**Video Article**

# Whole-cell recordings of light evoked excitatory synaptic currents in the retinal slice

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## Abstract

We use the whole-cell patch clamp technique to study the synaptic circuitry that underlies visual information processing in the retina. In this video, we will guide you through the process of performing whole-cell recordings of light evoked currents of individual cells in the retinal slice preparation. We use the aquatic tiger salamander as an animal model. We begin by describing the dissection of the eye and show how slices are mounted for electrophysiological recordings. Once the slice is placed in the recording chamber, we demonstrate how to perform whole-cell voltage clamp recordings. We then project visual stimuli onto the photoreceptors in the slice to elicit light-evoked current responses. During the recording we perfuse the slice with pharmacological agents, whereby an 8-channel perfusion system allows us to quickly switch between different agents. The retinal slice preparation is widely used for patch clamp recordings in the retina, in particular to study amacrine or bipolar cells, which are not accessible in a whole-mount preparation.

## Protocol

### Solutions

- Intracellular solution for ganglion cells (in mM): 100 K-gluconate, 8 KCl, 1 $MgCl_2$, 1 EGTA, 10 HEPES, 4 ATP, .5 GTP, 90 µM Sulforhodamine B (for staining), adjusted to pH = 7.4 with KOH
- Intracellular solution for bipolar cells (in mM): 90 K-gluconate, 8 KCl , 1 $MgCl_2$, 10 BAPTA, 10 HEPES, 4 ATP, .5 GTP, 90 µM Sulforhodamine B (for staining), adjusted to pH = 7.4 with KOH
- Extracellular Ringer's solution (in mM): 112 NaCl, 5 Glucose, 5 HEPES, 2 KCl, 2 $CaCl_2$, 1 $MgCl_2$, adjusted to pH = 7.75 with NaOH. Oxygenation of the solution is not necessary.

### Prepare recording chambers

Our recording chamber consists of a microscope slide with an adhesive well in which small silicone blocks cut out of another adhesive well are placed to provide stability for the tissue slices.

### Preparation of retinal slices

To avoid bleaching of the photoreceptors the dissection is carried out in IR or dim red light light. If one is interested in recording responses from the rod pathway, only IR light should be used (IR dissecting microscope and no room light. Additional IR goggles should be used for procedures not done under the microscope). If one is mainly interested in recording cone light responses, dim red room light or a red flash light can be used. The microscope, however, should still have IR oculars.

If photoreceptor input is irrelevant for the experiment, procedures can be carried out in normal room light using a regular microscope.

1. Prepare two microscope slides with an adhesive silicone well: create two parallel bands of silicone grease (about .5 cm apart) on each slide and place a rectangular piece of filter paper on one side of the slide, crossing both grease bands (push the filter paper with part of a razor blade so that it is well attached to the slide)
2. Sacrifice a salamander according to established protocols: The animal is placed in an ice bath for 30 minutes, then decapitated and double-pithed.
3. Remove eye from salamander head and place it under the dissecting microscope on a piece of tissue paper moistened with ice-cold Ringer's solution
4. Remove connective tissue from the eye
5. Use razor blade to make a small cut in the cornea
6. Hold cut cornea with forceps while removing it with scissors along its attachment to the eye
7. Remove the lens with forceps
8. Hold the iris with forceps and cut along the ora serrata to remove the iris
9. Cut the eyecup into two rectangular pieces
10. Pick up one of the pieces and place it sclera side up on the filter paper (make sure to flatten out the piece while pushing it down very carefully)
11. Gently lift the sclera off the retina, which now should be attached to the filer paper
12. Quickly add ice-cold Ringer's solution to the well
13. Repeat the same for the second piece
14. Use a razor blade placed in a custom made slicer to cut 200-300µm wide slices.

15. Use a pair of forceps to pick up the slices at the side of the filter paper, turn them over so the layers of the retina become visible, and place them on the vacuum grease bands
16. Pick a slice that looks undamaged and shows horizontal bands indicating the layers of the retina (the layers are sometimes difficult to see under the dissection microscope as the magnification is not high enough, however if one can recognize some layering the slice is usually good enough. The final decision on whether to use the slice, however, is made once it is viewed under the upright microscope). Move it to the recording chamber slide by building a Ringer bridge between the two slides
17. Make sure the filter paper is pushed against the rubber blocks in the recording chamber so that the slice is not tilted (ganglion cells and photoreceptors should be in focus at the same time)

## Placing the tissue in the recording rig

1. The recording slide is placed under the microscope and perfused with Ringer's solutions through a gravity perfusion syste
2. The preparation is visualized with an upright microscope with a 10x and 40x water immersion objective.
3. Again, we only use IR light to prevent bleaching of the photoreceptors. An IR camera is attached to the side port of the microscope which is connected to a TV screen.

## Visual Stimulation

Visual stimuli are programmed in Matlab using the Psychtoolbox and are projected onto the retina through the top port of the microscope using a microscopy image injector (Mbf bioscience). A Bits++ Digital Video Processor (Cambridge Research Systems) is used to obtain a 14bit luminance scale and to synchronize stimulus presentation with data acquisition. The visual stimuli used in this video are bright or dark bars (width = 460µm) of 100% contrast, which were presented for two seconds on a steady uniform background (luminance = 8 * $10^4$ photons/µm/s, size: 1.84 x 1.38mm).

## Electrophysiology

- Patch electrodes are pulled from borosilicate glass (OD: 1.5mm, ID. .84mm) with a micropipette puller. For ganglion cells, resistances are between 2 and 4 MOhms. For bipolar cells, higher resistances between 4 and 8 MOhms are used.
- Voltage Clamp recordings are performed using a Multiclamp 700A (Molecular Devices), amplifier and lab internal software programmed in Labview.
- Drugs are perfused through an 8-channel perfusion system. The microperfusion electrode is placed close to the slice (the tissue should visibly move when you turn the perfusion on and off, but one needs to be careful that the pressure is not too strong so that the slice could become detached from the filter paper). During experiments the microperfusion should be constantly turned on (perfusing control solution if no drugs are needed) so that the recording does not get disturbed by switching the perfusion on and off.

## Discussion

Benefits:

1. All cell types are accessible
2. Easy identification of cell types, in particular if a fluorescent dye is added to the electrode solution
3. Pharmacological agents can easily reach target cells
4. The patch clamp technique allows to investigate the role of different ion channels in retinal computations. The same information can not be obtained through extracelluar spike recordings or recordings with sharp electrodes.
5. This technique can be applied to other animal models

Disadvantages:

1. Processes might get cut off in the process of slicing. Therefore, studies that focus on the spatial receptive field might be difficult.
2. As in most retinal in vitro preparations used for electrophysiology, the pigment epithelium is removed, which can lead to bleaching of photoreceptors. Studies that require stimulation with high light intensities or need to study the retina under many adaptation states might run into difficulties with this technique.

## References

1. Cook, P.B, Lukasiewicz, P.D, McReynolds, J.S.  GABA(C) receptors control adaptive changes in a glycinergic inhibitory pathway in salamander retina. Journal of Neuroscience. 20(2), 806-812 (2000).

2. Heflin, S.J., Cook, P.B. Narrow and wide field amacrine cells fire action potentials in response to depolarization and light stimulation. Visual Neuroscience. 24(2). 197-206. (2007).

3. Ichinose, T., Shields, C.R., Lukasiewicz, P.D. Sodium Channels in the Transient Retinal Bipolar Cells Enhance Visual Responses in Ganglion Cells. 25(7). 1856-1865. (2005).

 