

MEA Application Note:
Retina Recordings
(Micro Electroretinograms)
from *Rattus norvegicus*



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1 Introduction

1.1 About this Application Note

The intention of the MEA Application Notes is to show users how to set up real experiments with the MEA-System on the basis of typical applications that are used worldwide.

The documents have been written by or with the support of experienced MEA users who like to share their experience with new users.

In this document we describe the preparation and handling of rat retina for MEA recordings. This is widely used as an assay to monitor drug effects on the electroretinogram (ERG).

1.2 Acknowledgement

Multi Channel Systems would like to thank all MEA users who shared their experience and knowledge with us, especially

Alfred Stett, Thoralf Herrmann, Elke Guenther

from the *NMI* Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany.

2 Retina Preparation

2.1 Biological Materials

- 4 to 6 weeks old rats (Long Evans)

Note: All experiments can be performed with adult animals as well, however as visual properties change with the age of the animal, it is crucial to ensure that animals within one set of experiments are of the same age.

2.2 Technical Equipment

- MEA-System (with amplifier and data acquisition, see "Suggested MEA-System")
- MEAs (microelectrode arrays)
- Stereo microscope
- Inverted microscope
- Light sources to stimulate the retina
 - a) Light Emitting Diode (LED) - driven by stimulus generator - OR -
 - b) Tungsten halogen lamp with neutral density filters and a filter holder, shutter controlled by a stimulus generator
- Micropipettes and pipette tips (20 μ l and 1000 μ l)
- P-1000 Pipetman with 0.78 mm blue tips
- Cut-open wide Pasteur pipette
- Perfusion setup
- 15 ml BD Falcon tubes
- Sharps forceps
- Curved forceps
- Small scissors
- Surgical eye scissors



2.3 Chemicals

- L-Glutamate
- Phenol red
- Carbogen gas (95 % O₂, 5 % CO₂)
- NaCl
- KCl
- CaCl₂
- NaHCO₃
- Glucose
- MgCl₂

2.4 MEA Coating

Depending on the type of selected MEA, various coatings may be applied. For retina experiments, we recommend MEAs with transparent ITO tracks and contact pads. For good surface properties we prefer a treatment of the MEA in a plasma cleaner combined with a coating of Poly-L-Lysine (PLL).

Suggestions for coating methods can be found in the MEA manual available in the “Download section” of the MCS web site.

2.5 Preparations

2.5.1 Ringer’s solution with glutamate and phenol red

NaCl	120.0 mM
KCl	5.0 mM
CaCl ₂	2.0 mM
MgCl ₂	1.0 mM
NaHCO ₃	30.0 mM
Glucose	15.0 mM
L-Glutamate	0.2 mM
Phenol red	10 ppm
pH = 7.5	

Aerate 40 ml of this solution at 30 °C for at least 30 min with carbogen (95 % O₂, 5 % CO₂).

Tip: A simple setup how to aerate a small volume is displayed in the preceding picture on the right.



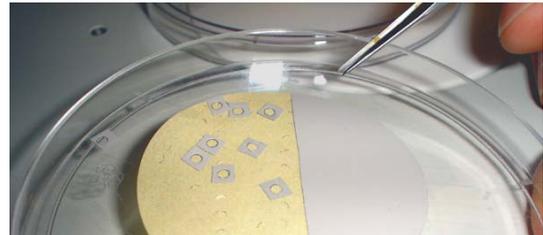
2.5.2 Ames' Medium

Ames' Medium (Sigma-Aldrich)	8.8 g/l
NaHCO ₃	1.9 g/l

Add to distilled water and aerate the solution for at least 30 min with carbogen (95 % O₂, 5 % CO₂).

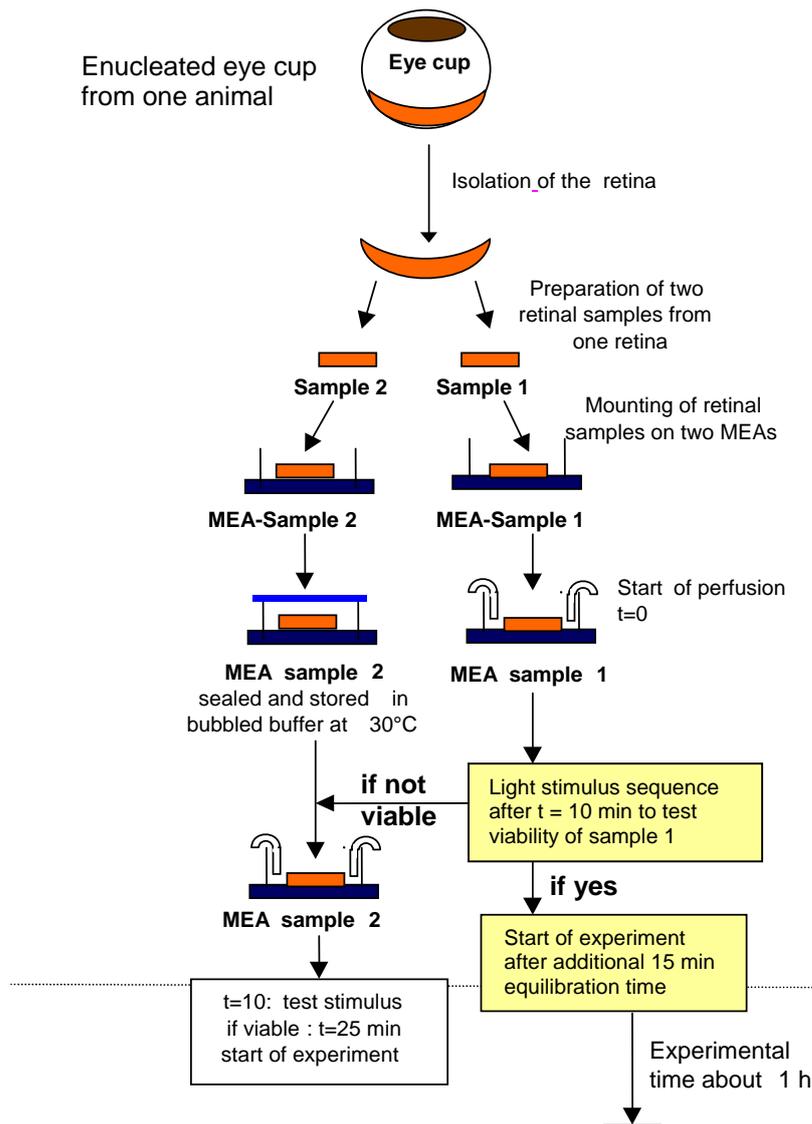
2.5.3 Filter paper carriers

1. Cut thin filter paper in squares of 5 mm by 5 mm.
2. Punch holes of 1.8 mm diameter in the center of the filter paper with a hole punch.



2.6 Experimental Outline

The following illustration outlines the preparation and recording experiment.



2.7 Dissection

2.7.1 Removing the eye-cups

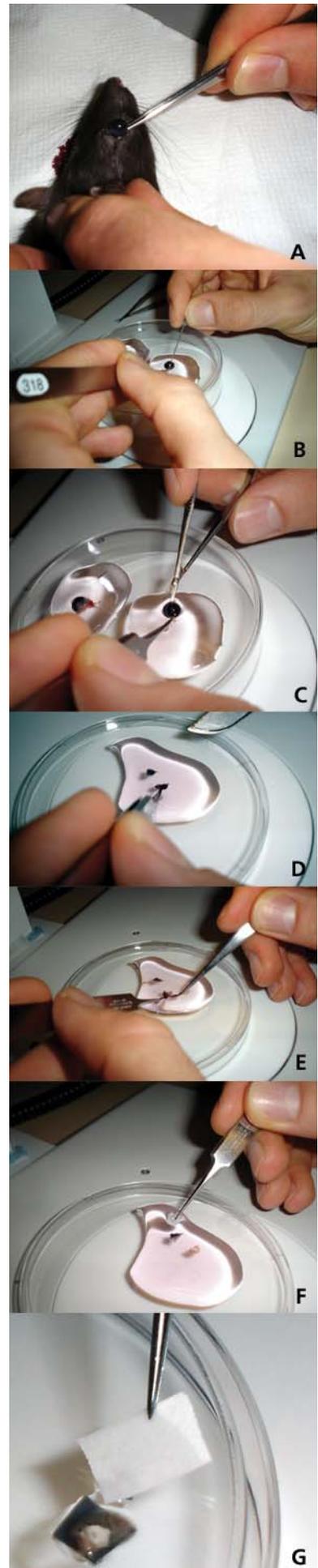
Note: The dissection is performed under dim red illumination to minimize photo pigment bleaching.

1. Euthanize the animal with carbon dioxide.
2. Both eyes are excised with a large straight forceps (see fig. A on the right).

2.7.2 Retina isolation

Note: All following steps are carried out in Ames' Medium or standard Ringer solution bubbled with 95 % O₂ / 5 % CO₂ at a temperature of 30 °C. The dissection is performed under a binocular or dissection microscope.

1. Remove excess tissue.
2. Perforate the eye-cup with an injection cannula (fig. B).
3. Open the eye-cup along the ora serrata with a fine eye scissor. Use the perforation hole as an access point for the scissors (fig. C).
4. Remove the lenses with fine straight forceps.
5. Dissect two retinal segments that will be recorded separately (MEA sample 1 and 2) (fig. D). Proceed the following steps with both samples.
6. Loosen the neural retina from the sclera with very fine curved forceps (fig. E).
7. Take a filter paper carrier and lubricate it with buffer (fig. F).
8. Transfer the retina onto the carrier.



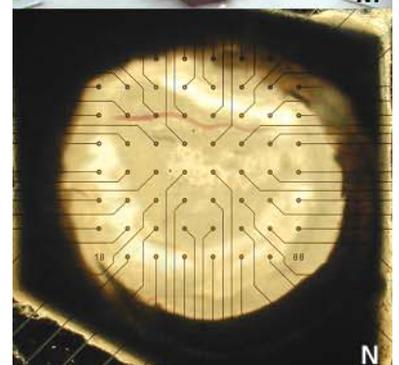
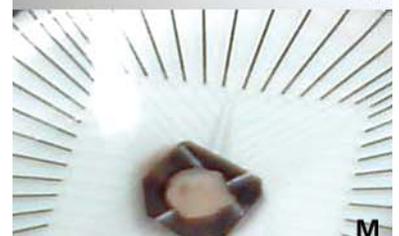
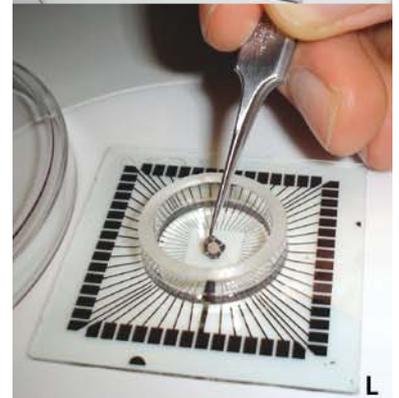
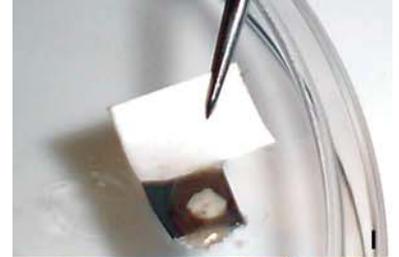
2.7.3 Preparing and transferring the retina

1. Place the carrier with the retina onto a dry part of the preparation dish.
2. Remove any excess buffer with small filter paper pieces (fig. G).
3. Position and unfold the retina on the carrier.

- Carefully remove the vitreous body with straight forceps (fig. H).

Important: This step is very critical. Insufficient removal of the vitreous body will result in bad recordings. Be careful not to damage the retina in this step.

- Remove excess buffer with a small piece of filter paper (fig. I).
- Trim the filter carrier; there should be sufficient carrier left to grasp it with the forceps.
- Transfer samples 1 and 2 with the retina upside down onto the recording fields of two MEAs (fig. J to L). The filter paper is left on the retina samples. Figures M and N show a close-up view of the retina on the MEA.
- Fill the dishes with aerated Ringer's solution.



Light Stimulation and Recording

- MEA sample 2 is sealed and stored at 30 °C as a replacement for sample 1 in case that this fails the viability and adherence test.
- Mount the MEA with the sample 1 directly onto the MEA amplifier.
- Start the perfusion at a flow rate of 1 ml/min with Ames' or Ringer's. The start of perfusion is referred to as t_0 and should take place about 15 min after the enucleation.
- 10 min after t_0 , a series of flashes is applied to the sample for testing its viability and adherence to the MEA. If the test result is positive, the experiment is started according to the experimental protocol. If the test on MEA sample 1 fails, sample 2 is used for the experiment.
- A typical experiment will need about 1 hour time.

3 Suggested MEA-System

3.1 System Configurations

Depending on the throughput and the analysis requirements desired in your laboratory, different system configurations are recommended for the recording from retina samples.

- **MEA2100-System:** For the MEA2100-HS60(HS2x60)-System, the same MEAs can be used as for the MEA1060 amplifiers, with the same advantages and disadvantages. In addition, for the MEA2100-HS120-System a 120-channel MEA layout is available (120MEA200/30iR), which provides a larger electrode field with a resolution identical to the 60MEA200/30iR. This might be interesting for a mapping applications. The MEA2100-System allow to operate a maximum of two headstages with up to two 60-channel MEAs each.
- **USB-MEA60-System:** 60-channel MEA recording system for inverted or upright microscopes. The temperature controller **TC02** regulates the temperature of the MEA and of the perfusion fluid via the perfusion cannula **PH01**. A MEA1060 amplifier allows recording up to 60 channels from a MEA. This is the standard configuration for low-throughput academic research.
- Filter settings for **spike-only recording:** 300 Hz to 3 kHz.
- **Filter setting** for slow potentials (electroretinograms): 0.5 Hz to 3 kHz.

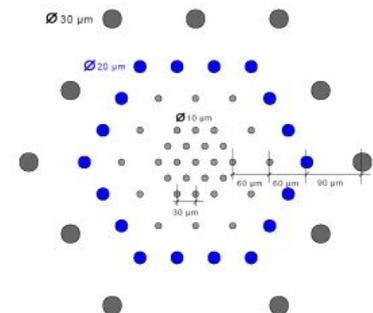
3.2 Microelectrode Arrays

Available MEAs differ in electrode material, diameter, and spacing. For an overview on available MEA types please see the Multi Channel Systems web site (www.multichannelsystems.com) or contact your local retailer.

The microfold structures formed by titanium nitride (TiN) result in a large surface area that allows the design of small electrodes with a low impedance and an excellent signal to noise ratio. For recording from retina, a high spatial resolution with an electrode diameter of 10 μm and a spacing of 100 μm or even 30 μm can be necessary.

Recommended MEAs include:

- **60HexaMEAs** feature a hexagonal layout, perfect for recording from retina. 60 electrodes are aligned in a special configuration with varying electrode diameters (10, 20, 30 μm) and interelectrode distances (see picture). The specific layout resembles ideally the regularity of the retina's architecture. The density of neurons is more important in the center than in the peripheral. This is matched by the density of electrodes on the MEA, which is also higher in the center than in the peripheral. The flat, round electrodes are made of titanium nitride (TiN). Tracks and contact pads are made of opaque Ti or transparent ITO; insulation material is silicon nitride. Electrodes in the center have a diameter of 10 μm with an interelectrode distance of 20 μm , where the peripheral electrodes have a diameter of 20 μm and 30 μm .
- **60MEA100/10iR-Ti:** standard 8 x 8 layout, TiN electrodes for recording and stimulation.
- **60ThinMEA100/10-ITO** for high-resolution imaging and combination with intracellular calcium measurements. ThinMEAs are only 180 μm "thick" and mounted on a robust ceramic carrier. Tracks and contact pads are made of transparent ITO.
- **60HDMEAs:** These High Density MEAs feature two recording areas of 5 x 6 electrodes. In either area the spacing between the 10 μm electrodes is only 30 μm . The two recording fields are 500 μm apart. This MEA type is also available as a thin MEA.
- **60MEA200/30iR-Ti** with a medium spatial resolution and a substrate-integrated reference electrode, recommended for microERG recordings with full-field stimulation if a spatial resolution of the slow potentials is not required.



4 Multisite microERGs on MEAs

4.1 Theoretical Background

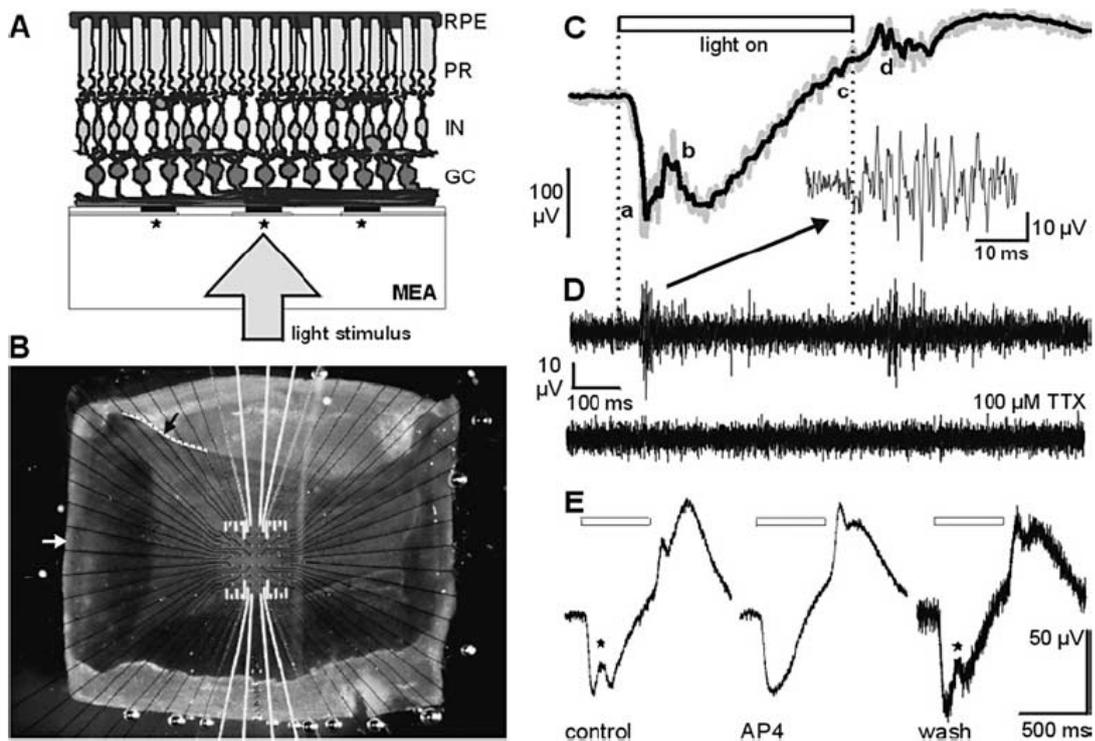
Adapted from Stett et al., 2003

The retina is a peripheral, easily accessible part of the central nervous system. Stimulation with light pulses results in a complex signalling by neurons within the layers of the retina (see figure A). The retinal ganglion cells transmit retinal information to higher visual centers in the brain via their axons that form the optic nerve. Retinal function can be affected by acute injuries, intoxications, or retinal diseases, either inherited or acquired, resulting in visual impairment or even blindness.

In clinical practice, the so-called electroretinogram (ERG) is a widely used ocular electrophysiological test to diagnose impaired vision. Light impulses falling on the retina synchronously activate a large number of neurons and Müller glia cells, which regulate the extracellular potassium concentration. The resulting change in trans-retinal voltage is measured as the ERG.

The ERG has a multiphasic waveform. Its shape mainly depends on the stimulus conditions, the state of the retina's adaptation, and the species. The full-field ERG of a dark adapted retina in response to a bright flash of white light consists of four major components: The fundamental a-, b-, and c-wave at light onset, and the d-wave at light offset. Each of the components can be attributed to the activity of certain retinal cells. Under pathophysiological conditions the shape and amplitude of these components is altered and can be influenced by pharmacological compounds.

A retina sensor, based on multisite recording of local ERGs in vitro, has been developed to easily and effectively assess effects of pharmacological compounds and putative therapeutics, drug side effects, and consequences of degeneration-related processes on retinal signalling. For the recording of light-evoked activity, a retinal segment with the pigment epithelium, dissected from an explanted rat retina, is placed ganglion cell site down on an MEA. Local ERGs (microERGs; Fig. C) with the typical components and ganglion cell spikes (Fig. D) can be recorded with appropriate filter settings. The prominent components of the microERG can be pharmacologically identified as shown in Fig. E for the b-wave, which is smaller in recordings from isolated retinas than in recordings from intact eyes.



MEA retina sensor.

A An explanted chicken retina is placed onto an MEA with the ganglion cell side (RPE retinal pigment epithelium, PR photoreceptor, IN interneurons, GC ganglion cells). The light stimulus is projected through the transparent MEA and retina onto the photoreceptors. Ganglion cell activity and retinal field potentials (microERG) are recorded by 60 substrate-integrated microelectrodes (*).

B View through an MEA on a retinal segment (white arrow) from a chicken retina. The broken line marks the border of the pigment epithelium.

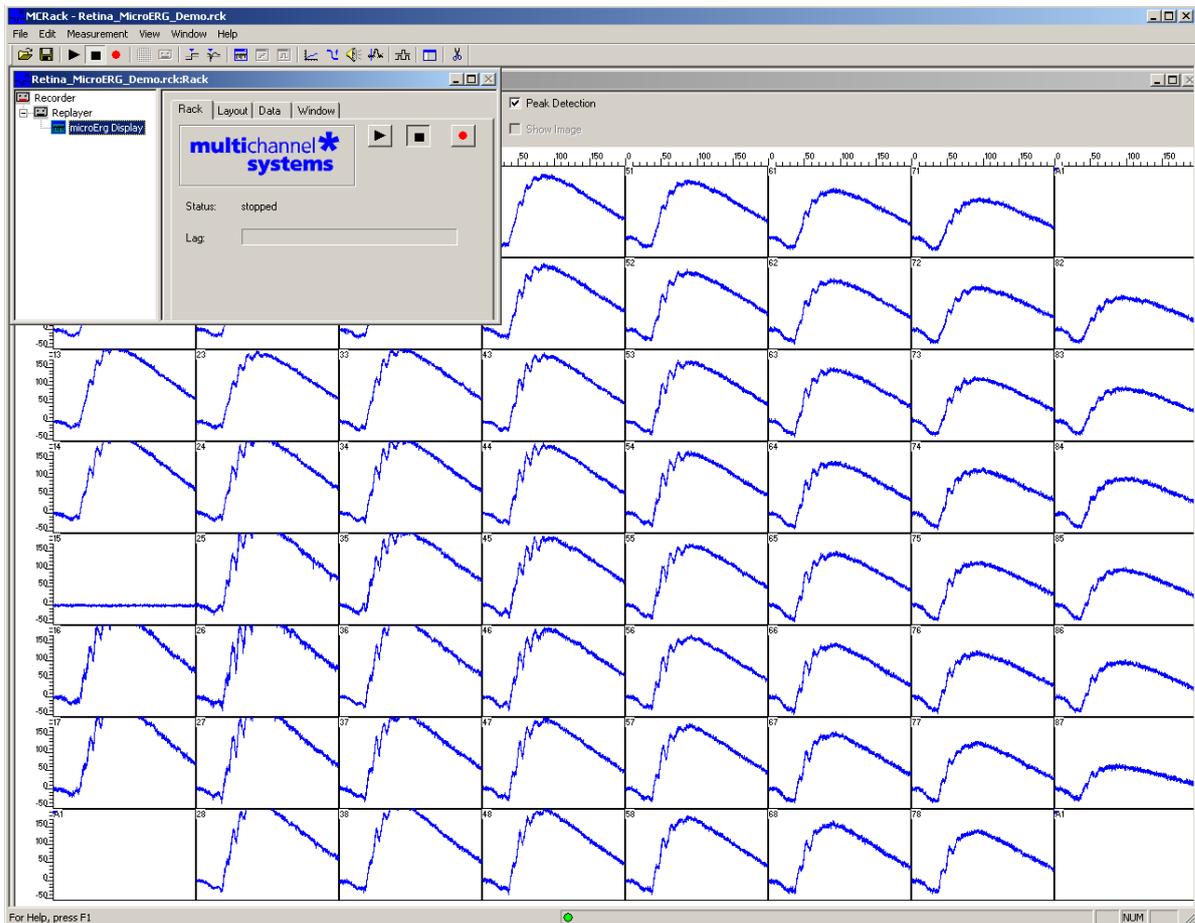
C microERG with a-, b-, c-, and d-wave of a chicken retina, evoked by full-field white light, impulse duration 500 ms, 0.5 Hz. Gray curve single sweep, 0.5 Hz to 2.8 kHz. Black line 5 sweeps averaged, filtered 0.5–100 Hz.

D Spike activity (insert), extracted from gray curve shown in C by off-line filtering at 200 Hz–2.8 kHz. The spikes were extinguished by 100 μ M TTX.

E Drug action on a light-evoked microERG. 2-Aminophosphonobutyric acid (AP4), a blocker of the on-signal pathway in the retina, resulted in the disappearance of the b-wave (*) that mainly reflects retinal Muller cell and bipolar cell activity. B-wave amplitude is restored after washing out the drug. Light pulses 500 ms, 0.5 Hz (Stett et al., *Anal Bioanal Chem* (2003) 377 : 486–495)

4.2 MicroERGs (Field Potentials)

The following screen shot shows typical microERGs recorded with the MEA-System viewed in the MC_Rack program.



Micro ERG on a standard MEA from Long Evans rat, female, age 39 days.

Full-field stimulation, temporal pattern: 1 pulse (1.5 s) per min

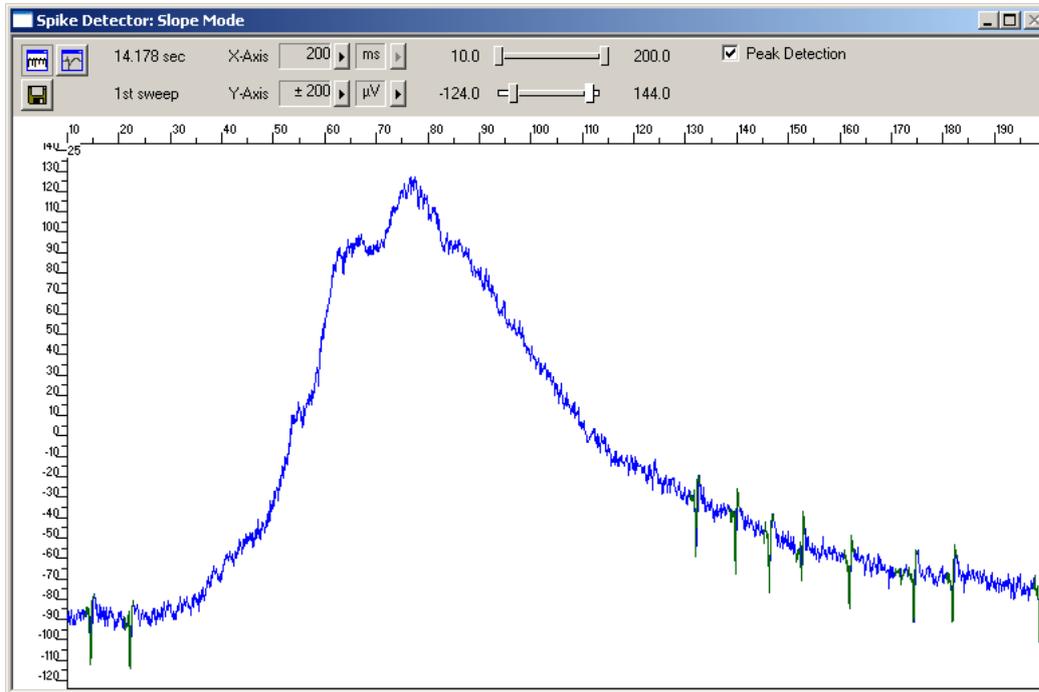
Illumination: Halogen lamp 100 W, filtered (BG28), intensity 310–8200 Lx

Perfusion: RRET EZL 02, T = 30 °C

(Data kindly provided by NMI Reutlingen, Germany)

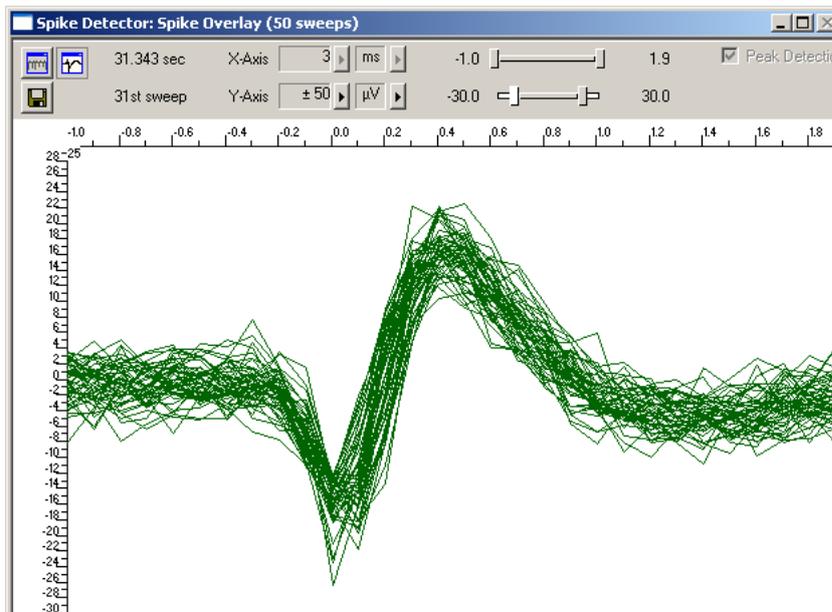
4.3 Spike Extraction

The next picture shows the waveform-based online spike detection on a single channel. In **Slope** mode, the **Spike Sorter** reliably extracts spikes from the underlying field potential.



Spike waveforms extracted from a microERG.

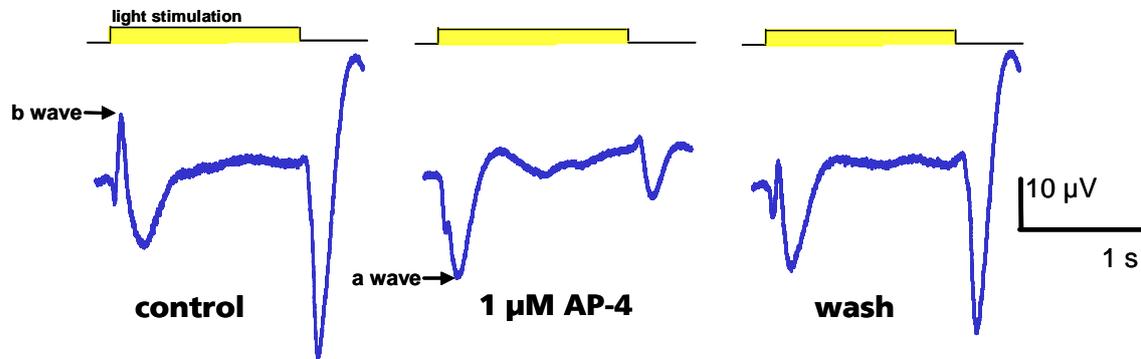
Spikes were detected with the **Slope** mode of the MC_Rack **Spike Sorter** ($\Delta V = -8 \mu V$, min slope = 0.6, max slope = 5). Spike cutouts of 2 ms are shown in green. (Data kindly provided by NMI Reutlingen, Germany)



Spike cutout overlay of 50 sweeps from the same electrode.

4.4 Drug Testing

By superfusion of the retina explant with drugs in defined concentrations, specific alterations of the ERGs can be monitored. In its present form, the MEA retina sensor is suitable for drug testing up to one hour, depending on the reversibility of tested drug effects.



Drug action on a light-evoked microERG. Application of the glutamate analog AP4, a blocker of the on-signal pathway in the retina, resulted in the separation of the a-wave by elimination of the b-wave. The b-wave amplitude is restored after drug wash out.

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