter, 480 nm, 10-nm bandwidth	Newport	10BPF10-480
Bandpass filter, 530 nm, 10-nm bandwidth	Newport	10BPF10-530
Bandpass filter, 580 nm, 10-nm bandwidth	Newport	10BPF10-580
Bandpass filter, 590 nm, 10-nm bandwidth	Newport	10BPF10-590
Filter wheel	Newport	FWM1X12
Flaming/Brown micropipette puller	Sutter Instrument	P-97
Benchtop Faraday cage	ALA Scientific Instruments	N/A
Shade cloth	N/A	N/A

**Note:** 1) Adult flies of the same age should be used in both the control and experimental groups, regardless of age and sex, except when comparing the signals of the same genotype at different ages. 2) All chemicals are stored at room temperature (20°C–26°C).

**MATERIALS AND EQUIPMENT**

**Equipment**

**Note:** This table lists the instruments in [Figures 3](#) and [4](#).

Number	Equipment
1	Electronic shutter controller
2	Electronic shutter
3	Digitizer
4	Intracellular electrometer
5	Constant current lamp power supply
6	Low voltage halogen lamp
7	Condensing lens assembly
8	Optical fiber
9	Collimating beam probe
10	Computer
11	Stereo microscope
12	Tilting stand with magnetic base
13	Micromanipulator
14	Microelectrode holders
15	Bandpass filter, 480 nm, 10-nm bandwidth
16	Bandpass filter, 530 nm, 10-nm bandwidth
17	Bandpass filter, 580 nm, 10-nm bandwidth
18	Bandpass filter, 590 nm, 10-nm bandwidth
19	Filter wheel
20	Flaming/Brown micropipette puller
21	Benchtop Faraday cage
22	Shade cloth

△ **CRITICAL:** 1) The low voltage halogen lamp (Equipment 6) can be replaced with a Xenon lamp that is ozone-free and 300-W (Newport Corp. 6258). This lamp was chosen because the spectrum of the lamp has no UV emission, which is critical given most retinal photoreceptors are UV sensitive. 2) Filters (neutral density and spectral) and a filter exchange apparatus are also recommended to measure intensity-response curves and perform PDA (prolonged depolarization after potential) protocols. 3) An OG 590 nm (long pass) filter should be used to prevent net Rhodopsin to Metarhodopsin conversion (see ‘before recording 3, 4’).



**Figure 3. The equipment for ERG recordings, Part 1**

(A) The number corresponds to the list of materials and equipment: 1, 3, 4. (Wu et al., 2019). (B) The number corresponds to the list of materials and equipment: 2, 5-8, 15-19 (Wu et al., 2019). (C) The number corresponds to the list of materials and equipment: 10, 21-22 (Wu et al., 2019).

## Buffer

### Ringer's Solution

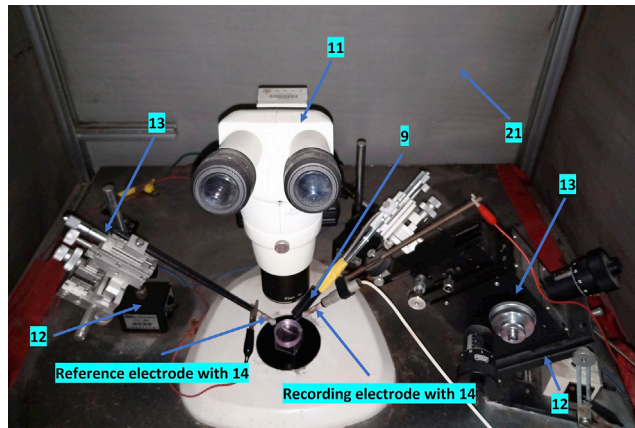
Reagent	Final concentration	Amount
NaCl	150 mM	4.39 g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	2.25 mM	0.165 g
KCl	4 mM	0.15 g
NaHCO <sub>3</sub>	48 mM	2 g
Phenol red	0.0002%	0.001 g
ddH <sub>2</sub> O	N/A	Up to 500 mL
<b>Total</b>	<b>N/A</b>	<b>500 mL</b>

Phenol red (as a color indicator)

### Conductive solution

Reagent	Amount
Electrode jelly	600 μL
Ringer's Solution	600 μL
<b>Total</b>	<b>1,200 μL</b>

**Note:** Store all solutions at room temperature (20°C–26°C) for up to two weeks.



**Figure 4. The equipment for ERG recordings, Part 2**

The number corresponds to the list of materials and equipment: 9–14, excluding 10, and 21 (Wu et al., 2019).

△ **CRITICAL:** Phenol red is useful for showing the position and condition of the solution compared to a colorless solution. Also, the color allows us to easily move the electrodes during the experiment without damaging the electrode tip.

## STEP-BY-STEP METHOD DETAILS

### Before recording

⌚ **Timing:** ~2 h

These procedures involve immobilizing flies, applying conductive solution to the fly. After placing the electrodes, it's ready for recording.

1. Anesthetize flies with carbon dioxide (CO<sub>2</sub>), then use high precision tweezers to hold the fly wings and gently glue the wings on a plastic board with double-sided adhesive tape.

**Note:** Tilt the body of the flies approximately 45° to completely expose one compound eye to the light beam (Figures 5 and 6).

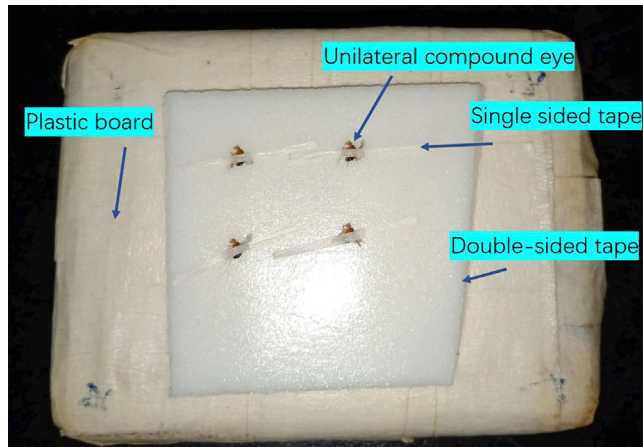
2. Fix the thorax and abdomen of the flies with single-sided adhesive tape with a width of 1/4 of fly body length.

**Note:** This not only ensures the integrity of the compound eye structure but also mitigates movement of the eye during electrode placement (Figures 5 and 6).

△ **CRITICAL:** 1) To keep the eye intact, do not touch the compound eye during this step. Exposure of one whole compound eye is important for recording. 2) The number of immobilized flies at one time should not exceed six to ensure the recording time will not be too long, which results in decline of vitality or even death of the flies. Preferably, the time for fixing and recording steps should be less than two hours.

3. Place the flies under a microscope.
  - a. With an objective lens at 20×, take a small amount of conductive solution with an electrode and daub thinly over the entire surface of the compound eye and the thorax.
  - b. Apply the appropriate amount of conductive solution, once the surface becomes transparent and bright.





**Figure 5. Immobilized flies**

Blue arrows point to the location of each material (Wu et al., 2019).

**Note:** Carry out these operations in a Faraday cage covered with a shade cloth, and filter the source light using an OG 590 nm filter.

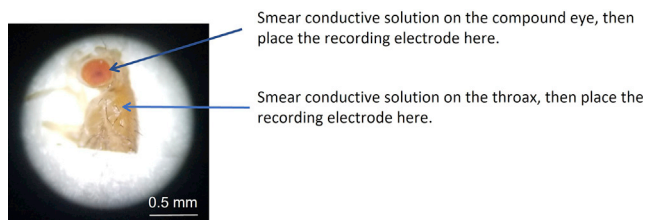
△ **CRITICAL:** Pay attention to the amount of conductive solution smeared: too little solution will dry during the experimental process, whereas too much solution will electrically bridge the compound eye and thorax, which will prohibit the acquisition of physiological signals (Figure 6).

4. Place the electrodes.

- a. Press the reference electrode gently onto the thorax at the position coated with conductive solution by manipulating the left-hand three-axis micromanipulator.
- b. Next, carefully place the recording electrode onto the surface of the compound eye by using the right-hand three-axis micromanipulator.
- c. Manually change the position of the light beam to point directly at the compound eye of the fly.

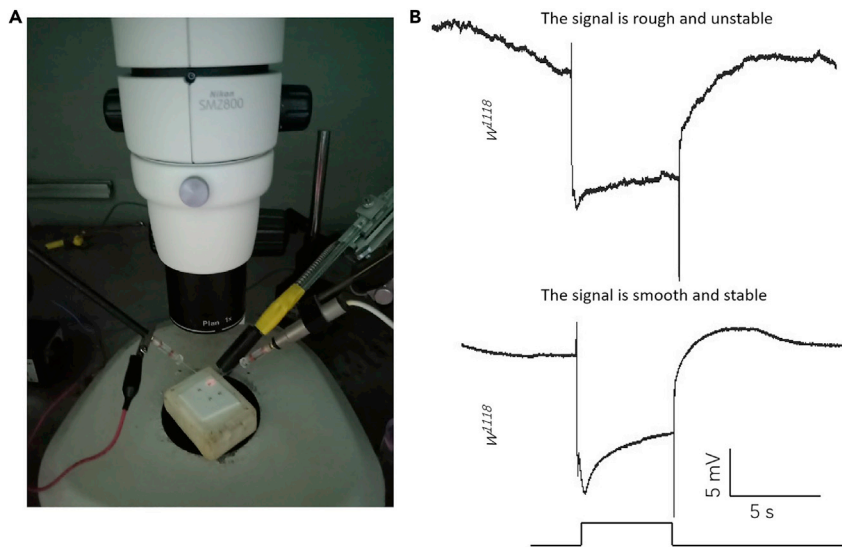
**Note:** Carry out these operations in a Faraday cage covered with a shade cloth, and filter the source light using an OG 590 nm filter (Figure 7).

△ **CRITICAL:** Pay attention to the electrodes' pressure on the respective body parts. Keep the compound eyes and thorax intact without any dents, to ensure the normal state of the flies. Fix approximately six flies to keep the experiment as short as possible and to ensure free breathing.



**Figure 6. Fly before recording**

Conductive solution is smeared on the surface of the compound eye and thorax, then two glass microelectrodes are placed on the thorax and compound eye (Wu and Han, 2019).



**Figure 7. Pre-test ERG measurement**

(A) The state of flies for pre-recording to evaluate the ERG signal.

(B) Pre-recording data. Upper, rough, and unstable recording trace. Examine the condition, electrodes, etc., then redo the pre-recording; Lower, an ideal smooth and stable pre-recording trace (Wu et al., 2019).

## Pre-recording

⌚ Timing: ~7 min

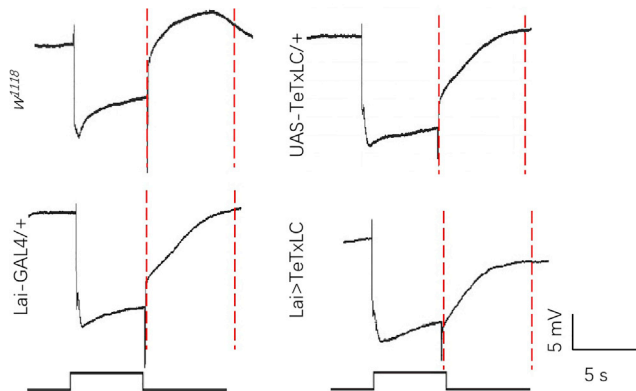
These procedures include checking whether the equipment is working well and whether the flies are in normal physiological condition.

5. Set up the equipment and prepare flies for recording.
  - a. Cover the Faraday cage with shade cloth completely to protect the compound eye from ambient light.
  - b. Run Axoscope 10.6 software, control the light on/off stimulation via the electronic shutter controller to assess the compound eye's light response.
  - c. Use the shutter output simultaneously as the second channel that records a high or low stimulus signal to tag the exact time point at which lights are turned on or off.

**Note:** Signals are not amplified by an Intracellular Electrometer. The signal is sampled at 4 kHz.

- d. Deliver light flash trains by a collimating beam probe. Position this probe approximately 4–6 cm away towards the fly's eye. Adjust the light intensity and spectrum based on experimental purpose (see details in 'ERG recording') (Figure 7).
6. If a signal with proper amplitude that depends on the intensity and spectrum of light, is observed, proceed to the next step. If not, please redo steps 4–5 (Figure 7).
7. Turn off the light, stop the software, and let the flies adapt to the darkness for a few minutes to ensure that the light-dependent translocations have enough adaptation processing.

⚠ **CRITICAL:** Dark adaptation is necessary for reliable measurements. We usually allow flies to adapt to darkness for at least 5 mins to ensure that  $\text{Ca}^{2+}$  dependent fast light adaptation mechanisms such as rhodopsin and pigment granule migration.



**Figure 8. Traces of ERG recording**

ERG recording traces of  $w^{1118}$ ,  $Lai-GAL4/+$ ,  $UAS-TeTxLC/+$ , and flies expressing  $TeTxLC$  using a  $Lai-GAL4$  driver. The first red dashed lines indicate the light offset and the second red dashed lines indicate the cut-off time, the lines define the window for data to analysis. The bottom trace represents the light monitor (Wu et al., 2021).

## ERG recording

⌚ Timing: ~1 h

These procedures below outline four ERG recording protocols that satisfy various experimental needs.

8. Run Axoscope 10.6 to record the retinal potential of flies.
  - a. Normal ERG recording.
    - i. Before ERG recording, perform a pre-test to determine the stimulus intervals that are sufficient for dark adaptation.
    - ii. Present stimuli with increasing intervals to find an interval where the response to the onset of the second light is not attenuated compared to the one to the first light.

**Note:** As this interval is invariant to genotypes, the same interval (10 s) is used in the following experiments. In the protocol, the stimulus sequence is as follows: 10 s darkness, 5 s light stimulus with a light intensity of 400 lux that is measured by an illuminometer, 10 s darkness, and 5 s light stimulus.

- iii. Record flies twice in succession with the same stimulus.

**Note:** For example, we used this protocol to test these flies  $w^{1118}$  (white eye color),  $Lai-Gal4/UAS-TeTxLC$  (orange eye color),  $Lai-Gal4/+$  (orange eye color), and  $UAS-TeTxLC/+$  (orange eye color) flies (Figure 8).

**Note:** For complete details on the use and execution of this protocol, please refer to these publications (Hu et al., 2012; Wu et al., 2021).

- b. Testing for light intensity.
    - i. Recording flies with different light intensity stimulations can examine the excitability and light sensitivity of photoreceptors. Use Stimulations to measure intensity-response curves.
    - ii. Before recording, perform a pre-test at all light intensity levels to determine the level at which the amplitude is not attenuated compared to the maximum amplitude that can be achieved.

**Note:** Measure the light intensity with an illuminometer.

- iii. Determine the stimulus sequence according to the results of the pre-test.

**Note:** Set the first light intensity as 1/10,000 of the maximum intensity, then gradually increase the light intensity until the flies produce detectable physiological responses of the eyes. At this point, mark the light intensity, then gradually increase the light intensity to record the flies' response to the highest light intensity. The stimulus sequence is as follows: 5 s darkness, 1 s light stimulus with different light intensity levels, and 5 s darkness.

**Note:** For complete details on the use and execution of this protocol, please refer to this publication (Hu et al., 2015).

- c. Testing for inactivation.

**Note:** The receptor potential of the *trp* mutant appears normal in response to a flash but quickly decays close to baseline during prolonged illumination. Therefore, we need to judge and determine the different durations of a light stimulus for different genotypes or ages of flies.

- i. Before recording, perform a pre-test for the duration of light stimulus in which the response is not decaying.
- ii. Determine the stimulus sequence according to the results of the pre-test.
- iii. For *trp* mutant flies, the stimulus sequence is as follows: 90 s darkness, 1 s orange light stimulus with a light intensity of 400 lux, 90 s darkness, and 1 s orange light stimulus. Record the responses twice to this stimulation.

**Note:** As an example, this protocol can be used to test the following flies: *w*<sup>1118</sup> (white eye color) and *trp*<sup>343</sup> (white eye color), etc.

**Note:** For complete details on the use and execution of this protocol, please refer to this publication (Han et al., 2006).

- d. Testing with different wavelength stimuli.

**Note:** The photoreceptors of *Drosophila* have distinct spectral sensitivities that allow them to distinguish stimuli of different wavelengths. Photoreceptors R1–R6 have the same spectral sensitivity, and these cells express a blue absorbing visual pigment, Rhodopsin 1 (Rh1). In contrast, photoreceptors R7 and R8 exhibit heterogeneity, and these cells express four different spectra absorbing visual pigments. R7 cells express one of two different UV absorbing visual pigments, either Rhodopsin 3 (Rh3) or Rhodopsin 4 (Rh4). R8 cells express either blue absorbing Rhodopsin 5 (Rh5) or green absorbing Rhodopsin 6 (Rh6). Despite the fact that the ERG signal is mostly composed of R1–6 photoreceptor responses, it represents the voltage created by all of the light-evoked responding cells in the head. The phenotypes of R7/R8-related phototransduction mutants may not be significant enough to detect the reduction of amplitude through normal ERG which reflects the field potential of photoreceptors R1–R8. Mutants connected to different photoreceptor cells can be screened using various spectrum stimuli that can evoke a depolarizing receptor potential in the dark. If a sufficiently bright light is used to photoconvert >>20% of rhodopsin to Metarhodopsin, the depolarizing potential persists in the dark (PDA); it is then terminated by orange (580 nm) stimulus (Pak et al., 2012). As a result, mutants with more than 20% residual rhodopsin can be identified by PDA. We can improve the efficiency and efficacy of ERG-based screening for identifying phototransduction-defective mutants by using PDA. Before recording, perform a pre-test at all light intensity levels and different spectra to ensure that the PDA is occurred.

- i. When a colored light stimulus is applied to compound eyes, the photoreceptors undergo substantial photoconversion. When the light stimulus is removed, the photoreceptors elicit a depolarizing receptor potential in the dark. We can determine the stimulus sequence that generates PDA in dark conditions based on these properties.

- ii. For PDA, the stimulus sequence is: 1–5 s darkness, 1–5 s 580 nm light stimulus with high-intensity, 1–5 s darkness, 1–5 s 480 nm light stimulus with high-intensity or other spectra, 1–5 s darkness, 1–5 s 480 nm light stimulus with high-intensity or other spectra, 1–5 s darkness, 1–5 s 580 nm light stimulus with high-intensity or other spectra, 1–5 s darkness, 1–5 s 580 nm light stimulus with high-intensity, and 1–5 s darkness.

**Note:** For complete details on the use and execution of this protocol, please refer to this publication (Han et al., 2006).

9. Sample the signal at 4 kHz.

**Note:** During recording, the compound eye signal is not amplified by the Intracellular Electrometer high impedance amplifiers.

10. Save the data in .abf format, which can store several data points based on sampled frequency, to conveniently use the fitting function of Clampfit software to analyze data and record the corresponding information about the flies (genotype, age, gender, etc.).
11. Group size should vary depending on the context of the experiment, but the sample size needs to achieve statistical power.

**Note:** During the experiment, the temperature should be maintained at 25°C, and the humidity should be controlled in the appropriate range of 60%–80%. A stable and uniform environment helps to obtain reliable and consistent results.

### After recording

⌚ Timing: ~4 h

The following steps will give you a brief description of how to analyze the ERG traces you got from the previous steps.

12. Analyze the recorded data files with Clampfit 10.6.
13. Select the trace at any given time you want to analyze.
  - a. For example, measure the response decay after stimulus offset using the trace between the light offset and the light on.
  - b. Analyze the ERGs of control flies and *Lai-Gal4/UAS-TeTxLC* flies and compare the trace characteristics, according to a previous publication (Wu et al., 2021).
14. Select the light off time as the start time point depending on the parallel second channel that tags the light on/off time, then select all 5 s recorded data of control flies and *Lai-Gal4/UAS-TeTxLC* flies to compare the decay trace difference with Graphpad Prism 7. See “[quantification and statistical analysis](#)” for the calculation of decay time constants (Figure 8).
15. Export data from the selected trace to an Excel sheet.

**Note:** The detailed analysis method is described in the “[quantification and statistical analysis](#)” section below.

### EXPECTED OUTCOMES

In the ‘Normal ERG recording’ protocol, we chose  $w^{1118}$ , *Lai-GAL4/+*, *UAS-TeTxLC/+*, and *Lai-GAL4/UAS-TeTxLC* flies and used the ERGs recording to compare their decay traces. To quantitate the contribution of fast decay and the speed of response termination, the time required for 1/4 recovery of amplitude ( $t_{1/4}$ ) was measured. For the three controls, we found no difference in  $t_{1/4}$ . However, the response decay of  $w^{1118}$  was different from that of *Lai-Gal4/UAS-TeTxLC* flies. Therefore,

we used GraphPad Prism7 to further analyze the traces of  $w^{1118}$  and *Lai-GAL4/UAS-TeTxLC* flies' after light off. The ERG recordings of  $w^{1118}$  flies were well-fitted with a double exponential function, tau (slow) = 7,087 ms and tau (fast) = 80.96 ms, while the recordings of *Lai-GAL4/UAS-TeTxLC* flies were well-fitted with a single exponential function, tau = 6,686 ms. This indicated the fast decay of ERG recording is disrupted in *Lai-Gal4/UAS-TeTxLC* flies. *Lai-GAL4/UAS-TeTxLC* flies specifically express tetanus toxin light chain in lamina amacrine cells, which blocks their chemical neurotransmission. Therefore, this result suggests amacrine cell signal transmission is essential for fast decay in ERG recording. A representative result is shown in this publication (Wu et al., 2021).

In the 'Testing for light intensity' protocol, we can analyze the amplitude during different light intensity levels (Hu et al., 2015). Additionally, in the 'Testing for inactivation' protocol, we can analyze the activation speed and maintained component to elucidate the detailed process of the molecular activation-inactivation transition (Han et al., 2006). In the 'Testing with different wavelength stimuli', we can analyze the amplitude, on/off transient, termination speed, and more (Han et al., 2006). By analyzing ERG recording traces, we can elucidate details of ion channels, photoreceptor signal transduction, and synaptic transmission between the photoreceptor and downstream lamina neurons.

### QUANTIFICATION AND STATISTICAL ANALYSIS

Use GraphPad Prism 7 to fit the decay traces of the  $w^{1118}$  and *Lai-GAL4/UAS-TeTxLC* flies ERG recordings. First, check whether the traces conform to double exponential decay fittings. Continue if there are two decay time constants that are reasonable in statistical analysis. Second, compare decay time constant tau to determine if there is a difference between genotypes. The  $R^2$  is compared between single exponential decay and double exponential decay fittings to determine which is the better fitting. Two-tailed Student's t-tests are used to compare the decay constants.

### LIMITATIONS

The response characteristics of ERGs are influenced by the selective absorption of spectral light by different eye pigments. White-eyed flies such as  $w^{1118}$  has the red pigment eliminated in the compound eye, which makes their eyes more sensitive to light than red-eyed flies because the red pigments strongly attenuate by absorbing incident light, which changes the spectrum of the light reaching the photoreceptors. The ERG presents a complex signal generated by many cell types and the neural transmissions between them. Therefore, we need to consider the genetic background and carefully select the control group for each experiment.

During the experiment, the flies are firmly fixed to limit their behavioral motion. When only one compound eye is exposed, pixel size and optical cross-talk between compound eyes are reduced. As a result, ERGs may not be able to accurately reflect the natural physiological condition of flies. It is ideal to test relevant behavioral paradigm to support and replenish electrophysiological results.

### TROUBLESHOOTING

#### Problem 1

It is easy to destroy the integrity of the compound eye (Before recording steps 1 and 4).

#### Potential solution

- Only grasp the wings with tweezers to immobilize flies on the plate. The flies are oriented to the correct position under the microscope.
- The single-sided tape should be on the thorax and away from the reference electrode (Figure 6).
- Ensure the angle of tilt to fully expose one side of the compound eye.

- When placing the electrodes, begin with a low 5× magnification of the microscope to place the electrode near the compound eye, then increase the magnification of the microscope to 20× and slowly, gently place electrodes on the cornea and the thorax.
- Place the electrode in the center of the compound eye with the least possible pressure.

### **Problem 2**

Low signal-to-noise ratio (Pre-recording steps 5 and 6).

#### **Potential solution**

- Keep the electrode from drying. Immerse the electrode in Ringer's solution until recording or fill the electrodes with Ringer's solution right before the experiment.
- Replace the recording electrode if the noise is obvious.
- Maintain the temperature and humidity of the experimental environment to ensure the vitality of flies.
- A benchtop Faraday cage will increase the signal-to-noise ratio.

### **Problem 3**

The recorded ERG signal of flies is unstable, for example changes in the signal baseline, and the same genotype has various ERG results (Pre-recording steps 5 and 6).

#### **Potential solution**

- Select healthy and active flies.
- Maintain the temperature and humidity of the cultivation and experimental environment.
- Make sure the flies are fixed correctly and that their heads and legs are unable to move.

### **Problem 4**

Under some recording conditions, such as too low/high light intensity or blue or UV color light, it is hard for researchers to see the flies. Furthermore, it is dangerous for flies and researchers and may even damage their retinas (ERG recording step 8).

#### **Potential solution**

- View the flies only through a filter or safety glasses that absorb harmful wavelengths and wear appropriate protective glasses.
- When placing electrodes, use the acceptable light intensity and spectra. After closing the shade cloth, the light intensity and spectra are changed to the test conditions.
- When fixing flies, it is recommended to use dim lights with rhodopsin-insensitive spectra (such as red or orange 580 nm).

### **Problem 5**

Because of the high gain and rapid transmission of the phototransduction cascade, defects in the ERG signal are often not evident. Researchers will be unable to detect mutations with partly functional phototransduction if they use the standard light intensity or light interval. (ERG recording step 8).

#### **Potential solution**

- Use neutral density filters to attenuate the stimulating light and reduce the illumination intensity.
- Construct a light intensity and interval ERG response curve during the 'pre-recording' step. Then choose an intensity that brings the response amplitude to saturation and a sufficiently large interval that maintains the response amplitude for subsequent experiments.

- In the case of an ambiguous mutation, reduce the light intensity or change the interval to check if there is a difference in ERG response between the sets of experiments.

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Junhai Han ([junhaihan@seu.edu.cn](mailto:junhaihan@seu.edu.cn)).

#### Materials availability

This study did not generate new unique reagents.

#### Data and code availability

This study did not generate or analyze datasets or code.

### ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China grants (31771171 to J.H. and 31771136 to Y.T.), a Guangdong Key Project 2018B030335001 grant to J.H., a Jiangsu Natural Science Foundation for outstanding young scientists grant BK20180061, and Zhishan Youth Scholar Program of SEU to Y.T. We thank Dr. Aljoscha Nern for *Lai-GAL4* flies, Bloomington Stock Center for *w<sup>1118</sup>* flies, and members of the J.H. laboratory for critical comments on the protocol.

### AUTHOR CONTRIBUTIONS

J.W. and Y.T. designed the research. J.W. performed the research. J.W., W.D., and J.H. analyzed data. J.H. and W.D. edited the paper. Y.T. and J.W. wrote the paper.

### DECLARATION OF INTERESTS

The authors declare no competing interests.

### REFERENCES

- Gu, Q., Wu, J., Tian, Y., Cheng, S., Zhang, Z.C., and Han, J. (2020). *Gαq* splice variants mediate phototransduction, rhodopsin synthesis, and retinal integrity in *Drosophila*. *J. Biol. Chem.* *295*, 5554–5563.
- Han, J., Gong, P., Reddig, K., Mitra, M., Guo, P., and Li, H.S. (2006). The fly CAMTA transcription factor potentiates deactivation of rhodopsin, a G protein-coupled light receptor. *Cell* *127*, 847–858.
- Hu, W., Wan, D., Yu, X., Cao, J., Guo, P., Li, H.S., and Han, J. (2012). Protein Gq modulates termination of phototransduction and prevents retinal degeneration. *J. Biol. Chem.* *287*, 13911–13918.
- Hu, W., Wang, T., Wang, X., and Han, J. (2015). Ih channels control feedback regulation from amacrine cells to photoreceptors. *PLoS Biol.* *13*, e1002115.
- Katz, B., and Minke, B. (2018). The *Drosophila* light-activated TRP and TRPL channels - targets of the phosphoinositide signaling cascade. *Prog. Retin. Eye Res.* *66*, 200–219.
- Pak, W.L., Shino, S., and Leung, H.T. (2012). PDA (prolonged depolarizing afterpotential)-defective mutants: the story of *nina's* and *ina's-pinta* and *santa maria*, too. *J. Neurogenet.* *26*, 216–237.
- Sayeed, O., and Benzer, S. (1996). Behavioral genetics of thermosensation and hygro-sensation in *Drosophila*. *Proc. Natl. Acad. Sci. U S A* *93*, 6079–6084.
- Wu, J.L., Hu, W., and Han, J.H. (2019). Intracellular recording of *Drosophila* photoreceptors. *Bio-101 e1010269*. <https://doi.org/10.21769/BioProtoc.1010269>.
- Wu, J., Ji, X., Gu, Q., Liao, B., Dong, W., and Han, J. (2021). Parallel synaptic acetylcholine signals facilitate large monopolar cell repolarization and modulate visual behavior in *Drosophila*. *J. Neurosci.* *41*, 2164–2176.
- Wu, J.L., Hu, W., and Han, J.H. (2019). Electroretinogram recording of *drosophila* eye. *Bio-101 e1010280*. <https://doi.org/10.21769/BioProtoc.1010280>.