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MEA Application Note:
Organotypic Cultures of
Hippocampal Slices: Preparation,
Recording and Data Analysis



Imprint

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Introduction

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.

This application note includes a complete protocol for the dissection of rat or mouse brain, the preparation of hippocampal slices for organotypic cultures (OTCs), and a guide how to run a standard experiment and analyze the data afterwards. Suggestions for suitable MEA-System configurations, and support files are also included.

Several options to combine OTCs with recordings on microelectrode arrays will be described in this application note.

It is assumed that the reader is familiar with the functions of the software MC_Rack and MC_Stimulus II and the general terminology in the field.

Acknowledgement

Multi Channel Systems would like to thank all MEA users who shared their experience and knowledge with us. A part of this document is based on the instructions provided by Dr. Andrea van Bergen.

Aim of the Experiment

Electrophysiological experiments on brain slices are established since about 40 years and have the main advantage that the brain tissue is more accessible in a slice than *in vivo*. Hippocampus brain slices are the most frequently used slice type in electrophysiology because the hippocampal structure is easily identified in the brain, and also because its main organotypical characteristics are preserved in the slice and also in culture. Of course, it is also possible to record from other brain areas such as cerebellum or cortex. This application note focuses on the hippocampus preparation from rat or mouse.

In an acute experiment, the slice is used directly after preparation and discarded after the experiment. Slices from neonatal animals can also be kept in prolonged culture, over several weeks or even months, as so called organotypic cultures (OTC). Even though the synaptic organization is not exactly the same as in native tissue, the main characteristics and functions are preserved. OTCs have the advantage that they allow to observe the electrophysiological activity over a longer period of time on the same slice with repeated recordings on consecutive days.

OTC experiments are especially useful for longterm experiments, for example, for monitoring the development of the neuronal networks and its electrophysiological activity, the behavior of cocultured slices from different regions and the regeneration of tissue, or longterm drug effects.

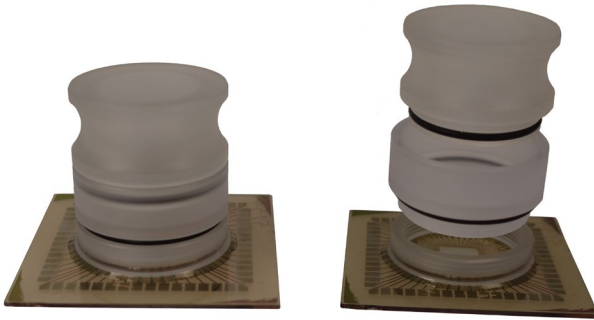
Material

Animals

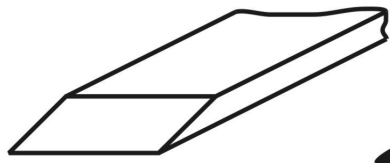
Neonatal rat or mice, age P3 (postnatal day 3) to P10. P6 animals are most often used. In younger animals, the hippocampal formation is harder to see, in older animals the regenerative potential of the tissue decreases, which makes cultivation increasingly difficult. It is important that the animals are not prepared directly after a stress situation, for example, transportation, as this may impair the quality of the tissue.

Technical Equipment

- MEA-System (with amplifier and data acquisition, please see chapter 7).
- MEAs (microelectrode arrays). For the Gähwiler method, MEAs with a threaded plastic ring and culture chamber are needed. They also should have an internal reference electrode (please refer also to chapter 7.3).



- Stimulus generator (optional).
- Stereo microscope.
- Microscope to document the slice position in relation to the electrodes, preferably with attached camera. A picture of the slice on the electrode field can then be loaded into the MC_Rack program for aligning the data traces to the electrodes.
- Incubator, for example Heraeus Cytoperm from Kendro Laboratory Products, www.kendro.com, set to 35 °C, (5 % CO₂), adapted with a tilt mechanism for culturing slices. Degree of tilt and speed: -70° / +70° per minute, with a resting time of 2 min inbetween rotations. The tilting mechanism is only necessary for Gähwiler cultures.
- Cooled metal block.
- Tissue chopper (for example, Mcllwain from Campden Instruments Ltd.).
- Razor blades, ideally breakable carbon steel blades (F.S.T, Heidelberg).
- Adjustable pipettes and pipette tips (10 µl and 1000 µl).
- Surgical instruments: Large sharp scissors for decapitation, smaller sharp scissors for brain preparation, narrow flat spatula for removing the brain, two narrow sharp spatulas with very smooth surface for separating the slices (sharpen and sand smooth the spatula thoroughly before each use), blade holders and breakers (F.S.T, Heidelberg), wide spatula to scoop up the chopped slices.



sharpened spatula to separate slices, 3 mm wide



bend spatula to scoop up slices, 6 mm wide

- Sterile dishes.
- Multi well dishes with fitting filter inserts, for example Millicell cell culture inserts from Millipore.
- Slice grid (optional).
- Peristaltic pump and tubing (optional).

Chemicals

- Chicken plasma (Gibco/Invitrogen)
- Thrombin (50 U/ml, Sigma-Aldrich, Inc.)
- Gey's Salt Solution (Laboratoires EUROBIO, CS1GEY00)
- Glucose D (+) (Merck KGaA, 108337)
- Kynurenic acid (Sigma-Aldrich, Inc., K3375)
- HCl
- Isopropanol (for cleaning the MEA contact pads)
- Basal Medium Eagle contains Earle's salts, but no L-glutamine. (Gibco/Invitrogen)
- Hanks' Balanced Salt Solution (HBSS) without Calcium / Magnesium (Gibco/Invitrogen)
- Horse serum (Donor Equine Serum from HyClone)
- L- Glutamine (Gibco/Invitrogen)
- Carbogen gas (95 % O₂ / 5 % CO₂, optional)

Media

Dissection Buffer

Gey's Salt Solution

Glucose D (+) 0.5 % (w/v)

Kynurenic acid 1 mM

Mix and adjust pH to 7.2 with HCl. Chill to 4 °C and carbogenate with 95 % O₂ / 5 % CO₂.

Culture Medium (Gähwiler-Type Cultures)

Basal Eagle medium	50 % (v/v)
HBSS	25 % (v/v)
Horse serum	25 % (v/v)
D-Glucose	0.5 % (w/v)
L-Glutamine	1 mM

Store at 4 °C for up to two weeks.

Culture Medium (Stoppini-Type Cultures)

50 ml of BME medium (Basal Medium Eagle without L-glutamine Cat: 01-015-1A Bioind.com)

25 ml of HBSS (Hanks' balanced salt solution 1X, 14175-053 GIBCO)

25 ml of inactivated Horse Serum (Sterile GIBCO 26050)

2 ml of D-(+)-Glucose at 45 % (Sigma G87691)

1 ml of GlutaMAX -VP (29.23 mg/ml) (Final Conc. = 1.303 mM)

250 µl of Penicillin/Streptomycin 100X Sol. (GIBCO 15140)

Total volume: 103.25 ml, please mix and filter sterile.

ACSF Buffer

	MW	mM	gram/2 l 10x stock	gram/5 l 10x stock	gram/1 l final ACSF	gram/2 l final ACSF	gram/3 l final ACSF
NaCl	58.4	125	146	365			
KCl	74.56	3.5	5.22	13.05			
NaH ₂ PO ₄ .H ₂ O	138	1.2	3.312	8.28			
CaCl ₂ .2H ₂ O	147.02	2.4	7.06	17.64			
MgCl ₂ . 6H ₂ O	203.3	1.3	5.29	13.21			
Glucose	180.2	25			4.5	9.0	13.5
NaHCO ₃	84.0	26			2.18	4.36	6.55

pH 7.35, 310 mOsmol

10x stock solution without glucose and NaHCO₃ can be kept at 4 °C for about a month. Prepare fresh final ACSF for each day by adding glucose and NaHCO₃. complete ACSF should only be stored overnight.

Preparation and Cultivation

MEA Pretreatment

MEAs should be hydrophilized to improve tissue attachment, for example with a plasma-cleaner treatment (see also MEA manual). If slices are going to be cultivated directly on the MEAs (Gähwiler method), the arrays should be sterilized before use by autoclaving at 121 °C for 40 min. After autoclaving, MEAs should be set up under the sterile hood next to the preparation instruments.

Setting up the Slice Preparation

All necessary instruments must be sterilized and set up under a sterile hood.

- Binocular for preparation with proper illumination; for cooling during preparation, put an ice bag on the binocular platform, or work on a metal block taken out of the freezer.
- Tissue chopper with blade and Teflon platform; set slice thickness to 300 - 400 μm as desired.
- Surgical instruments: large sharp scissors for decapitation, smaller sharp scissors for brain preparation, narrow flat spatula for removing the brain, narrow sharp spatula with very smooth surface for separating the slices, blade holders and breaker with razorblade fragment, wide spatula to scoop up the chopped slices.
- Dishes with preparation buffer on ice.
- Thrombin and chicken plasma on ice.
- Disposal bag for the animals.
- MEAs - OR - multi well dishes with fitting filter inserts.



Decapitation and Brain Removal

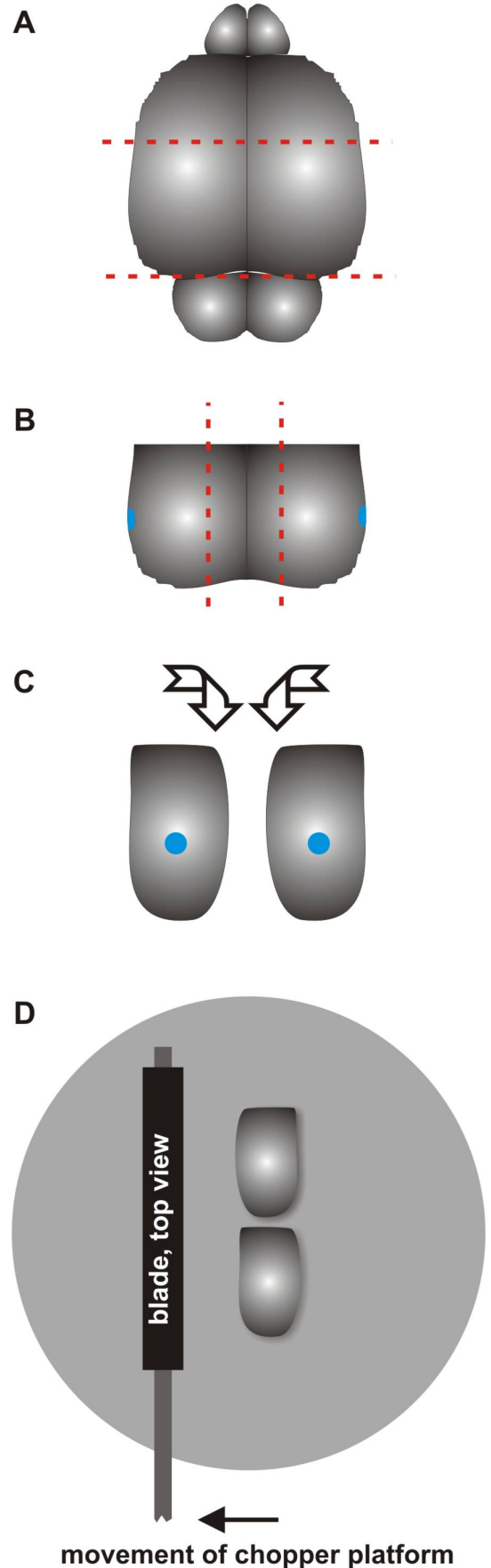
- Decapitate the animal with large sharp scissors.
- Cut the scalp left and right from caudal to rostral from the back of the head to the ears.
- Pull the skin flap to the front to expose the skull.
- Cut the cranium carefully with a scissor on ice as follows. This method is simple and fast. It has the disadvantage that the neocortex may get damaged during the procedure. This is generally not a problem as the hippocampus is located inside the temporal lobe and should be safe.
 - a) Midline all the way from foramen magnum to the front end of the brain.
 - b) Perpendicular to cut between forebrain and cerebellum.
 - c) Perpendicular to (a) along the front end of toward the base of the brain.
- Fold the two skull segments toward the sides.
- Quickly remove the brain with the blade of a narrow spatula.



Dissection

The dissection should be performed both well and quickly. Try to keep the tissue as cool as possible during the whole procedure. Prepare only one animal at a time until the separated slices are in the fridge.

- Place the brain with the dorsal side up onto a wetted filter paper in a Petri dish filled with cold dissection buffer. The Petri dish should be kept on an ice-cold metal block to ensure a stable cool temperature.
- With the razor blade fragment, cut off cerebellum and frontal pole (**A**).
- Cut the brain in the sagittal plane in three approximately equally wide pieces. Keep both hemispheres and discard the middle part (**B**).
- Flip both hemispheres onto the sagittal cutting surface (**C**, mind the blue markers).
- With the bend spatula, move the hemispheres to the Teflon platform of the chopper. Arrange the cutting edge of the frontal pole in a 90 ° angle to the blade (**D**).
- Remove access buffer with a tissue to improve adhesion to the platform. Put some buffer on the blade to prevent slices from sticking to the blade.
- Chop the hemispheres to 300 - 400 μm thick slices. Use a high blade force and a medium speed.
- Scoop up the chopped tissue carefully with the bend spatula and put them in a fresh dish with cold preparation buffer.
- Separate the slices with the small sharp spatulas and select the ones with the hippocampal formation clearly visible. Dissect out the hippocampus with the razor blade or the sharp spatulas. From a P6 rat, about six good slices can be expected, from a P6 mouse four.
- Store the slices for about 1 h in the fridge before putting them on the MEA or membrane.



Mounting Slices onto MEAs (Gähwiler Method)

Important: Do not touch the slice directly. The slice should not be folded to avoid damage to the tissue. Be careful not to touch the MEA surface with the transfer pipette to avoid damage to the electrodes.

- Remove the lid and middle ring from the MEA.
- Pipette 12 μ l of chicken plasma onto the recording field of the MEA.
- Take the slice out of the buffer with a broad spatula and let it slide right into the drop of plasma.
- Distribute the drop with a pipette tip. Position the slice very carefully by gently pushing it with a pipette tip from the sides into place. The area of interest should cover the electrode area.
- Pipette 12 μ l of thrombin onto the slice. It is not necessary to mix it with the plasma.
- Incubate for 5 -10 min to allow a rigid clot that glues the slice onto the MEA.
- Add the middle ring to the MEA.
- Add 1.4 ml of Gähwiler culture medium.
- Close the lid, wipe the MEA with 70 % ethanol, and put it in the incubator.

Mounting Slices onto Membranes (Stoppini Method)

- Put the filter inserts into the wells of the multi well plate.
- Take the slice out of the buffer with a broad spatula and let it slide right onto the membrane. Depending on the size of the membrane insert, one or more slices can be positioned on one membrane.
- Remove access buffer with a filter paper.
- Add Stoppini culture medium to the well until it reaches the membrane and the membrane becomes transparent. The insert must not float.
- Close the multi well plate, wipe it with 70 % ethanol, and put it in the incubator.

Culturing Slices (Gähwiler Method)

The MEAs with mounted slices can be cultured in an incubator at 35 °C that was adapted with a tilt mechanism. Recommended degree of tilt is -70° to $+70^\circ$ per minute, with a resting time of two minutes in-between rotations. The tilting is necessary to ensure that the air and the medium cover the slices in alternating cycles. No humidity or CO₂ is needed. The incubator shown below was adapted by the user for culturing organotypic slices on MEAs. Half the volume of the culture medium should be replaced about twice a week.



The plasma-thrombin clot gets digested over time and the tissue comes down to the electrodes. First recordings can be done two days after preparation in the ideal case. After one week, all cultures should be ready for recording. Maximum culture time can be up to a month or longer. However, with increasing time in culture, more and more cultures either detach or holes appear due to tensions in the plasma clot. The ideal recording period is between one and two weeks after preparation.

Culturing Slices (Stoppini Method)

MEAs on Stoppini membranes can be cultured in a regular incubator with humidity and CO₂ together with cell culture preparations. Medium should be changed every second day.

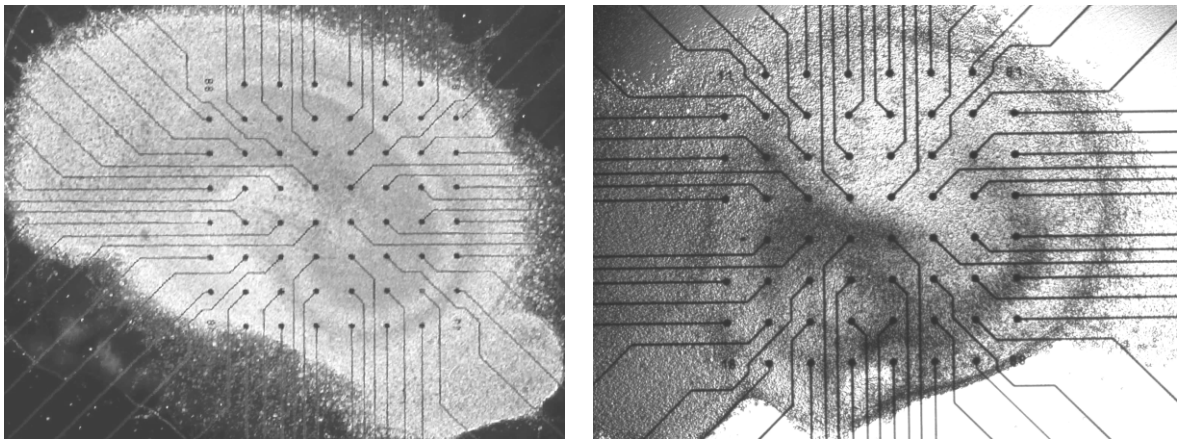
Alternative Methods

A third method to cultivate slices has been proposed by Ken Shimono (Shimono et al., *Journal of Neuroscience Methods*, 120 (2002) 193-202). Shortly, slices are positioned on a coated MEA, and medium is filled up only to an interface level, to expose the upper surface of the slice to the air. This method has the advantage that no tilting mechanism is needed. However, medium changes are necessary every day and the cultures are very sensitive to drying. Yet another method which can be used without tilting device using PDMS inserts was devised by Yevgeny Berdichevsky and colleagues (Berdichevsky et al., *Journal of Neuroscience Methods*, 178 (2009) 59-64). An overview of publications using organotypic slices on MEAs can be found on the MCS web site (<http://www.multichannelsystems.com/publications>).

Images of Organotypic Cultures

While the general structure of the tissue remains widely intact in OTCs, changes do happen during cultivation time. Generally, the slices become much thinner over time. After a week in culture, slices are usually around 100 μm thick. At the same time, they become a bit wider, cell layers become broader, and cells migrate out in all directions. A healthy culture therefore looks more transparent than an acute slice, even if the initial cutting thickness was the same. The hippocampal cell layers, which are clearly visible in an acute slice, become broader and partly disintegrate during culture. They are still obvious in a DAPI stain, but not as easily recognizable in a phase contrast image. Furthermore, a healthy slice culture is usually surrounded by a halo of migrated cells. Below you can see two hippocampal organotypic cultures after ten days of cultivation.

The culture on the left is a Gähwiler culture, directly grown on the MEA. The culture on the right is a Stoppini culture grown on a filter membrane and flipped upside down on the MEA.



Recording

Advantages and Disadvantages of Different Culture Types

Depending on the type of culture, OTCs can be recorded only once or several times over an extended period of time. Stoppini cultures on membranes are much easier to prepare. No specific hardware is needed, and there are no problems with a plasma-thrombin clot. However, they can be recorded only once for a few hours under perfusion, like an acute slice.

Gähwiler cultures on the other hand need a tilting device, and problems with holes in the plasma-thrombin clot often occur with increasing culture time. But the advantage is that repeated recordings of a few minutes can be done without perfusion and without opening the culture dish over the period of days and weeks. This allows investigating long lasting effects and processes.

Recording Stoppini Cultures

Note: The following steps have to be carried out as fast and careful as possible.

Set up everything for a recording with perfusion (please see chapter 5.4 below). Remove the membrane insert with the Stoppini culture from the incubator and cut the membrane around the tissue with a scalpel or fine scissors. Carefully lift the culture by the membrane with forceps and put them upside down on the electrode field of a dry MEA, so that the tissue faces the electrodes. Put a slice grid on the tissue. Hold the slice grid down carefully with forceps and add oxygenated ACSF, otherwise the surface adhesion of the ACSF might lift off grid and slice. Transfer the MEA to the amplifier and start perfusion with ACSF as fast as possible. The position of the tissue can be adjusted by gently pushing the slice grid with forceps or a pipette tip.

Recording Gähwiler Cultures

With perfusion

Set up everything for a recording with perfusion (see chapter 5.4 below). Remove MEA with the culture from the incubator and open the lid. Remove the culture medium and the middle ring of the culture dish and fill up with oxygenated ACSF. Transfer the MEA to the amplifier and start perfusion with ACSF as fast as possible.

Without perfusion

Remove MEA with the culture from the incubator and transfer it to the amplifier without opening the lid. Cultures will tolerate not being tilted longer the older they get, because the tissue becomes thinner. No recordings are possible at the first 1 - 2 days *in vitro*, because there is still plasma-thrombin clot between electrodes and tissue. The clot gets digested over time, and the tissue gets in contact with the electrodes. Five minutes recording are possible during the first week, later on 10 - 20 min should also be safe. After recording, wipe the MEA with 70 % ethanol and put it back in the incubator. To be able to do recordings with closed lid, you need to use MEAs with integrated reference electrode.

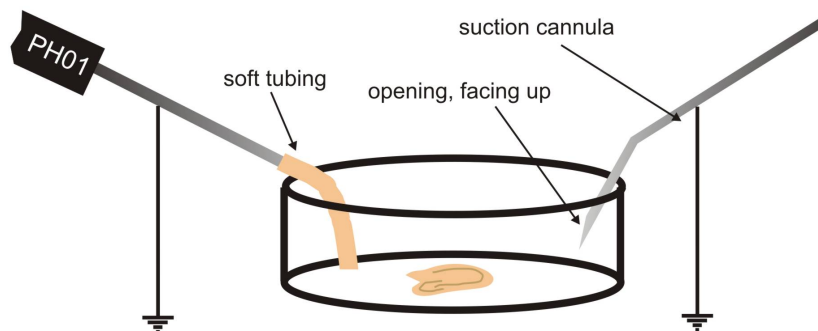
Perfusion

Note: We recommend the perfusion cannula with temperature control (PH01) for optimal environmental conditions. A two-channel temperature controller (TC02) allows controlling both the MEA temperature (via the heating integrated into the amplifier) and the buffer temperature.

Prepare a sufficient volume of ACSF as listed above and aerate the buffer with carbogen gas for 15 min. Control pH and osmolarity. Before mounting the MEA, make sure the perfusion is ready and all tubing is already filled with oxygenated ACSF.

Carefully transfer the MEA to the amplifier without tilting it. It is also possible to mount the slice on the MEA with the MEA already on the amplifier. This saves time, but is sometimes unpractical due to space restrictions, if the amplifier is mounted on a microscope. Start perfusion as fast as possible at a rate of 2 - 5 ml/min. Control position of the slice under the microscope. Check the noise level and wait 25 - 20 min before starting the experiment. Noise level of the electrodes should be around 15 μ Vpp. Healthy slices usually show spontaneous spiking activity. Spikes are relatively small compared to spikes from cultured neurons, due to the larger distance between firing cells and electrodes.

Low frequency fluctuations are most often caused by the perfusion. Shortly switch off the pump to see whether the fluctuations disappear if the pump is off. 50 Hz noise can also be caused by the perfusion but is independent of the pump running or not. Perfusion in and out should contain a piece of metal that can be connected to the amplifiers ground to remove 50 Hz noise. The easiest way is to use a bend cannula for suction. The opening of the cannula should be positioned in a way that it always sucks air and liquid at the same time, possibly resulting in a constant slurping noise. This prevents the fluid level from going up and down, which also causes noise. See a suggested perfusion setup on the picture below.



If you experience low frequency noise from the perfusion, try to optimize the suction as described above. Additionally, a drip chamber can be used to interrupt the fluid flow between pump and recording chamber. A 10 Hz high pass filter in the software can also be used to remove low frequency fluctuations in the baseline, if the amplitude is not larger than about 200 μ V.

50 Hz noise can be caused by the perfusion or by external noise sources. Remove perfusion in and out from the bath. If the noise persists, check for external noise sources, like microscope lamp, power supplies close by and so on. If the noise is caused by the perfusion, check the grounding of the perfusion in and out. If the magnetic perfusion holders (MPH) from MCS are used, the perfusion should already be grounded via the magnet which fixes the perfusion holder on the metal plate of the amplifier.

MC_Rack Setup

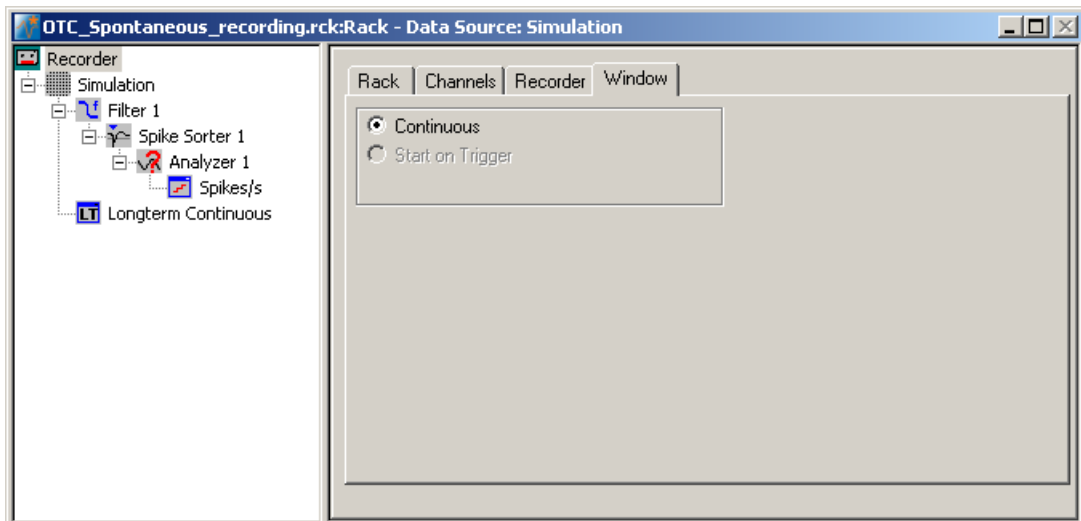
The setup of MC_Rack is very much dependent on the type of experiment. For racks and stimulation files for LTP experiments, please refer to the application note "LTP experiments on Acute Hippocampus Slices" available on the Multi Channel Systems MCS GmbH web site.

In this chapter two exemplary racks are presented, one for the recording and analysis of spontaneous activity, and another one for evoked activity by electrical stimulation. You may have to recreate the rack to fit your specific data source, but the basic structure of the rack remains the same.

Spontaneous activity

Open the rack "OTC_Spontaneous_recording.rck". The rack contains a long term display to monitor spontaneous activity continuously. A digital filter removes field potentials. A spike sorter tool detects the spikes, and the spike rate is analyzed and displayed in one second time bins.

In the recorder, select the "Raw Data" stream to be recorded continuously. Sampling frequency in the "Data Source" should be 20 kHz or more, the input voltage range should cover approximately signals up to $\pm 800 \mu\text{V}$.

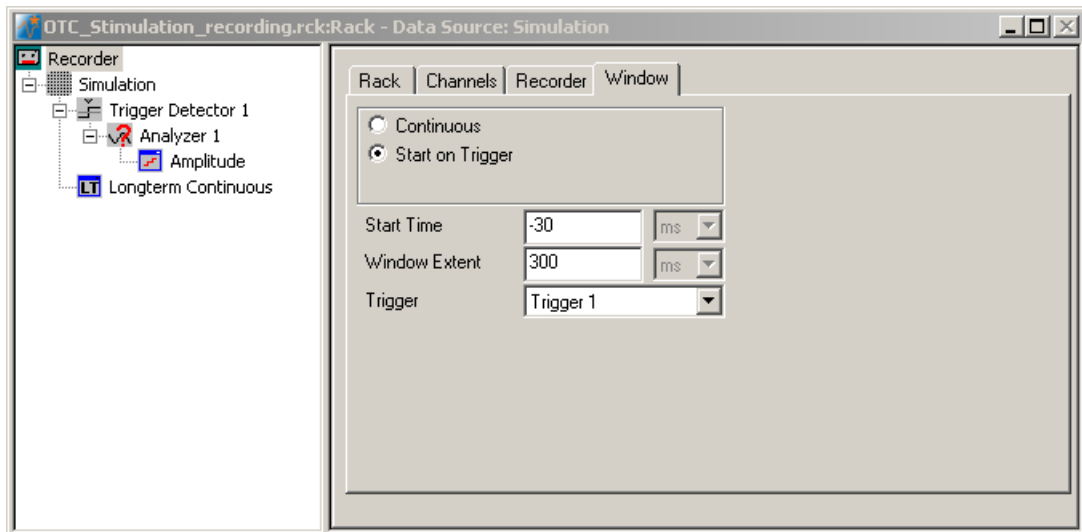


The long term display shows the unfiltered spontaneous activity. A 200 Hz high pass filter removes field potentials and leaves only the spikes. The spike sorter detects the spikes and the analyzer determines the spike rate in 1 s time bins. Please adjust the spike detection level according to your experiment.

Evoked activity

Open the rack "OTC_Stimulation_recording.rck". The rack is very simple, it contains a trigger detector, a long term display to monitor spontaneous activity continuously, and an analyzer to display and analyze evoked responses after electrical stimulation. In the recorder, select the "Raw Data" stream and the trigger to be recorded, and "Start on Trigger" mode on trigger one. A 300 ms time window starting 30 ms before the trigger covers all stimulation evoked response. In addition, the relatively long time window before and after the stimulation pulse allows to access possible spontaneous activity before the stimulation, as well as epileptiform bursting induced by the stimulation.

Sampling frequency in the “Data Source” should be 10 or 20 kHz, the input voltage range should cover approximately signals up to ± 4 mV.



A continuous long term data display can be used to check the noise level and possible spontaneous activity. The trigger detector reacts on TTL signals coming in on bit 0 of the digital channel. It is therefore necessary that the stimulator generates a TTL signal together with the stimulation pulse. The “Analyzer” is also starting on Trigger 1 and displays signals evoked by stimulation only.

EPSPs evoked by stimulation usually have their minimum within the first 10 ms after stimulation. Consequently, the “Analyzer” is set to analyze a region of interest (ROI) between 1 and 8 to 10 ms after the trigger. The peak to peak amplitude is analyzed and plotted in the parameter display versus time. Even if maybe not the best choice for final data analysis, the peak to peak amplitude is a good parameter to monitor signal stability over time during the experiment.

Stimulator Setup

Three preprogrammed stimulation files are provided. One channel of the stimulator is used, as well as the corresponding trigger output. Voltage or current pulses can be used. Voltage pulses are a safer, because when a current pulse is programmed and an electrode with high impedance is selected for stimulation, the compliance voltage to drive the current may go very high, possibly destroying the electrode. All example files are programmed as voltage pulses. The safe range for 30 μm electrodes and 2 x 100 μs long pulses is approximately up to ± 3 V or ± 150 μA , respectively. See safe charge injection limits in the MEA manual for more details. A TTL output is generated on Sync Out 1 of the STG together with every stimulation pulse. The Sync Out must be connected to the digital channel bit 0 of the data acquisition.

In any case, charge balanced, biphasic pulses should be used, always starting with the negative phase. Positive pulses can lead to the formation of a Titanium Oxide isolation layer on the electrodes.

Test:

A simple biphasic pulse with ± 2 V one channel, repeated every 10 s until manually stopped. This pulse can be used to check the viability of the cultured slice and probe for the best stimulation electrodes. If no response is seen at ± 2 V, the slice is probably dead. An EPSP signal amplitude of about 1 mV should be expected for a good quality slice.

IO-500-3000 mV:

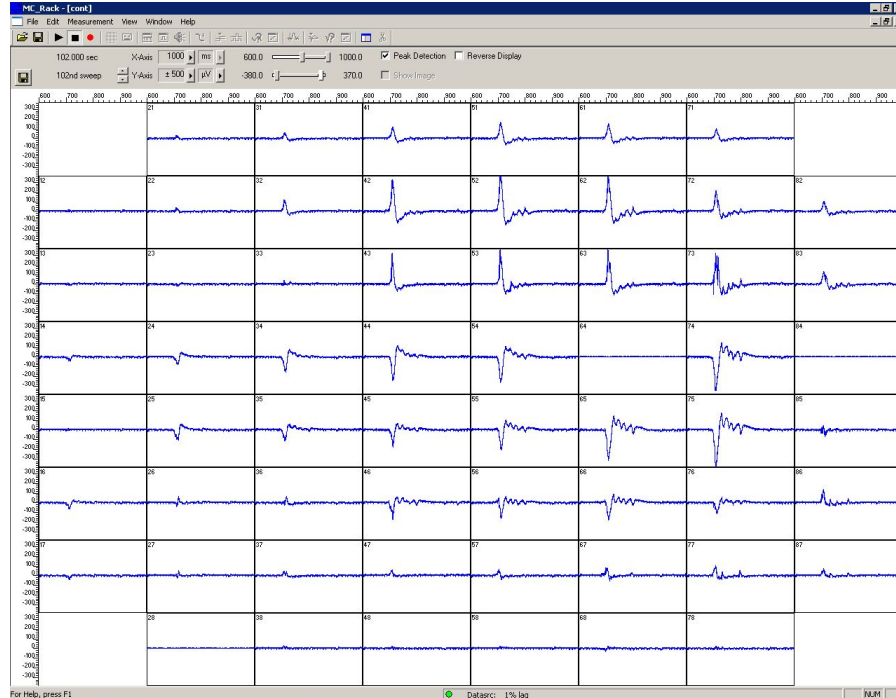
Pulses with increasing amplitude to generate an input output curve (IO curve). Each amplitude has three repeats, running from 500 mV to 3 V in steps of 250 mV. A response can be expected sometimes from the very beginning, most often starting at 750 mV or 1 V. The paradigm will run once and then stop automatically.

Paired-Pulse:

A double pulse one channel with 20 ms inter pulse interval on one channel, repeated every 10 s until manually stopped. This pulse can be used to check for short term plasticity. The amplitude should be adjusted for each experiment according to the results of the IO curve.

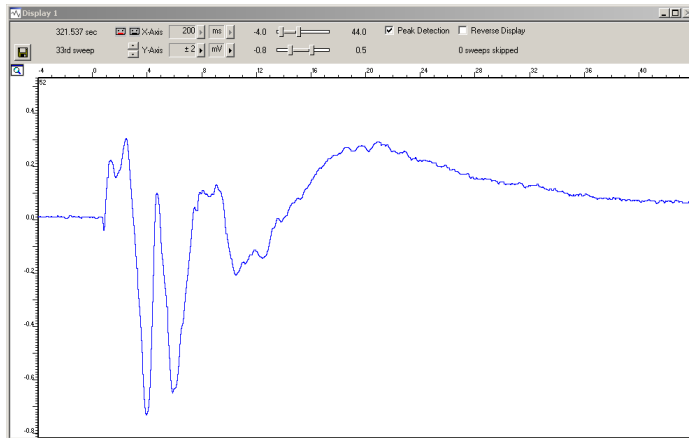
Expected Signals

The probability that any two neurons are directly connected is much higher in an organotypic culture than in an acute slice. Consequently, there is often more spontaneous activity, and the cultures are more likely to develop epileptiform activity. Large, synchronized field potentials can often be observed. Please see an example below.



When using electrical stimulation, this can lead to unstable signals. If a stimulation pulse is delivered during or shortly after a period of intense spontaneous activity, the evoked response is generally much smaller than usual.

In contrast to acute slice preparations, evoked responses often show multiple peaks, instead of one single signal. See below an example of a multiple population spike in CA1 after electrical stimulation of the Schaffer collaterals.

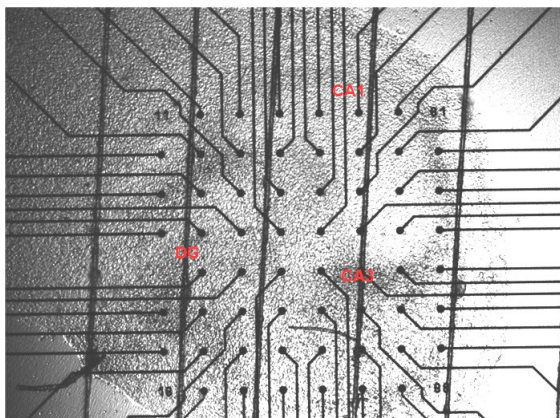


On the positive side, as there is usually a much better contact between active cells and electrodes. Signals, especially spontaneous spikes, are usually larger than in acute preparations.

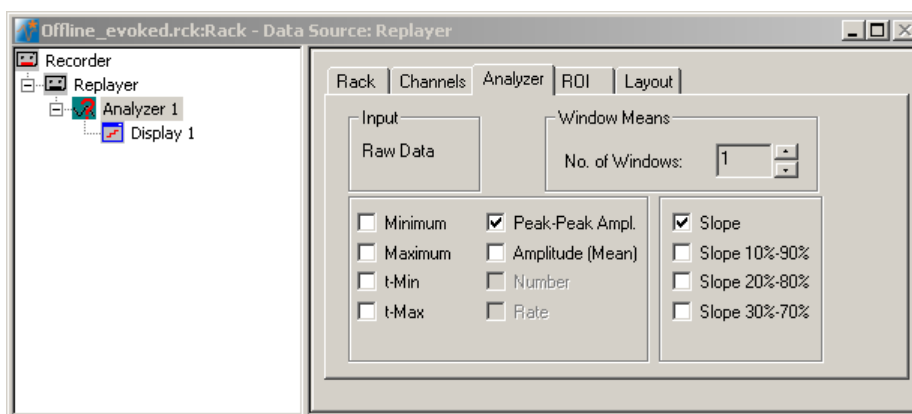
Data Analysis

Evoked Activity

After finishing the experiment, a set of useful information can be extracted from the data. Traditionally, the slope of the EPSP in the *stratum radiatum* is used as a parameter to assess for example LTP. However, data from MEA recordings allows extracting also additional information. The results can be exported directly from the parameter display as ASCII to be used in Excel, Origin or other programs. A sample analysis rack (Offline_evoked.rck) with a corresponding data file (Sample_triggered.mcd) is provided. The data was recorded from a Stoppini type culture of a P6 rat after seven days in culture. Please see image below.



The data file contains ten sweeps, stimulation electrode was #72. In comparison to acute slices, which usually display EPSPs with one minimum and single population spikes, less synchronized signals with multiple signal components often occur in OTCs, as can also be seen in the sample file. In addition, smaller bursts of field potentials can sometimes be observed some tens of milliseconds after the first evoked response is over, please see the 3rd sweep, for example.



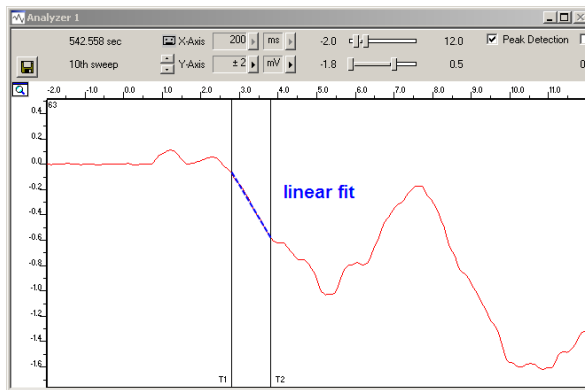
In the sample rack, an “Analyzer” is used to analyze slope and amplitude within the region of interest. The exact settings of the analyzer have to be adjusted depending on which parameters should be analyzed. The following paragraphs describe the analysis of different signal types and parameters.

EPSP Slope

The slope of the EPSP is the most often used parameter to monitor LTP, as it has a more direct correlation to the actual synaptic strength and is usually more stable than the EPSP amplitude. MC_Rack offers several options to analyze the slope of a signal. Inside the region of interest, the minimum and maximum is determined, and a straight line is fitted through the data points in the region of interest (Least Square Algorithm).

The slope of the straight line is then extracted. If the 10/90, 20/80 or 30/70 option is selected, the curve fit is limited to the data points within 80, 60 or 40 % between minimum and maximum (see MC_Rack help for details). The 20/80 slope for example is sometimes good to get an approximation for all electrodes at once. To get an exact slope value for one specific electrode, it is best to manually narrow down the region of interest to the linear part of the EPSP and use the regular slope as parameter.

In the analyzer tool, select "Slope" as parameter. Pick one electrode to analyze and zoom into the signal. Adjust the ROI to include only the linear part of the signal. Analyze the complete experiment with the same ROI and plot the results in the parameter display. From the parameter display, you can directly export the results as ASCII data (*.dat file) with the floppy disc icon in the header of the display, which will open with Excel, Origin or similar software.

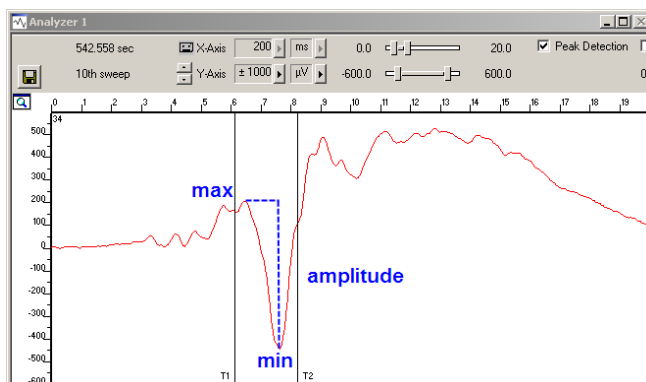


Of course, more than one electrode can be analyzed in this fashion, for example to investigate differences between different regions of the hippocampus. Signals with slopes below about 0.2 V/s should not be used, as very small absolute changes result in rather large relative changes, for example an increase of only 0.1 V/s will result in a 50 % increase after normalization, which might lead to distorting effects when compared to experiments with larger absolute signals.

Population Spike Amplitude

Population spikes, often simply called popspikes, are often present along the CA1 cell layer. They are more frequent in OTCs than in acute slices. Like EPSPs the popspikes are often less synchronized and display multiple peaks, though.

In the analyzer tool, select "Peak to peak amplitude" as parameter. Pick one electrode to analyze and zoom in on the signal. Adjust the ROI to include the local maximum before the popspike and the minimum of the signal. Analyze the complete experiment with the same ROI and plot the results in the parameter display. From the parameter display, you can directly export the results as ASCII data (*.dat file) with the floppy disc icon in the header of the display, which will open with Excel, Origin, or similar software. Same way as for EPSP slopes, more than one electrode can be analyzed, and very small signals should be avoided.

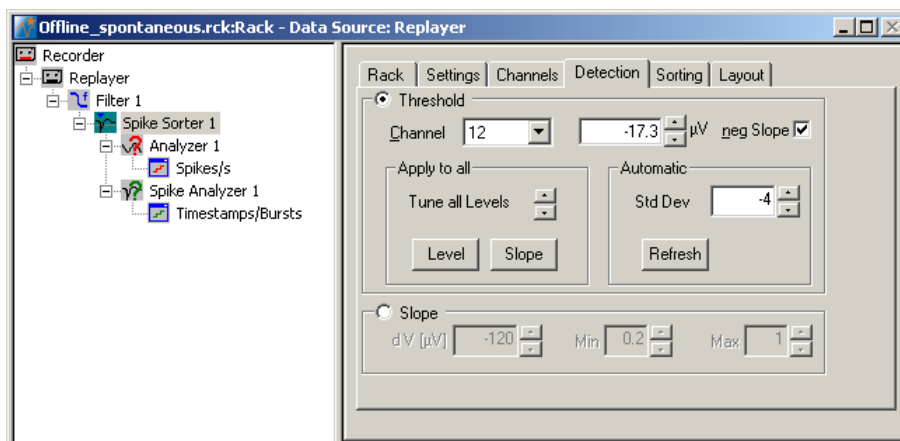


Spontaneous Activity

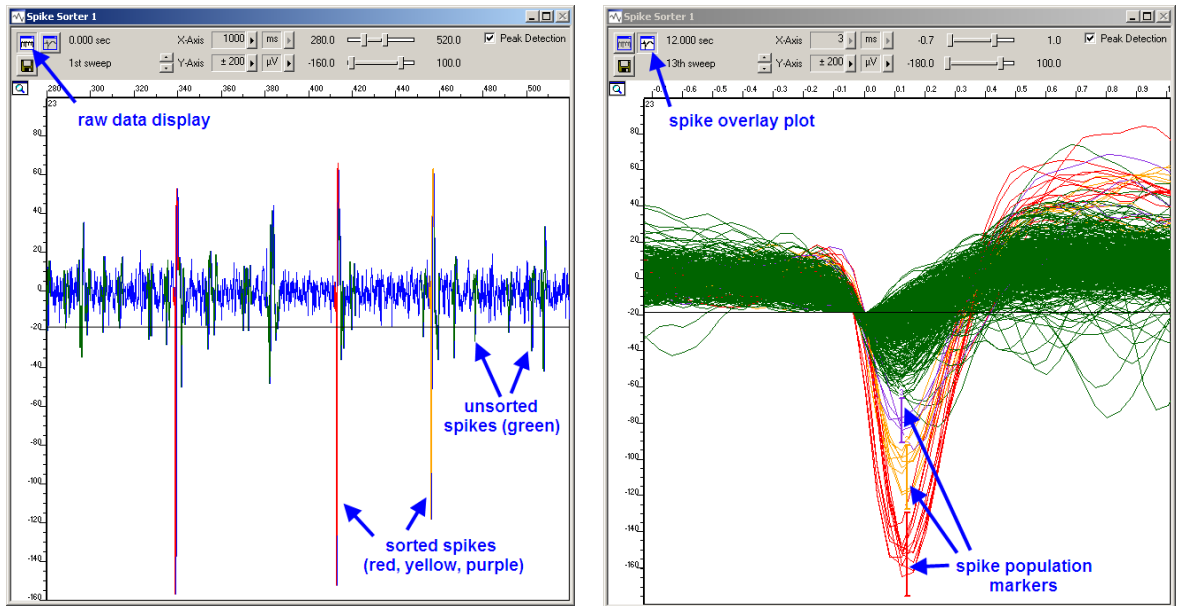
As mentioned before, spontaneous activity is rather prominent in organotypic cultures. While spontaneous field potentials are hard to interpret, spikes can also be extracted from spontaneous activity. A sample analysis rack (Offline_spontaneous.rck) with a corresponding data file (Sample_spontaneous.mcd) is provided. The data was recorded from a Gähwiler type co-culture of entorhinal cortex and dentate gyrus of a P6 rat after ten days in culture. Please see the image below.



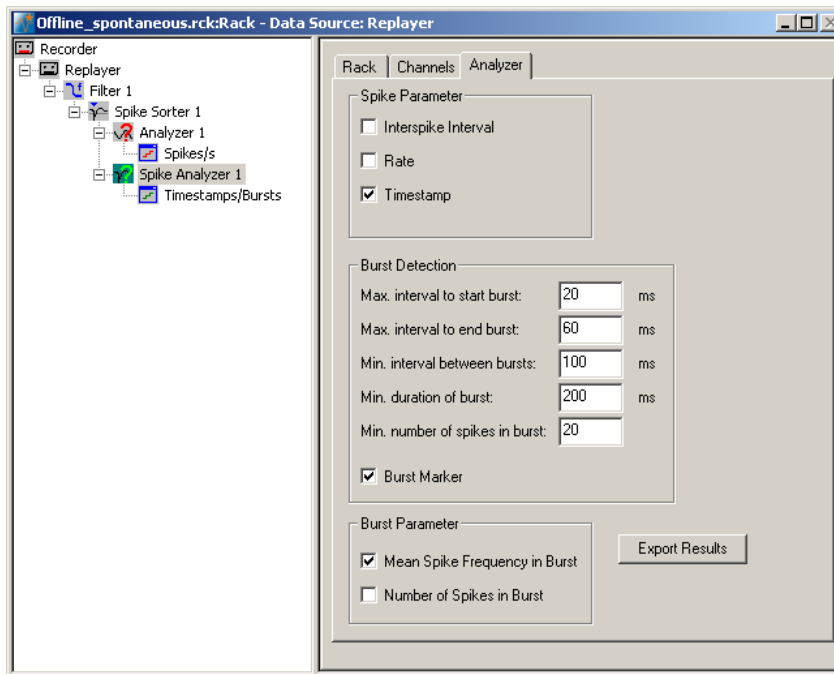
The data file contains 13 seconds of continuous data with spontaneous activity. A 200 Hz high pass filter removes slow frequency field potentials to improve spike detection. The spike sorter tool detects spikes on the filtered data with a threshold of four times the standard deviation of the noise. Spike sorting is demonstrated on four electrodes, #23, 44 and 72. The spike sorter display shows either filtered raw data or an overlay plot of detected events. The “Analyzer” shows the number of detected spikes per second versus time, and the “Spike Analyzer” shows time stamps color coded for sorted populations and spike burst markers.



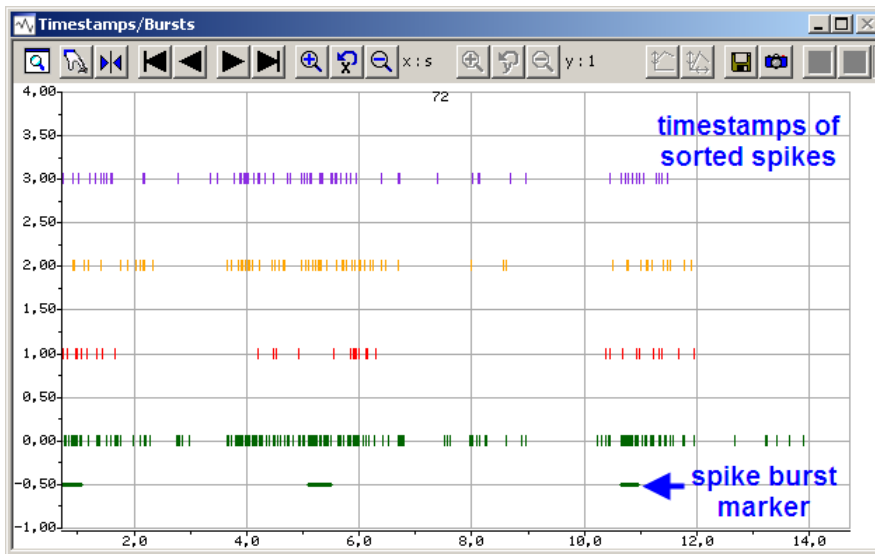
The spike sorter display can be switched between raw data display and spike overlay. In the spike overlay, three markers for different spike populations can be used to sort three populations of spikes individually on each electrode (1 = red, 2 = yellow, 3 = purple, everything else = green). For details, please refer to the MC_Rack manual for details.




While the "Analyzer" analyzes the detected spikes in 1 s time bins and plots the number of spikes per seconds versus time, the "Spike Analyzer" plots the time stamps of the sorted spikes in color code. Spike bursts are calculated according to parameters defined by the user in the "Spike Analyzer".



Spike bursts are labeled with a bar in the spike parameter display.



Analyzed parameters and data displays can be exported directly from the displays, either as

picture or in ASCII format with the camera respectively floppy disc icon in the toolbar  .

Suggested System Configurations

MEA2100-60-System-E

The MEA2100 is the most advanced system Multi Channel Systems MCS GmbH can offer today. It is as flexible as the USB-MEA60 and can use the same MEAs, in addition to unique 120 electrode arrays. Integrated current or voltage controlled stimulators can use any electrode as stimulation electrode. The system includes amplifier, data acquisition, and stimulators in one compact device, as well as floor and perfusion heating. The filter band of the DAQ can be changed by software. An additional box includes a unique freely programmable DSP for advanced closed loop experiments and many additional in and outputs for interface with other devices. The use of perforated MEAs is optional. More than ten electrode layouts with several additional options are available at the moment. The MEA2100 will fit equally well on upright and inverted microscopes. The system can be upgraded to operate up to four 60-channel or two 120-channel MEAs independently from one computer.

Included accessories: Temperature controller, perfusion heating, floor heating, magnetic perfusion holders.

Recommended accessories: Slice grids, peristaltic pump PPS2, tubing.

Optional accessories: Constant vacuum pump (CVP), perfusion element (PE), LTP-Director software, ALA MEA-Insert.

Pro: Very compact, suitable for inverted and upright microscopes, selectable filter band, programmable DSP.

Con: Electrodes not accessible for external stimulation devices, no direct access to analog raw data.

USB-MEA60-Inv/Up-System-E with STG4002

The USB-MEA60 uses 60 electrodes, each of which can be selected by software for stimulation and recording. The system includes amplifier and data acquisition in separate devices, floor and perfusion heating and an external two channel stimulator for current or voltage stimulation. A blanking circuit removes stimulation artefacts. The use of perforated MEAs is optional. More than ten electrode layouts with several additional options are available now. Amplifiers are available in "Up" or "Inv" configuration, optimized for upright or inverted microscopes. The data acquisition allows real-time feedback stimulation by a specific digital signal processor for fast online signal analysis. The data acquisition and STG can be upgraded to operate up to four amplifiers independently from one computer. Blanking circuit and the STG are optional, only if stimulation is intended.

Included accessories: Temperature controller, perfusion heating, floor heating, magnetic perfusion holders.

Recommended accessories: Slice grids, peristaltic pump PPS2, tubing.

Optional accessories: Constant vacuum pump (CVP), perfusion ground plate (PGP), LTP-Director software, ALA MEA-Insert.

Pro: Individual components (STG, DAQ) also usable for other experimental setups, very flexible applications.

Con: Many boxes and cables, relatively large footprint, especially in systems for parallel recordings from several MEAs.

Suggested Microelectrode Arrays

MEA2100-System

For the MEA2100, the same MEAs can be used as for the USB-MEA-60, with the same advantages and disadvantages. In addition, 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 combined cortex / hippocampus slice preparation.

USB-MEA60-System

MEAs with 10 or 30 μm electrodes are equally suited. If electrical stimulation is intended, 30 μm electrodes are better because stronger stimulation pulses can be applied. MEAs with internal reference electrode should be preferred for Gähwiler type cultures, as this will allow recordings with closed culture chamber. The material (TiN or ITO) does not matter; only if sophisticated imaging on the arrays is planned, ITO should be preferred. For the hippocampal formation, the 60MEA200/30iR can be used for rat and mouse slices. For mice, especially younger ones, the 60MEA100/10iR might be an alternative, but with the disadvantage of smaller electrodes.

Perforated microelectrode arrays were specifically designed for slice recordings. Perforated MEAs might be used for Stoppini type cultures, not for Gähwiler cultures. They can provide better signal to noise ratio, an improved viability and faster substance application to the slices. However, the additional perfusion cycle also introduces additional noise sources and is technically more demanding than regular perfusion on non perforated arrays. Also, the imaging possibilities are more restricted on perforated arrays. pMEAs provide a real advantage which is worth the additional effort if long lasting experiments are planned (> 2 h recording time), and / or if substances must be applied to the tissue during the experiment relatively fast.

Ring Options

For Gähwiler type cultures, the plastic ring with thread and the corresponding culture chamber is mandatory (please see the image). For Stoppini cultures, any ring option may be used. The standard glass ring is best for running a perfusion and will also allow using the ALA MEA-Insert.

