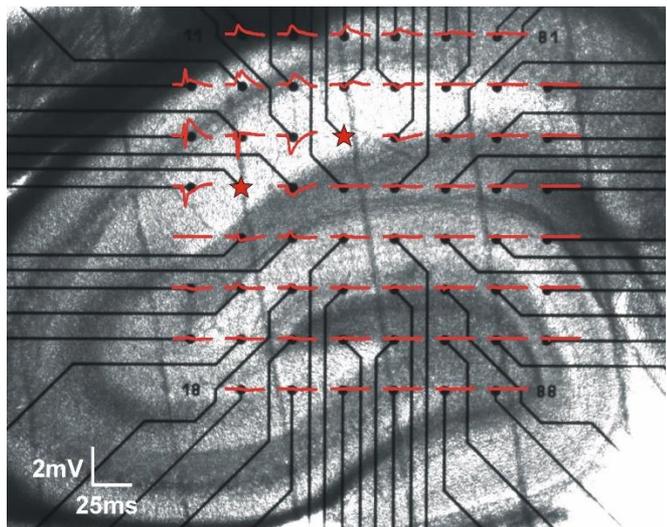




**MEA Application Note:
Acute Hippocampus Slices from
Rat or Mouse;
Preparation, Recording, and
Data Analysis**



Imprint

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1 Introduction

The intention of the MEA Application Notes is to show users how to set up experiments with a 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 acute experiments, and a guide how to run a standard LTP experiment, and analyze the data afterwards. Suggestions for suitable MEA-Systems configurations and support files are also included.

It is assumed that the reader is familiar with the functions of the software MC_Rack and MC_Stimulus or the Multi Channel Suite and the general terminology in the field of LTP experiments.

Important parts of the preparation procedure are also available as a [movie](#).

2 Material

2.1 Animals

One rat or mouse, suggested age P20 – P45. In younger animals, the tissue is less vulnerable to hypoxic and mechanical stress, so it's easier to obtain good slices. Use male animals to avoid influence of the oestrous cycle. Mouse brains are smaller than rat brains, so usually less usable slices can be obtained from a mouse brain. Apart from that, the preparation procedure is identical.

2.2 Technical Equipment

- MEA-System with internal or external stimulator and MEAs (microelectrode arrays)
- Peristaltic pump
- Optional: Suction pump or house vacuum
- Stereo microscope
- Inverted microscope for aligning the slice position to the electrodes, with a camera for documentation. Alternatively, a Video Microscope Table [MEA-VMTC-1](#) or [VMTC-2](#) can be used.
- Oscillating vibratome; suggested models: ThermoFischer, HM 650 V with cooling unit ([Catalog](#), p.51) or [Leica VT 1000 or 12000](#)
- Blades for the vibratome
- Adjustable pipettes and pipette tips (1000 µl)
- Transfer pipettes (cut Pasteur pipettes with wide opening, approximately 0.5 cm)
- Large sharp scissors or guillotine
- Surgical instruments: small scissors, narrow flat spatula, larger forceps, curved forceps, sharp forceps, scalpel, razor blade, spoon
- Filter paper
- 6 cm dishes
- Optional: Agar block (1% Agar)
- Optional: Fume hood and chamber for anesthesia
- Carbogen gas supply (95% O₂, 5% CO₂)
- Storage chamber for slices ("Bubble Chamber"); usually the slices rest on a net in ACSF, constantly oxygenated with Carbogen (see chapter 3.5)
- Slice grid (see chapter 4.3)
- Porous stones for oxygenating the solutions
- Optional: Hand blender to prepare ice slush
- Water bath

2.3 Chemicals

- Carbogen gas (95 % O₂, 5 % CO₂)
- NaCl
- KCl
- CaCl₂
- KH₂PO₄
- NaHCO₃
- D-Glucose
- MgSO₄
- Super glue (cyanoacrylate); suggested brand Loctite 401
- 100 % alcohol or acetone (for cleaning the MEA contact pads and the blades)
- Optional: Halothane or similar anesthetic

2.4 Media

ACSF Buffer

	MW	mM	g/2 l 10X stock	g/5 l 10X stock	g/1 l final ACSF	g/2 l final ACSF	g/3 l final ACSF
NaCl	58.4	125	146	365			
KCl	74.56	3.5	5.22	13.05			
NaH₂PO₄·H₂O	138	1.2	3.312	8.28			
CaCl₂·2H₂O	147.02	2.4	7.06	17.64			
MgCl₂·6H₂O	203.3	1.3	5.29	13.21			
Glucose	180.2	25			4.5	9.0	13.5
NaHCO₃	84.0	26			2.18	4.36	6.55

pH 7.35, 310 mOsmol

10 x 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.

Optional: Sucrose Cutting Solution

	MW	mM	g/l 10X stock	g/500 ml final solution
Sucrose	342.3	150	513.45	
NaCl	58.4	40	23.36	
KCl	74.56	4	2.98	
NaH₂PO₄·H₂O	138	1.25	1.73	
CaCl₂·2H₂O	147.02	0.5	0.735	
MgCl₂·6H₂O	203.3	7	14.231	
Glucose	180.2	10		0.9
NaHCO₃	84.0	26		1.09

pH 7.4, 330 mOsmol

Sucrose solution can optionally be used instead of regular ACSF during preparation and slicing. The advantage is that activity in the tissue and thereby excitotoxicity is further reduced during preparation. However, the solution is very sticky and makes cleaning of the slicing equipment much more time consuming.

3 Preparations

3.1 Solutions

- Prepare the ACSF and optionally Sucrose Cutting Solution as listed above.
- Aerate the buffer with carbogen gas for 15 min. Control pH and osmolarity.
- Refrigerate 300 ml ACSF buffer (or Sucrose Cutting Solution, if used) at -80°C until it's partially frozen.
- Crush the frozen solution thoroughly and oxygenate on ice until use. Best results are achieved with a hand blender.

Note: To speed up the freezing and crushing of ACSF, you might consider preparing ACSF ice cubes in advance, and store the ice cubes at -80 °C until use.

3.2 Setting up the Animal Preparation

- If using Halothane or similar anesthetic, set up a box with Halothane under the fume hood.
- Lay out all instruments needed for decapitating the animal, opening the skull, and removing the brain: Guillotine or large scissors, small scissors, larger forceps and spatula, and a spoon to take the brain out of the beaker.
- Prepare a beaker with ice cold ACSF or Sucrose Cutting Solution in a box with ice. Mixing a spoon of salt with the ice will decrease the temperature in the beaker even more.

3.3 Setting up the Brain Preparation

- Lay out all necessary instruments: Razor blade (cleaned with Acetone), curved forceps, sharp forceps, scalpel
- Super glue and a pipette tip to spread it
- Petri dish with filter paper or agarose in the bottom
- Petri dish on ice with cold ACSF or Sucrose Cutting Solution
- Binocular
- Optional: Agar block
- Transfer pipette
- Mounting platform of the vibratome



3.4 Setting up the Vibratome

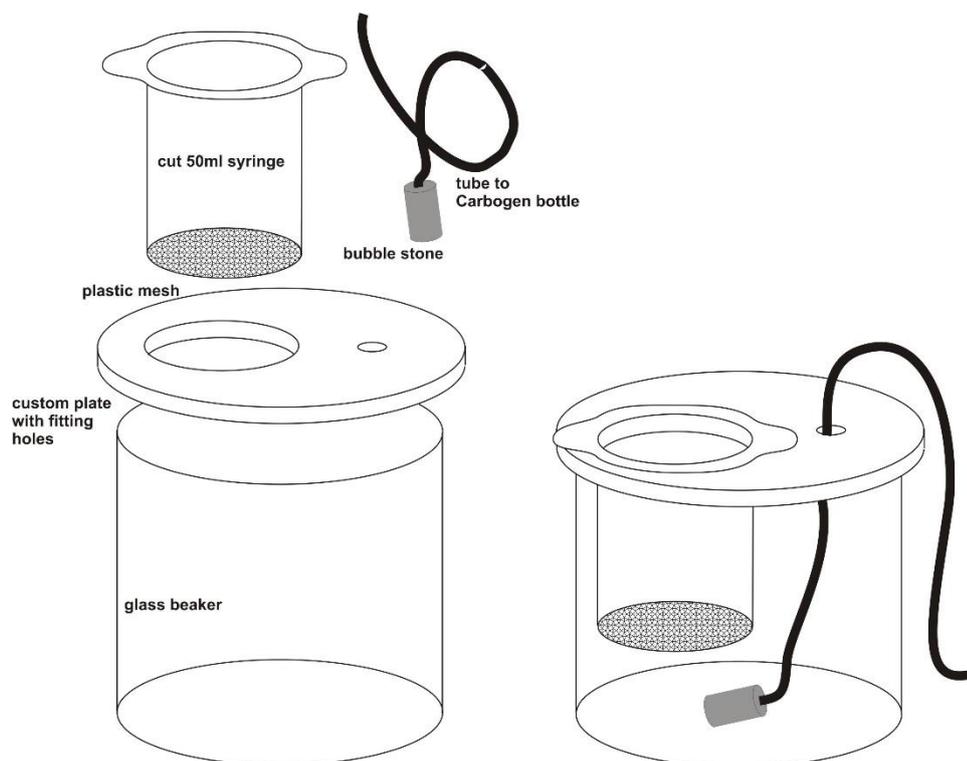
Note: As the design and handling of different vibratomes varies, please consult the manual of your vibratome for more details.

- Fill outer vibratome chamber with ice, add a spoon of salt to the ice.
- Fill inner vibratome chamber with crushed ice from ACSF or Sucrose Cutting Solution. Keep a free space in the middle and fill it with cold but liquid solution.
- Add carbogen tube to oxygenate the vibratome chamber.
- Clean the razor blade with acetone and fix it in the vibratome.

Note: Active cooling units are available for many vibratomes. Active cooling yields a more constant temperature during slicing and also a more reproducible slice quality than passive cooling with ice.

3.5 Setting up the Slice Storage Chamber

Set up the slice storage chamber with ACSF constantly oxygenated with carbogen next to the vibratome in a water bath at 32 °C. Commercial chambers are available for example from [Warner Instruments](#) or [Scientific Systems Design](#).



Concept for a simple, self-made storage chamber.

3.6 Setting up the MEA System

- Start the MEA-System and open the software.
- Mount the MEA inside the amplifier and start the perfusion
- Set the temperature of the MEA heating plate to 32°C, and the temperature of the PH01 approximately to 33.5°C to get 32°C in the MEA recording chamber. The temperature offset between PH01 and chamber is depending on the room temperature and flow rate, and should be determined with a thermometer. Increase the temperature on the PH01 with perfusion running till the thermometer reading inside the chamber is at 32°C. The offset is fairly constant for a given flow rate.
- Let the perfusion run for a few minutes with the empty MEA to flush any bubbles out of the system.

4 Slicing

4.1 Decapitation and Brain Removal

The steps after step 2 should be completed within a minute or less to avoid damage to the brain.

- Anesthetize the rat, for example with a tissue soaked in halothane in an appropriate chamber. Wait till the eyelids stop twitching.
- Remove the scalp with large scissors (ONLY if animal is anesthetized! Otherwise remove scalp after decapitation).
- Decapitate the animal with large sharp scissors or a guillotine.
- 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.
- Drop the brain in the beaker filled with ice-cold ACSF buffer or Sucrose Cutting Solution.

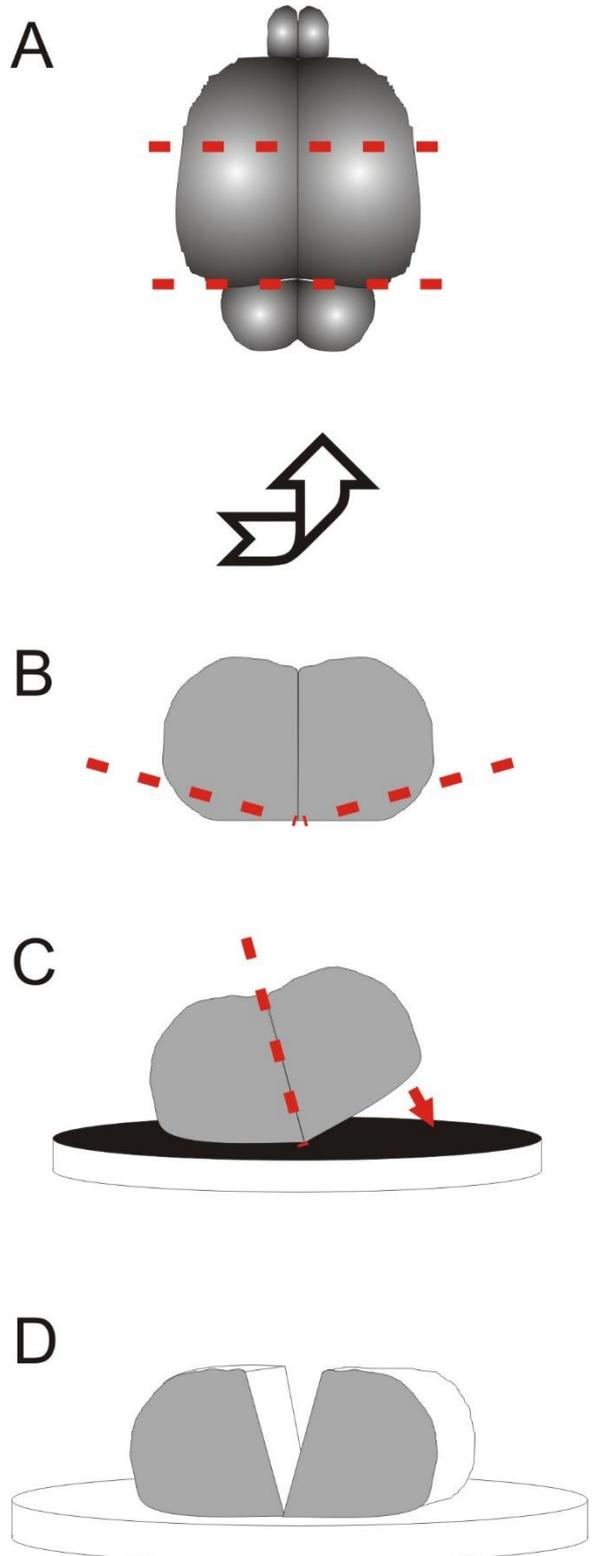


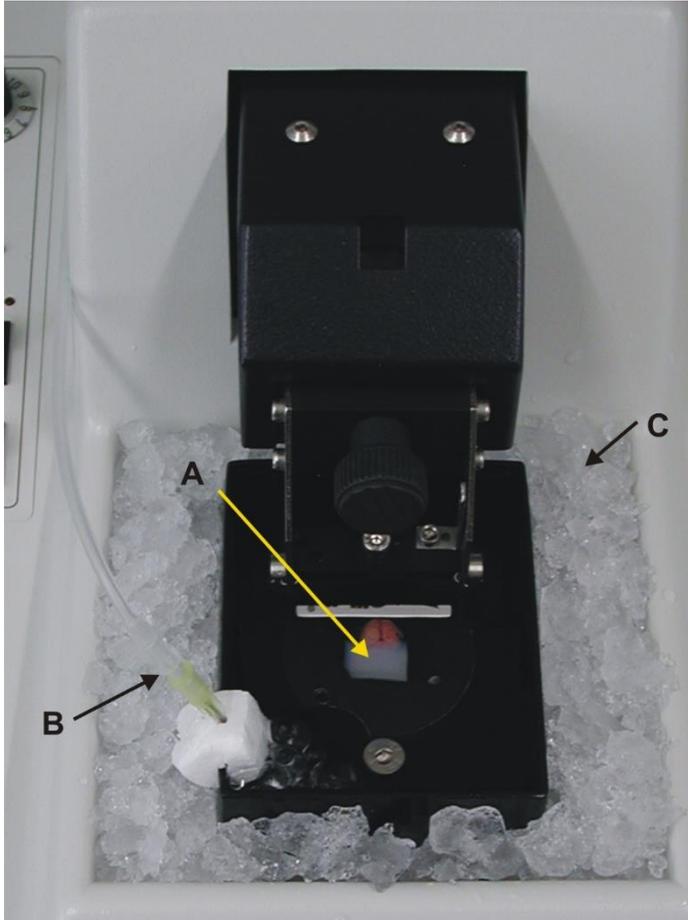
Opened rat skull with the fragments folded to the side.

4.2 Brain Dissection and Slicing

This part of the procedure is available as [video file](#).

- Spread superglue on mounting platform.
- Take the brain out of the beaker with the spoon and put it in the dish with filter paper. Fill up with ice cold ACSF, but the brain should **not** float.
- With the razor blade, cut off cerebellum and frontal pole. Flip brain 90 ° on cutting surface of frontal pole (**A**).
- Cut an approximately 15 ° piece off the bottom of each hemisphere (**B**).
- With the curved forceps, pick up the brain and drop it in the glue. The forceps must not touch the glue.
- Glue brain to platform on one side. Cut between hemispheres, and push down other side (**C, D**).
- Optional: glue Agar block behind the brain.
- Mount platform in vibratome.
- Remove about 3 mm with the first cut — the hippocampus should become visible now.
- Slice the brain according to the recommendations of the vibratome's manufacturer. Usually, maximum blade oscillation and amplitude and slow advance speed yield the best results. Typical hippocampal slices are about 300 – 400 μm thick.
- Pick the slices from the vibratome bath with the transfer pipette and put it in the dish with cold ACSF.
- Under the binocular, dissect out the hippocampus with scalpel and forceps.
- With the transfer pipette, move the hippocampal slice to the slice storage chamber. Store the freshly cut slices at least 1 h before further use.
- About 6 - 8 usable slices can be expected from a rat, about 4 - 6 from a mouse.





Vibratome overview after mounting:

- A Brain and agar block mounted on base plate.
- B Oxygenation for ACSF filled inner chamber.
- C Ice filled outer chamber.

4.3 Mounting the Slice onto the MEA

Important: Handle the slice gently. 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 of the electrodes.

The most common method to keep the slice down on the electrodes uses small weights, commonly called slice grids. Alternatively, perforated MEAs might be used to keep the slice in contact by suction. Please see the Application Note [Acute Hippocampal Slices on Perforated MEAs](#) for details.

You can use either self-made wire grids or a shim with a nylon mesh in the middle to apply the weight. Some steel carriers with nylon meshes as slice hold-downs are also commercially available from [ALA Scientific Instruments](#).

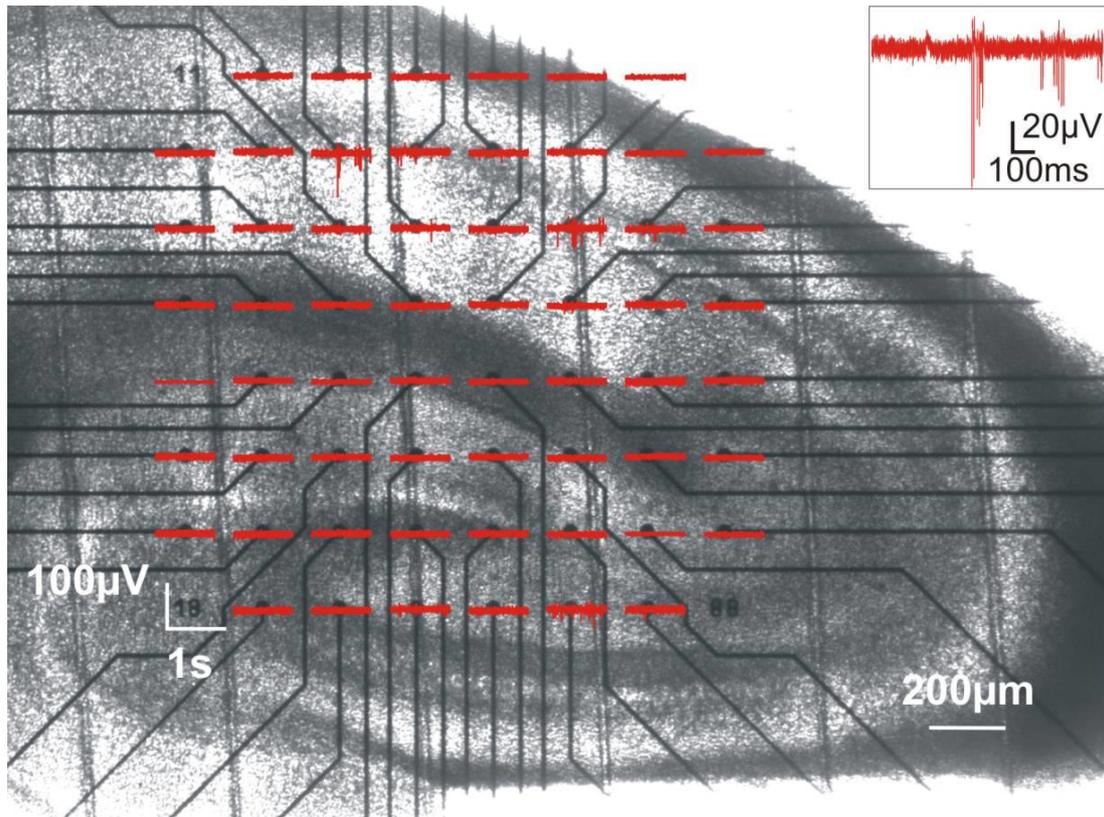
Commonly used custom made weights are U-shaped flattened pieces of platinum wire (80 – 120 mg) glued onto a mesh, a sort of nylon stocking or wedding veil, for example. You can also glue another platinum wire from the other side of the mesh, symmetrically to the first piece of platinum. This kind of grid is less damaging because you can vary the pressure on the slice by changing the thickness of the second wire. The thickness of the wire that is placed onto the slice should match approximately the thickness of the slice. If you use 350 μm slices, the wire should be around 300 μm and not more than 350 μm ; otherwise the grid will not hold the slice.

The following steps will have to be carried out as fast as possible to avoid hypoxic damage to the slice.

- Stop the perfusion (if running, see chapter 3.6). Remove ACSF from MEA chamber.
- Place the slice in a drop of ACSF with the transfer pipette onto the MEA; center it roughly on the recording area with a brush or a pipette tip.
- Remove ACSF until slice rests on the surface of the MEA.
- Position the slice by gently pushing it with a pipette tip or brush from the sides. The CA1 region should cover the recording area.
- Carefully put the slice grid on the slice.
- Hold the slice grid down carefully with forceps and add oxygenated ACSF with a pipette till the slice and the grid are submerged. Otherwise the surface adhesion of the ACSF might lift off grid and slice.
- Start the perfusion.

5 Recording

After the slice was mounted you can start recording. If the position of the slice in relation to the electrodes is acceptable, make a photo or at least a sketch of the relative positions for the experimental protocol. Noise level of the electrodes should be around $15\ \mu\text{V}$ peak to peak. Healthy slices usually show spontaneous spiking activity. Spikes are relatively small compared to spikes from cultured neurons, due to the larger distance between active cells and electrodes. See image below for a rat hippocampal slice on MEA with an overlay of spontaneous activity.



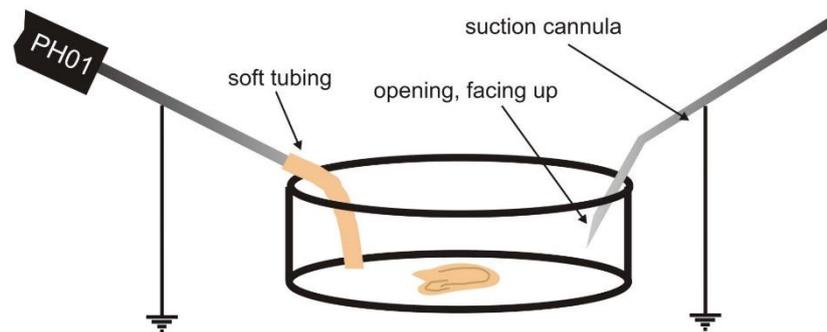
Hippocampal slice on MEA, traces with spontaneous activity in overlay.

5.1 Perfusion and Noise

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 below.



If you experience low frequency noise from the perfusion, try to optimize the suction as described above. Additionally, a **droplet isolator** can be used to interrupt the fluid flow between pump and recording chamber. The PPS2 pump from MCS is already equipped with droplet isolators. 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 maybe 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.

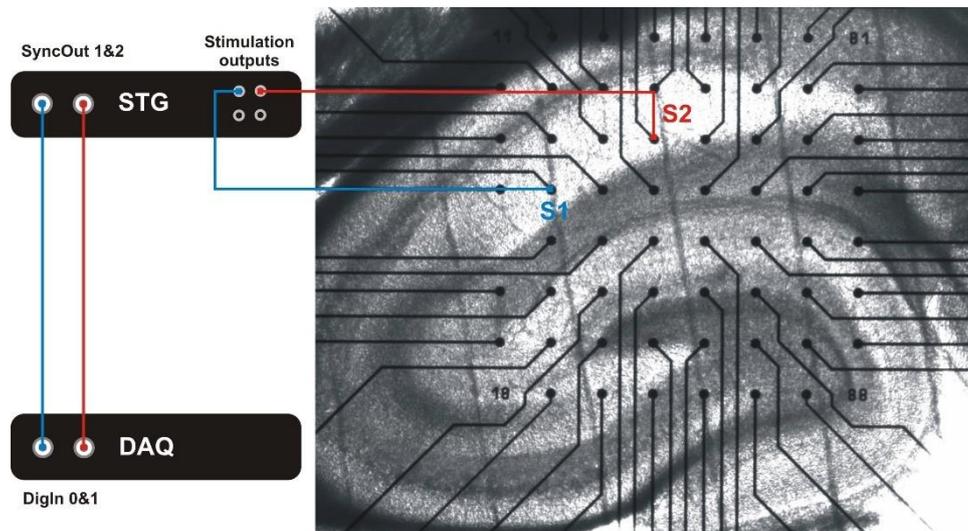
If you are unsure about grounding, use a Multimeter to check the connection between any part of the setup and the system ground. Ground connectors are on the back of the USB-ME data acquisition systems and on the back of the interface board of the MEA2100-System.

5.2 Software Setup

Recordings can be done using [MC_Rack](#) or the [Multi Channel Suite](#) with a MEA2100-System or an USB-MEA1060-System. Both options will be described below.

5.2.1 Recording with MC_Rack

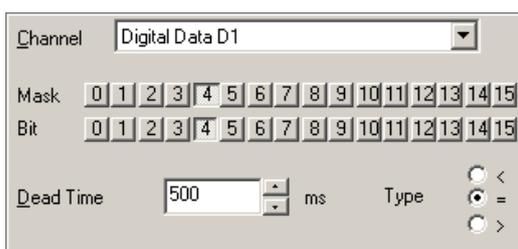
In this chapter, an exemplary setup for MC_Rack is shown to do a LTP experiment in a hippocampal slice with two independent stimulation pathways, S1 and S2. Two different stimulation electrodes are used to excite the two pathways, stimulation pulses are delivered alternately from two different channels of the stimulator. Each stimulation pathway must also be associated with a trigger output (one trigger for each stimulation pulse on S1, a different trigger for each stimulation pulse on S2).



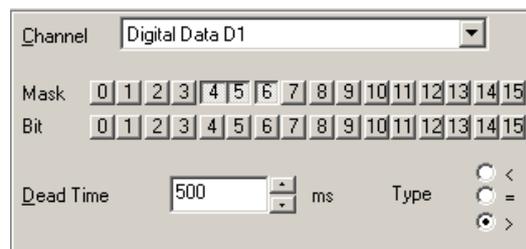
Experimental setup with stimulation pathways S1 and S2 for a **USB-ME-DAQ** and an external STG.

The rack and the stimulation paradigms are included in the download of this manual. Settings vary of course depending on the actual recording device used, but the general idea remains the same. To adjust the rack to your specific device, consult the respective manual or contact MCS support. If the internal Stimulator of an **MEA2100-System** is used, no external connections are needed. The internal Stimulators generate automatic triggers on the digital channel, bits 4, 5 and 6 for the Stimulators 1, 2 and 3, respectively.

It is possible to trigger on stimulation pulses from any of the three internal stimulus generators. The three internal STG units send trigger signals with each stimulation pulse on bits 4, 5 and 6 of the digital channel, Stimulus 1 on bit 4, Stimulus 2 on bit 5 and Stimulus 3 on bit 6. Please see chapter "Use of the Digital Channel in the MEA2100" in the MC_Rack manual for more details. So to trigger, for example on each pulse of Stimulus 1, a Trigger detector in MC_Rack must be set to the Digital Channel, bit 4. Please see screenshot below.



Trigger on Stimulator 1

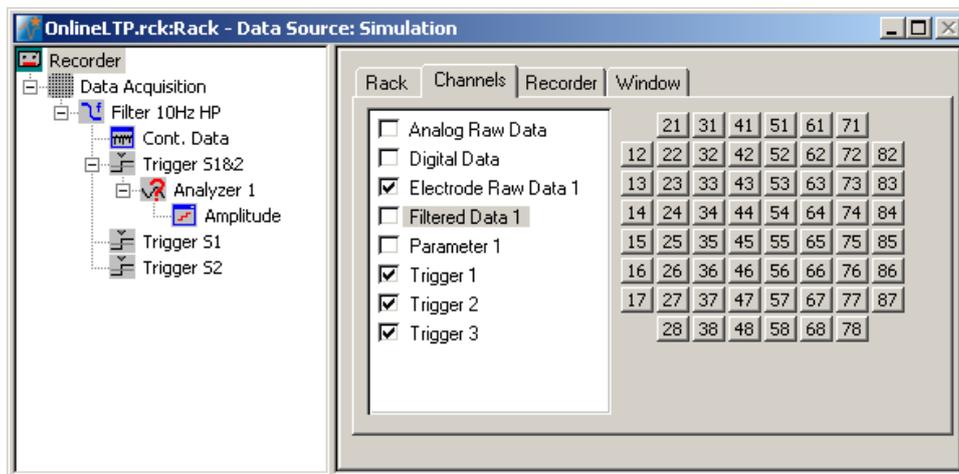


Trigger on Stimulator 1, 2 or 3

It is also possible to set the trigger detector to react on a signal from any of the three STG units. This might for example be useful to start a triggered recording if a stimulus is applied from any of the STG units. All three bits must be unmasked, and the bit value must be defined as > 0. See chapter “Triggering on TTL Pulses” of the MC_Rack manual for more information. Triggered recording and any instrument which is below the Trigger Detector in the virtual tree of MC_Rack can now work in relation to the selected STG unit(s).

Two demoracks are included, the rack **OnlineLTP.rck** will work in Demo mode on all computers. The rack **OnlineLTP_MEA2100.rck** will work only if a MEA2100 system is attached to the computer

In the recorder, select the “Raw Data” stream and all three triggers to be recorded, and “Start on Trigger” mode on trigger one. A 100 ms time window starting 10 ms before the trigger is usually sufficient to cover all stimulation evoked response. Sampling frequency in the “Data Source” should be 10 or 20 kHz, the input voltage range should cover approximately signals up to +/-4 mV.



The 10 Hz high pass filter is optional to remove low frequency fluctuations. On a MEA2100 system, set the hardware filter to 10Hz high pass instead, using the [MEA Filter Config Tool](#).

A continuous data display can be used to check the noise level and possible spontaneous activity. Three trigger detectors react on the two trigger inputs associated with the two stimulation pathways S1 and S2. Each simulation pathway is provided by one channel of the stimulator used, and should be associated with a separate trigger. In this example the two trigger inputs are supposed to be connected to the Digital In bit 0 and bit 1 of the data acquisition. Alternatively, it is also possible to use Analog In channels to accept triggers. Trigger detector 1 is set to react on trigger input one *and* two. This trigger controls the recording and the Analyzer tool. Trigger detectors 2 and 3 react only on trigger input one *or* two, respectively. These two triggers are thereby associated with signals belonging to stimulation pathways S1 or S2, and can later be used to analyze the two stimulation pathways independently. The Analyzer works on the filtered data, starting on Trigger 1. Hence, signals evoked by stimulation pathways S1 and S2 are analyzed both and the results plotted versus time in a parameter display.

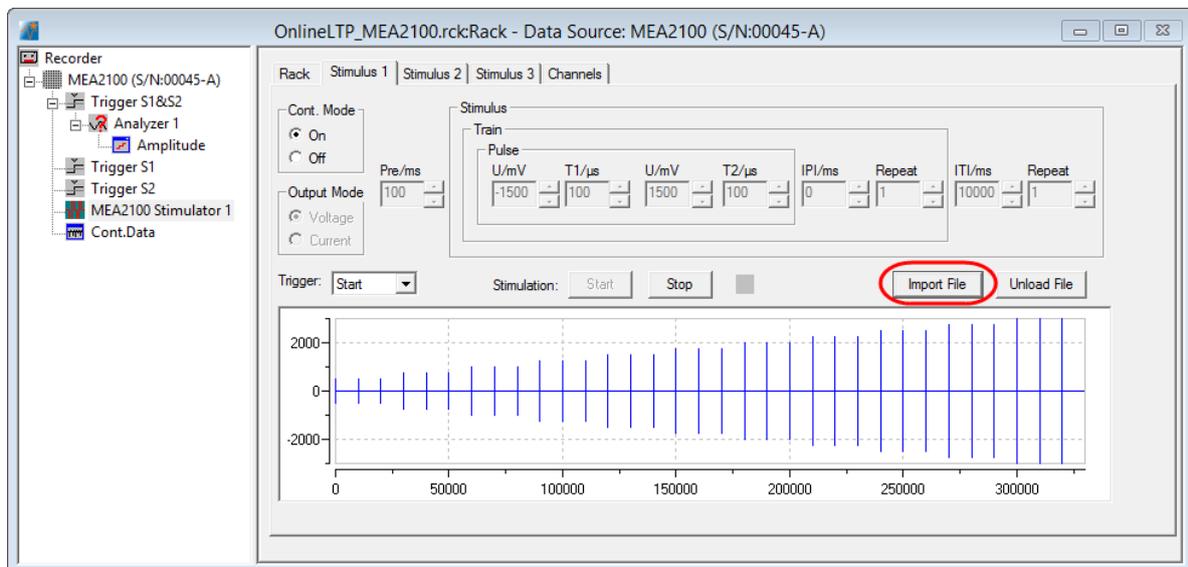
EPSPs evoked by stimulation usually have their minimum within the first 10 ms after stimulation. Consequently, the Analyzer is set to analyze a region of interest (ROI) between 1 and 10 ms after the trigger. The peak to peak amplitude is analyzed and plotted in the parameter display versus time. Even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

5.2.2 Stimulaton with MC_Rack

A number of preprogrammed stimulation files (*.stm) for **external stimulators** (STG2000 or 4000 series) are provided for this experiment. One or two channels of the stimulator are used, as well as the corresponding trigger outputs. Voltage or current pulses can be used. Voltage pulses are 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 $\pm 3\text{ V}$ or $\pm 150\ \mu\text{A}$, respectively. See safe charge injection limits in the MEA manual for more details.

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.

If a **MEA2100-System with internal stimulator** is used, you can use the ASCII export option of MC-Stimulus II (Edit/Export ASCII file) to export each stimulation pattern as ASCII data. This ASCII file can then be imported into the MEA2100 stimulator. This allows more complex stimulation patterns than the user interface of MC_Rack. All *.stm files are also included as **importable *.dat** files already. To change the stimulation pattern, just load the next ASCII file, or unload the current file to activate the manual controls again.



- **Test:**

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

- **IO-500-3000mV:**

Pulses with increasing amplitude on both channels 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.

- **Baseline-postLTP:**

Alternating pulses on channel one and two every 30 s. This paradigm can be used for the baseline and for recording after LTP induction. The amplitude should be adjusted for each experiment according to the results of the IO curve. The paradigm will run until manually stopped.

- **LTP-Induction-1x100Hz:**

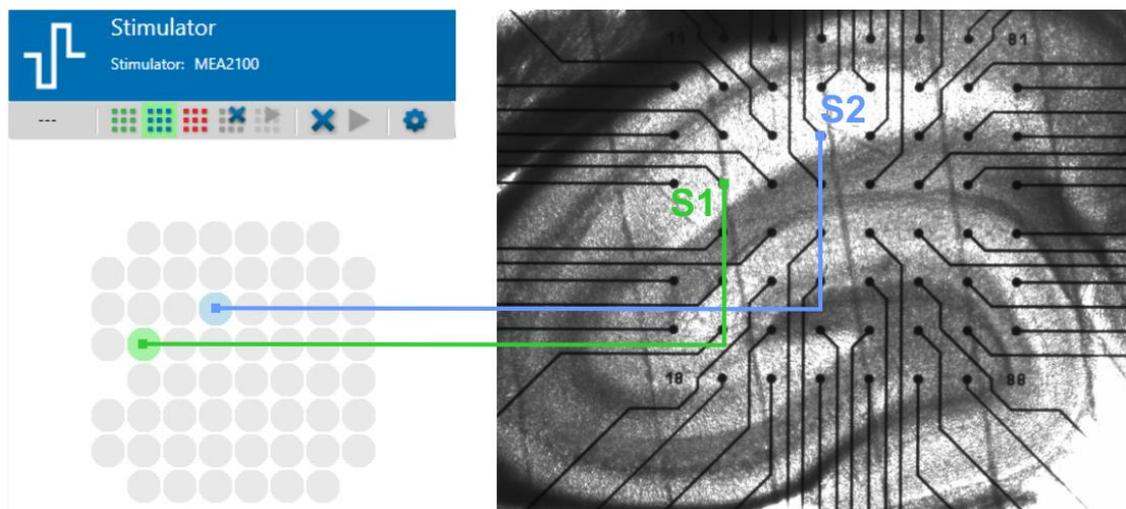
One hundred pulses at 100 Hz for one second on the first channel. Amplitude should be adjusted for each experiment to match the amplitude of the baseline stimulation. The paradigm will run once and then stop automatically. This paradigm will usually induce short term, decaying LTP.

- **LTP-Induction-4x100Hz:**

One hundred pulses at 100 Hz for one second on the first channel with four repeats at a 10 min interval. In each 10 min break, there is one test pulse at channel one and two. Amplitude should be adjusted for each experiment to match the amplitude of the baseline stimulation. The paradigm will run once and then stop automatically. This paradigm will usually induce long term, lasting LTP.

5.2.3 Recording with Multi Channel Experimenter

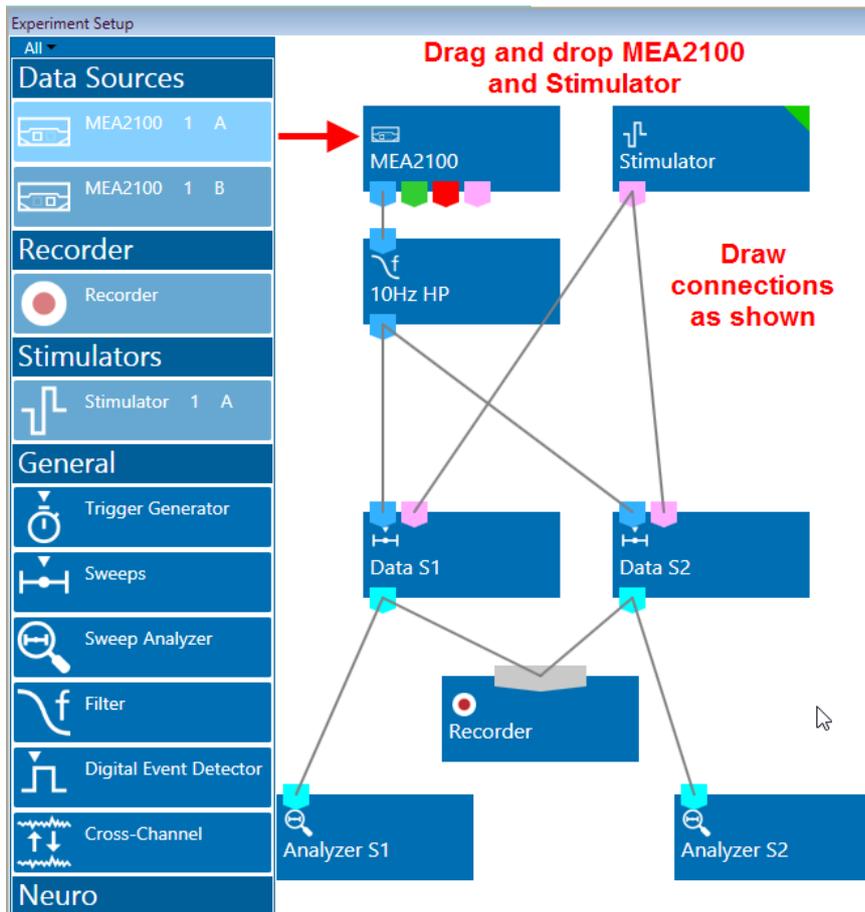
In this chapter, an exemplary setup for the Multi Channel Experimenter and a MEA2100-System is shown to do a LTP experiment in a hippocampal slice with two independent stimulation pathways, S1 and S2. Two different stimulation electrodes are used to excite the two pathways, stimulation pulses are delivered alternately from two different channels of the internal stimulator of the MEA2100-System. Each stimulation pathway must also be associated with a trigger (one trigger for each stimulation pulse on S1, a different trigger for each stimulation pulse on S2).



The experiment configuration (*.mse) and the stimulation paradigms (*.xml) are included in the download archive of this manual. For the internal Stimulator of the **MEA2100-System**, no external connections are needed. The stimulation electrodes can be selected via software, and the Stimulators generate automatic triggers which can be used to record evoked responses with the Sweeps tool.

The ready to go **OnlineLTP.mse** experiment file will open on all computers, but will only work properly if a MEA2100-System is attached to the computer. The experiment file comes **without data source** (MEA2100), to avoid hardware conflicts. Please drag and connect your data source and Stimulator as seen in the image below. Set the sampling rate to 20 kHz, lower sampling rates will result in problems with the markers in the predefined stimulation paradigms.

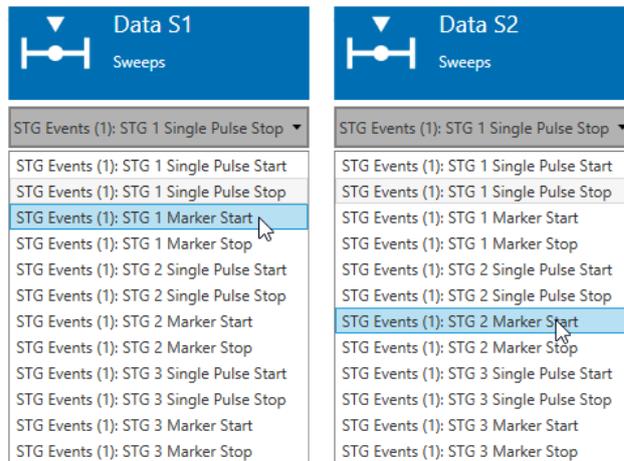




The continuous data display in the MEA2100 tab can be used to check the noise level and possible spontaneous activity. The raw data from the data acquisition (blue port) goes to a **filter**. The 10 Hz high pass filter is optional to remove low frequency fluctuations. You can alternatively set the hardware filter to 10 Hz high pass instead, using the [MEA Filter Config Tool](#). The filtered raw data goes to two **Sweeps tools**, one for pathway S1, and one for pathway S2. The sweeps generated by both instruments are recorded, and simultaneously analyzed by two Sweep Analyzers, respectively.

The two Sweeps tools are connected to the trigger port of the **Stimulator**, and set to react on the Stimulator units 1 and 2, which are stimulating pathways S1 and S2, respectively. These triggers initiate one sweep with 10 ms before and 90 ms after each stimulation.

After connecting the Sweeps instruments to the Stimulator, make sure to set the correct trigger condition in each Sweep instrument. Data S1 should be set to STG 1 Marker Start, and Data S2 to STG 2 Marker start respectively.



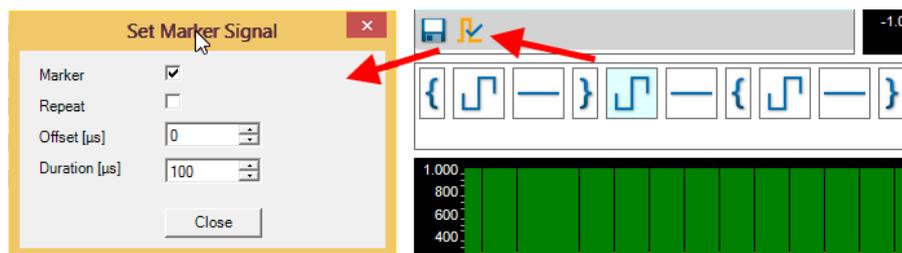
EPSPs evoked by stimulation usually have their minimum within the first 10 ms after stimulation. Consequently, the Analyzer is set to analyze a region of interest (ROI) between 1 and 10 ms after the trigger. The ROI for analysis can and should be adjusted **for each experiment**. The peak to peak amplitude is plotted in the Sweep Analyzer display versus time. Even if it may be not the best choice for final data analysis, the peak to peak amplitude is a good parameter to monitor signal stability over time and also the effect of LTP induction online during the experiment. Each Sweep Analyzer monitors the results from one stimulation pathway.

5.2.4 Stimulation with Multi Channel Experimenter

A number of preprogrammed stimulation files (*.xml) for **internal stimulators** of the MEA2100-System are provided for this experiment. One or two channels of the stimulator are used, as well as the corresponding trigger outputs. Voltage or current pulses can be used. Voltage pulses are 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 $\pm 3\text{ V}$ or $\pm 150\ \mu\text{A}$, respectively. See safe charge injection limits in the MEA manual for more details.

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.

Each stimulation file uses Markers (see Multi Channel Experimenter manual for details). These markers trigger the Sweeps instruments, as described above. One marker is generated for each test stimulation, and one marker at the beginning of each high frequency train, so there will be only one sweep at the beginning of each HFS train.



- **Test:**

A simple biphasic pulse with ± 2 V on one channel, starting with the DAQ start and repeated every 10 s until manually stopped. This pulse can be used to check the viability of the 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 on the best electrodes should be expected for a good quality slice.

- **IO-500-3000mV:**

Pulses with increasing amplitude on both channels 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.

- **Baseline-postLTP:**

Alternating pulses on channel one and two every 30 s. This paradigm can be used for the baseline and for recording after LTP induction. The amplitude should be adjusted for each experiment according to the results of the IO curve. The paradigm will run until manually stopped.

- **LTPcomplete_1x100Hz_S1 and _S2:**

A complete LTP experiment for two stimulation pathways, S1 and S2. Load LTPcomplete_1x100Hz_S1.xml to Stimulator one, and LTPcomplete_1x100Hz_S2 to stimulator 2. A test pulse will be applied every minute to each pathway, there is an offset of 30 s between the two pathways. A high frequency stimulation of one hundred pulses at 100 Hz for one second will be applied on **S1 only** after a baseline recording of 30 min. After the LTP induction, the experiment continues with the test pulse for four hours. Amplitude should be adjusted for each experiment according to the results of the I/O curve (see chapter 0). The paradigm will start automatically with DAQ start and stop automatically. This paradigm will usually induce short term, decaying LTP.

- **LTPcomplete_4x100Hz_S1 and _S2:**

A complete LTP experiment for two stimulation pathways, S1 and S2. Load LTPcomplete_4x100Hz_S1.xml to Stimulator one, and LTPcomplete_4x100Hz_S2 to stimulator 2. A test pulse will be applied every minute to each pathway, there is an offset of 30 s between the two pathways. A high frequency stimulation of one hundred pulses at 100 Hz for one second will be applied on **S1 only** after a baseline recording of 30 min. This high frequency pulse is repeated four times, with an interval of 10min in between. After the LTP induction, the experiment continues with the test pulse for four hours. Amplitude should be adjusted for each experiment according to the results of the I/O curve (see chapter 0). The paradigm will start automatically with DAQ start and stop automatically. This paradigm will usually induce long term, lasting LTP.

5.3 Experimental Design MC_Rack

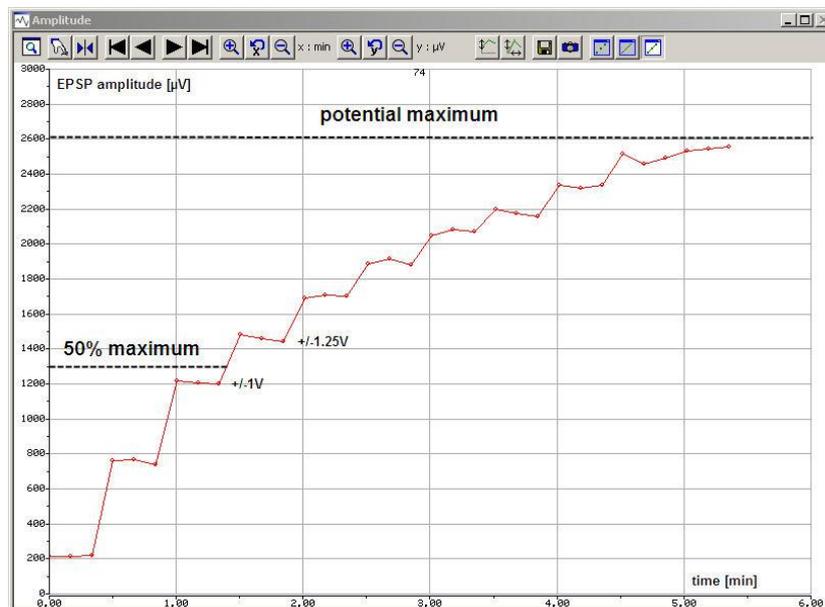
This chapter describes a typical LTP experiment without application of any compound. Data recording can be started approximately 20 min after mounting the slice on the MEA. This time can be used to check the viability of the slice and solve any noise issues.

After a picture of the slice was taken, check the spontaneous activity of the slice. Sporadic spiking activity is a sign of good health and a good tissue to electrode contact. Frequent, synchronized spontaneous bursting might be a sign of ongoing excitotoxic cell death.

Download the “**Test**” stimulation file to the STG and try a few stimulation electrodes in the *stratum radiatum*. Select one where stimulation with ± 2 V results in EPSPs on a few electrodes, and possibly also a population spike along the CA1 cell layer. Then look for a second stimulation electrode which will yield a response on the same electrodes. This is most easily achieved with retrograde stimulation, so picking a second stimulation electrode on the opposite side of the intended recording electrodes. Now connect the two selected stimulation electrodes to the stimulator outputs 1 and 2 (see chapter 5.2.1).

Download the “**IO-500-3000mV**” stimulation file. The stimulation channels one and two can be started individually (see MC_Stimulus manual for details). Run the IO curve first for pathway S1 (stimulation channel one) and then for pathway S2 (stimulation channel two).

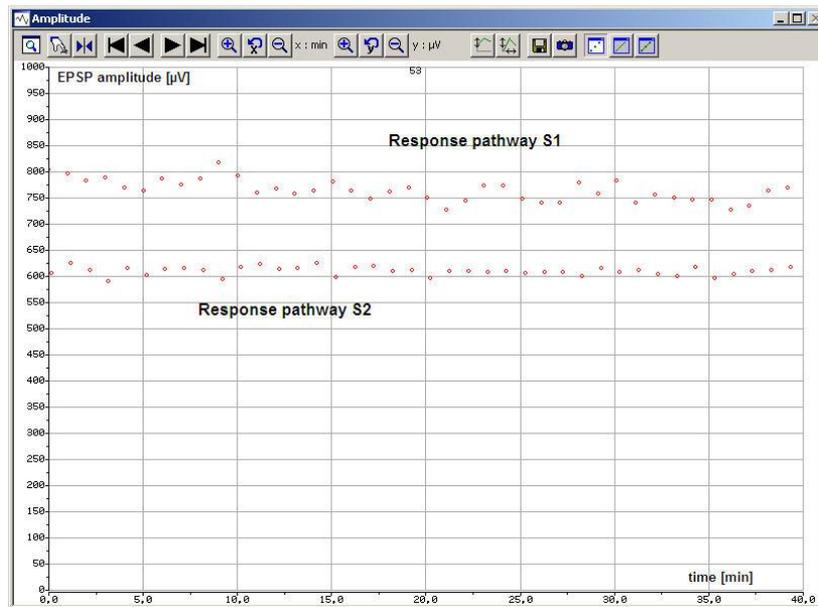
The response amplitude plotted versus time should in the ideal case result in a stepped curve approaching a maximum. There are three data points for each stimulation strength. Approximate the maximum response amplitude and take 50 % of that value. Use the stimulation strength resulting in 50 % of the maximum as standard for all other stimulation paradigms. In the example below, the 50 % stimulation strength would be about ± 1.1 V.



IO curve with stimulation from 0.5 to 3V in 0.25 V steps, three repeats each.

Optionally, you can now download the “**Paired-Pulse**” stimulation file to do some paired pulse experiments. This is not necessary to run a LTP experiments, though.

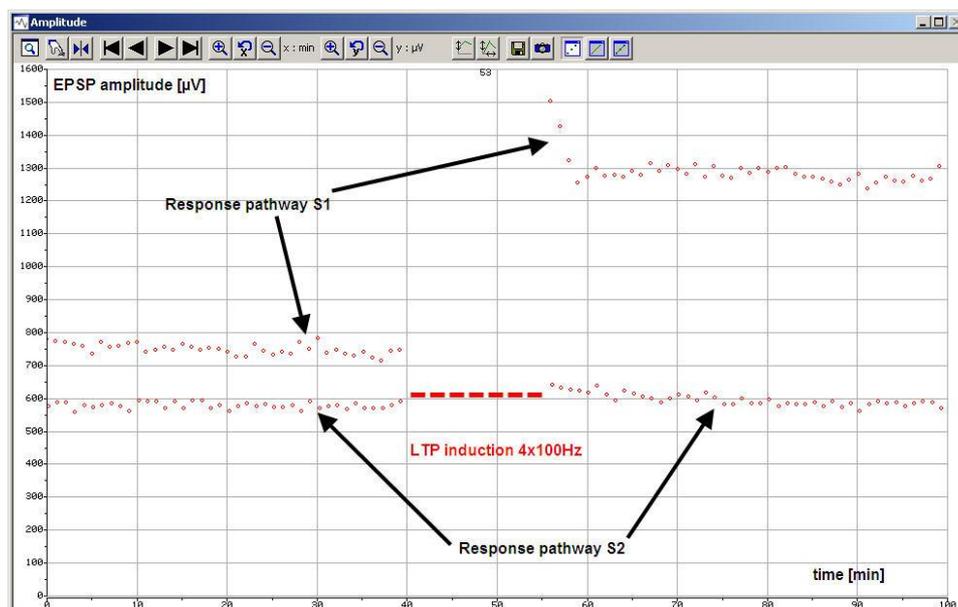
Download the “**Baseline-postLTP**” stimulation file and start the actual experiment. Make sure that the settings of the recorder and the file name are correct. Start the stimulation and monitor the signal stability over time. You should record at least 30 min of stable baseline before starting the induction of LTP.



40 min of baseline recording, one pulse per minute for each pathway.

After the baseline recording is finished, download the **"LTP induction"** paradigm. Depending on whether you want to induce lasting or decaying LTP, select either "LTP-Induction-4x100Hz" or "LTP-Induction-1x100Hz". You can either continue the data acquisition or record baseline, LTP induction or post LTP recording in three separate files. The latter is recommended, as it makes data analysis easier. If necessary, the files can be merged later with MC_DataTool.

After the LTP induction paradigm is finished, switch back to **"Baseline-postLTP"**, and continue the stimulation for as long as required. The signal amplitude on pathway S2, which did not receive a LTP inducing stimulus, should remain constant over the whole recording period. If it changes significantly, the experiment must be discarded. Optionally, a second set of IO curves can be recorded at the end of the experiment.



100 min of recording, with baseline LTP induction and post LTP. S1 gets potentiated, while S2 is stable and unaffected by the LTP induction.

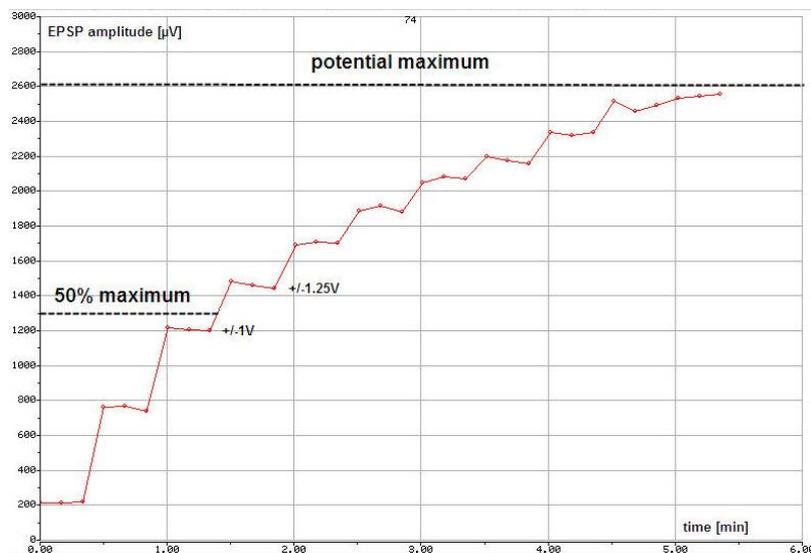
5.4 Experimental Design Multi Channel Experimenter

This chapter describes a typical LTP experiment without application of any compound. Data recording can be started approximately 20 min after mounting the slice on the MEA. This time can be used to check the viability of the slice and solve any noise issues.

After a picture of the slice was taken, check for spontaneous spiking activity. Sporadic spiking activity is a sign of good health and a good tissue to electrode contact. Frequent, synchronized spontaneous bursting might be a sign of ongoing excitotoxic cell death.

Open the “**Test**” stimulation file in Stimulator 1 and try a few stimulation electrodes in the *stratum radiatum*. Select an electrode where stimulation with ± 2 V results in solid EPSPs on a few electrodes, and possibly also a population spike along the CA1 cell layer. Then look for a second stimulation electrode which will yields a response on the same electrodes. This is most easily achieved with retrograde stimulation, so by picking a second stimulation electrode on the opposite side of the intended recording electrodes. Now selected these two stimulation electrodes as stimulation electrodes in Stimulator 1 and Stimulator 2, respectively (see chapter 5.2.3).

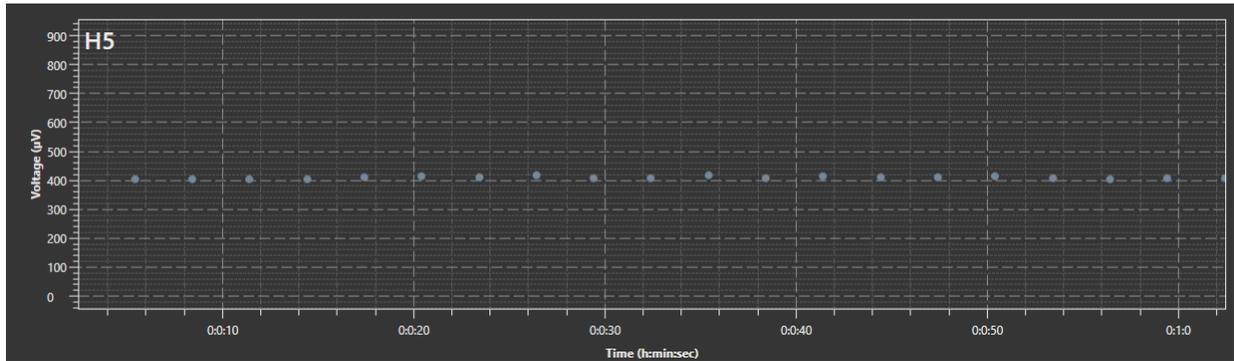
Open the “**IO-500-3000mV**” stimulation file in Stimulator 1, and run the IO curve first for pathway S1 (stimulation electrode one). **Clear stimulus on Stimulator 1** and open the “**IO-500-3000mV**” stimulation file in Stimulator 2, and run the IO curve also for pathway S2 (stimulation electrode two). The response amplitude plotted versus time should in the ideal case result in a stepped curve approaching a maximum. There are three data points for each stimulation strength. Approximate the maximum response amplitude and take 50 % of that value. Use the stimulation strength resulting in 50 % of the maximum as standard for all other stimulation paradigms. In the example below, the 50 % stimulation strength would be about ± 1.1 V (data shown from MC_Rack).



IO curve with stimulation from 0.5 to 3V in 0.25 V steps, three repeats each.

Optionally, you can now download the “**Paired-Pulse**” stimulation file to do some paired pulse experiments. This is not necessary to run a LTP experiments, though.

Open the “**LTPcomplete_1x100Hz_S1**” stimulation file to Stimulator 1 and the “**LTPcomplete_1x100Hz_S2**” to Stimulator 2 to start the actual experiment. If you want to induce lasting LTP, open “**LTPcomplete_4x100Hz_S1**” and “**LTPcomplete_4x100Hz_S2**” instead. Feel free to edit the duration of pre and post LTP induction times. By default, the experiments runs 30 min before and 240 min after LTP induction. Make sure that the file name in the Recorder tool is correct. Start Data Acquisition and Recording, both Stimulators will start automatically. Monitor the signal stability over time. You should record at least 30 min of stable baseline before starting the induction of LTP. Data of stimulation pathway S1 and S2 will show up in the respective Sweep Analyzer windows.



Baseline recording

After the baseline recording is finished, the LTP inducing high frequency stimulation will be applied to S1 only, while S2 is paused. For the 1x100 Hz paradigm, this will last only a second, while for the 4x100 Hz paradigm the high frequency stimulation is repeated four times, with 10 min in between. After the LTP induction, the test pulse identical to the baseline stimulation will continue on S1 and S2 for four hours. In the ideal case, the evoked signals on S2 stay unchanged for the whole experiment. Decreasing signals on S2 indicate a dying slice, or a decreasing tissue to electrode contact. If the signal on S2 changes too much during the course of the experiment, the experiment should be discarded. The evoked signals on S1 should increase after LTP induction. If 1x100 Hz was applied, the increase is temporal, if 4x100 Hz is applied, it should be stable over time.

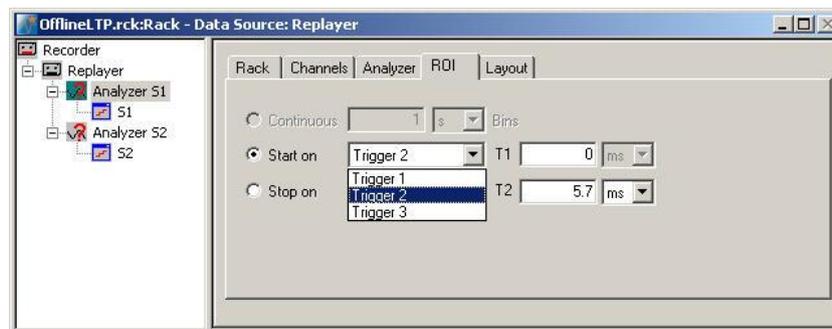
6 Data Analysis

After finishing the experiment, a set of useful information can be extracted from the data. The high number of electrodes usually allows extracting more than one parameter from one experiment. Parameters and the basic principle of extracting them is identical for MC_Rack and the Multi Channel Analyzer, but differences in the procedure apply.

6.1 Analysis with MC_Rack

The **three different triggers** allow the separate analysis of pathway S1 and S2. Traditionally, the slope of the EPSP in the *stratum radiatum* is used as a parameter to assess LTP. However, data from MEA recordings allows extracting 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 (OfflineLTP.rck) with a corresponding data file (Sample.mcd) is provided. The data file contains ten sweeps for each pathway, stimulation electrodes are #44 for S1 and #74 for S2.

Generally, to analyze only signals evoked by stimulation via S1 or S2, it is necessary to start the Analyzer tool on the corresponding trigger, in our example Trigger 2 for S1 and Trigger 3 for S2 (please see the image below).



In the sample rack, two analyzers are used to separately analyze signals from S1 and S2. The exact settings of the analyzer have to be adjusted depending on which parameters should be analyzed. The following chapters describe the analysis of different signal types and parameters.

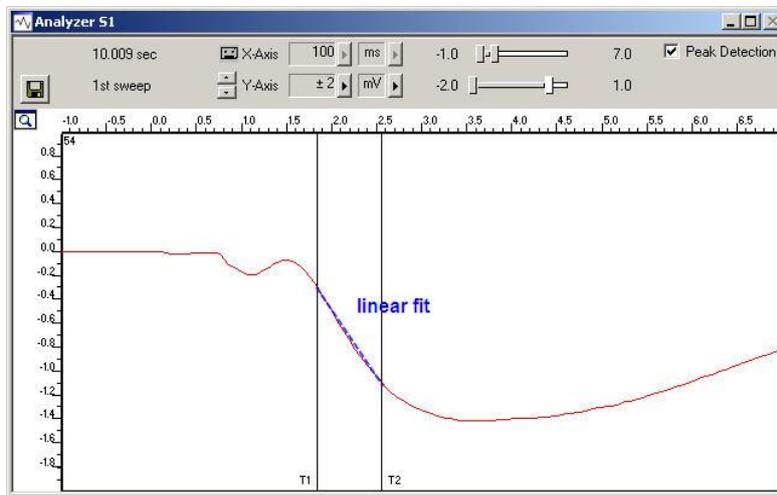
6.1.1 EPSP Slope

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 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 in on the signal. Electrodes where both stimulation pathways elicit EPSPs with roughly the same size are optimal, analyze the same electrode for both pathways. 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. The ASCII file will open with Excel, Origin or similar software.

Of course, more than one electrode can be analyzed in this manner, for example to investigate differences between different regions of the hippocampus. Signals with slopes less than 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 amplitudes.

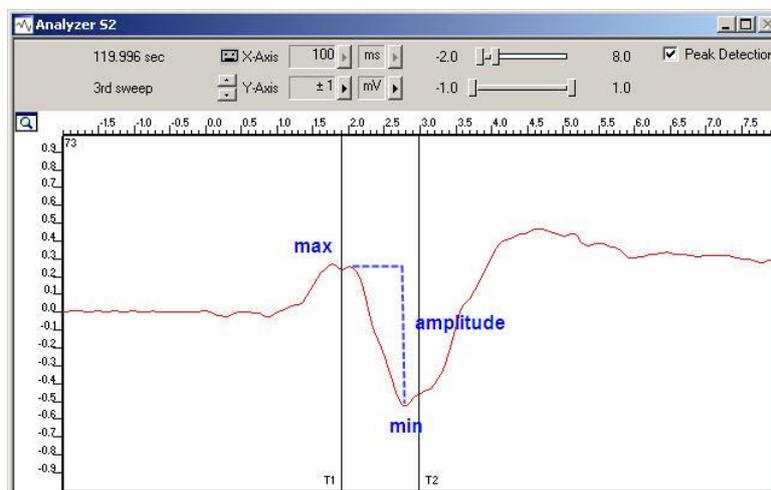


6.1.2 Population Spike Amplitude

Population spikes, often simply called **popspikes**, are often present along the CA1 cell layer, but sometimes emerge only after LTP induction, or not at all. If a popspike is present even before LTP induction, it is possible to analyze the popspike amplitude as additional parameter.

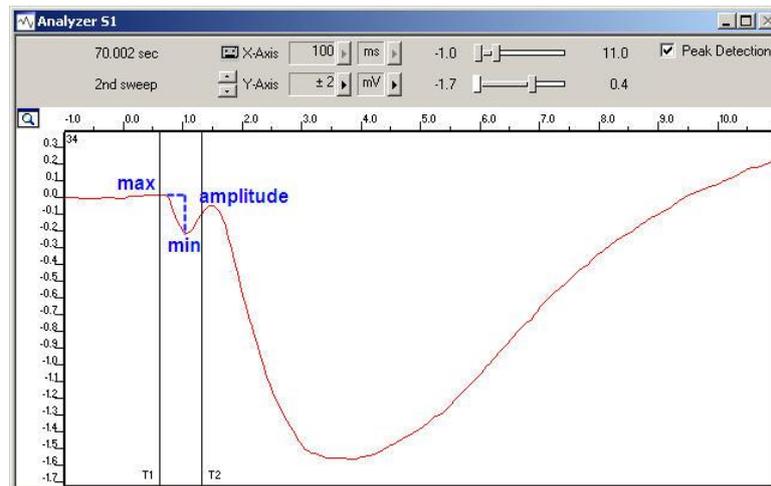
In the analyzer tool, select “Peak to peak amplitude” as parameter. Pick one electrode to analyze and zoom in on the signal. Electrodes where both stimulation pathways elicit pop spikes with roughly the same size are optimal, analyze the same electrode for both pathways. Adjust the ROI to include the local maximum before the pop spike 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. The ASCII file 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.



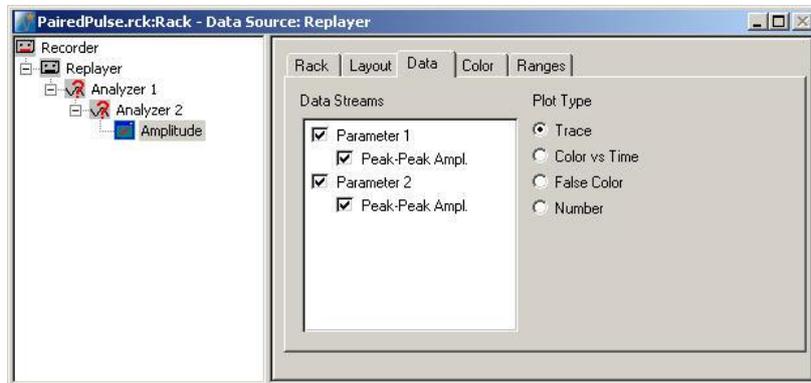
6.1.3 Fiber Volleys

Fiber volleys are **presynaptic signals** picked up directly from the axons. They are sometimes visible as relatively small negative peak before an EPSP. They can be used to measure the stability of the presynaptic input. The analysis works the same way as for pop spike amplitude select "Peak to peak amplitude" as parameter and pick an electrode with a fiber volley. Adjust the ROI only to include the local minimum and maximum of the signal. In the case of fiber volleys, it is not necessary that both stimulation pathways elicit a signal on the same electrode, because the volley serves as a control for the synaptic input for one individual stimulation pathway.



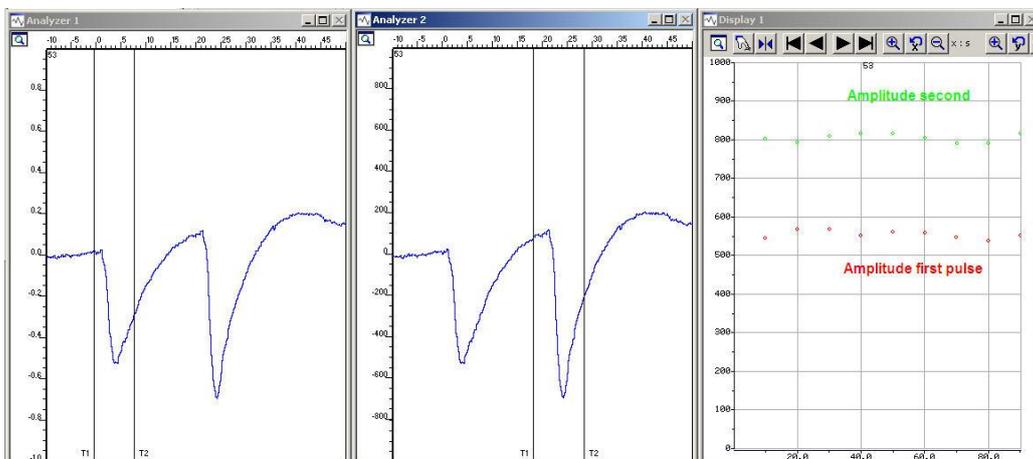
6.1.4 Paired Pulse Facilitation or Inhibition

Paired pulse facilitation is a form of short term synaptic plasticity which can also be analyzed before and possibly also after a LTP experiment. MEA recordings easily allow investigating the region specificity of facilitation and depression. For paired pulse facilitation and inhibition, please use the sample rack PairedPulse.rck and the data file PPFdata.mcd.



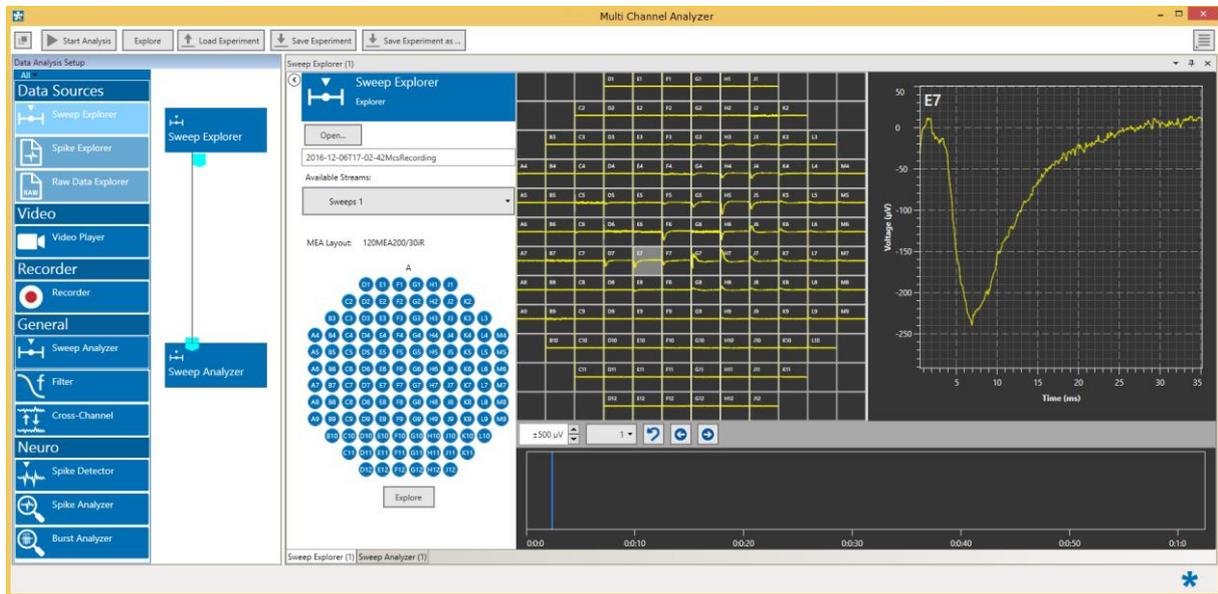
Two analyzers are connected in series, with one parameter display behind which can display the result of both. In both analyzer tools, select "Peak to peak amplitude" as parameter. Adjust the ROI in both analyzers to include the local maximum and minimum of the first and second EPSP, respectively. Both amplitudes will be plotted in the parameter display.

After the ASCII export, the ratio between the two can be calculated in suitable software.

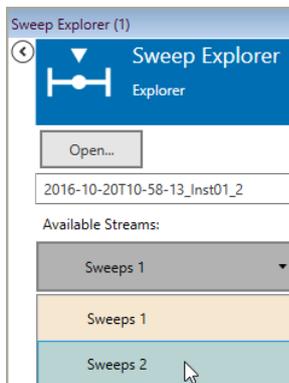


6.2 Analysis with Multi Channel Analyzer

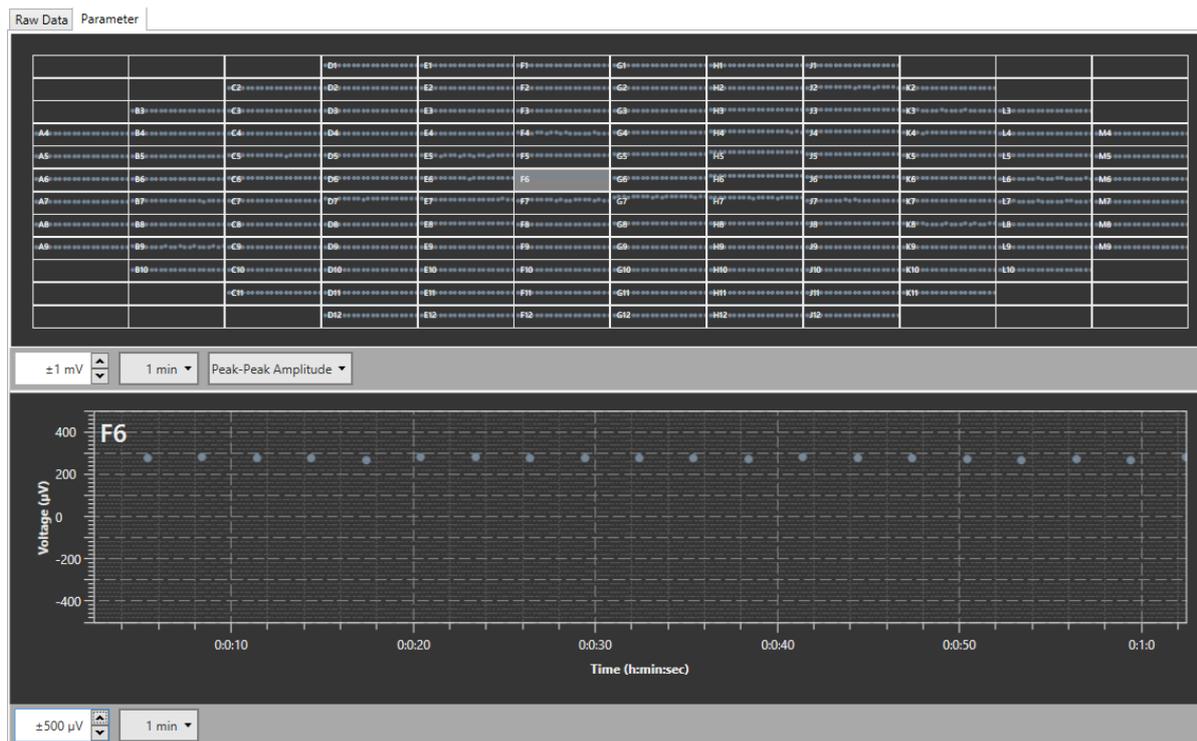
Traditionally, the slope of the EPSP in the *stratum radiatum* is used as a parameter to assess LTP. However, data from MEA recordings allows extracting additional information. The results can be exported directly as ASCII to be used in Excel, Origin or other programs. A **sample analysis rack** (OfflineLTP.msa) with a corresponding data file (Sample.msrd) is provided. The sample file contains only a single data stream.



Open a file with sweeps in the **Sweep Explorer** and connect it to a **Sweep Analyzer** instrument. Navigate through the data and select a sweep. Send the sweep to the Sweep Analyzer with the **Explore** function. Data from pathways S1 and S2 can be analyzed sequentially in the same Sweep Analyzer instrument. When analyzing a file with two Sweeps data streams, corresponding to stimulation pathways S1 and S2, you can simply select the stream to analyze from a drop down menu.



In the Raw Data tab of the Sweep Analyzer, adjust the **region of interest** (ROI) according to the signal you want to analyze. Press **Start Analysis** to extract all parameters from the complete file. Results can be exported as ASCII data.



The exact settings of the analyzer have to be adjusted for each data stream, and depending on which parameters should be analyzed. The following chapters describe the analysis of different signal types and parameters.

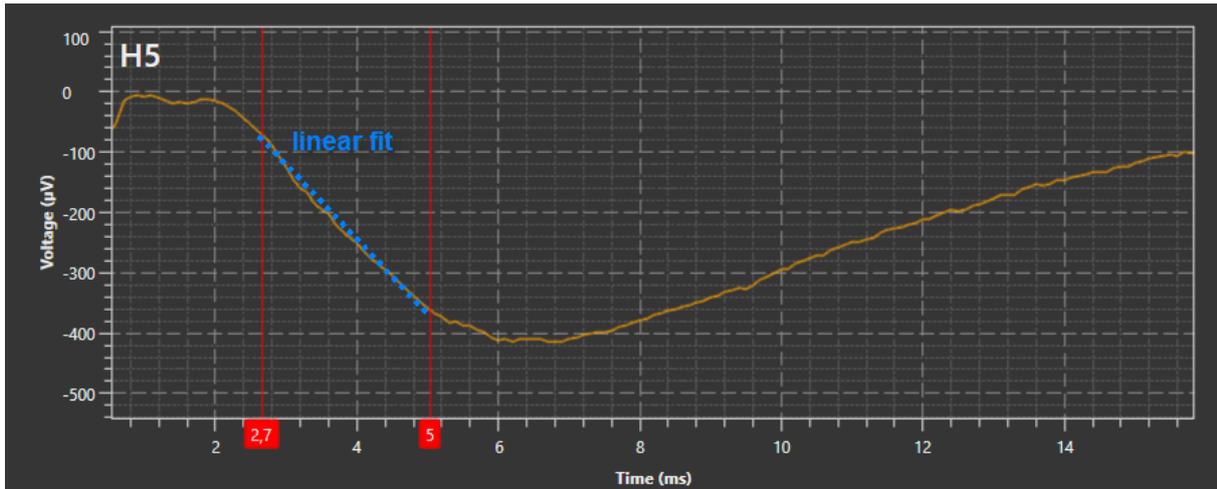
6.2.1 EPSP Slope

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. The Multi Channel Analyzer offers several options to analyze the slope of a signal.

Inside the region of interest (ROI) defined in the **Sweep Analyzer Raw Data tab**, 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. The 20/80 slope for example is 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 Sweep Analyzer, select "Slope" as parameter. Pick one electrode to analyze and zoom in on the signal. Electrodes where both stimulation pathways elicit EPSPs with roughly the same size are optimal, analyze the same electrode for both pathways. In the Raw Data tab, adjust the ROI to include only the linear part of the signal. Analyze the complete experiment with the same ROI and see the results in the Parameter tab. The results can be exported as ASCII data, which will open with Excel, Origin or similar software.

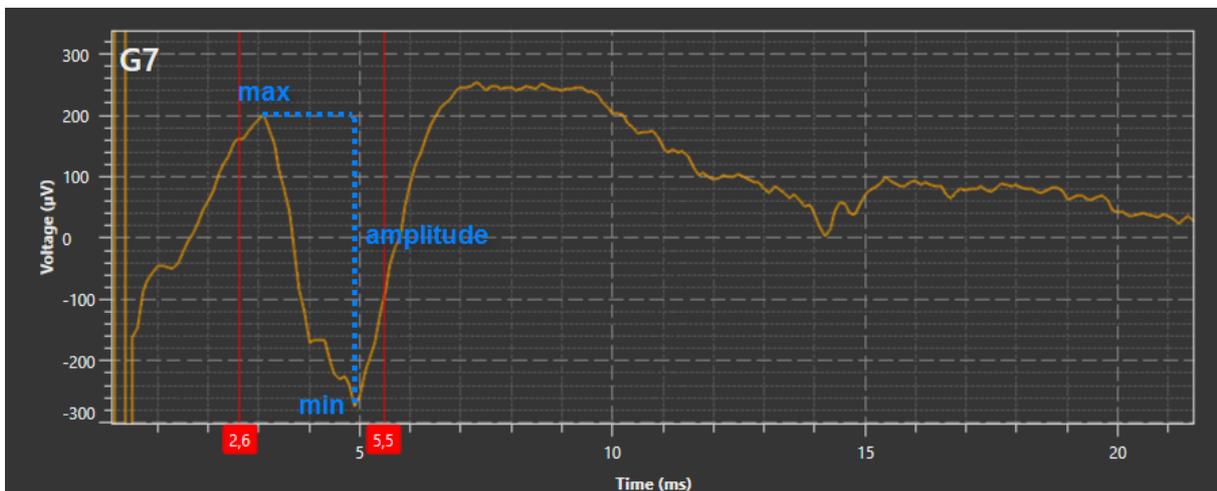
Of course, more than one electrode can be analyzed in this manner, for example to investigate differences between different regions of the hippocampus. Signals with slopes less than about -0.2 V/s should not be used, as very small absolute changes result in rather large relative changes (for example 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 amplitudes.



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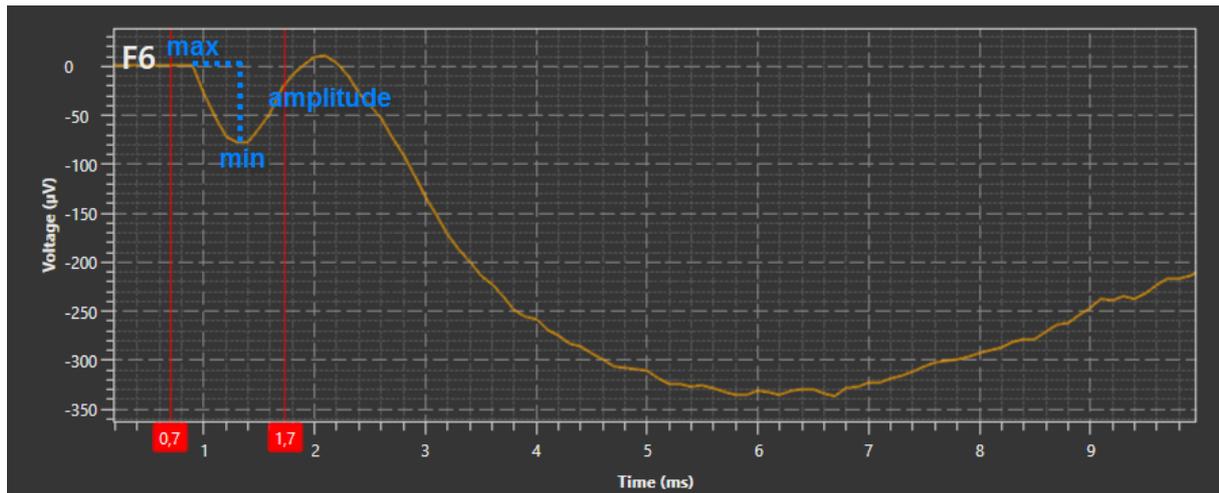
In the Sweep Analyzer, select “Peak to peak amplitude” as parameter. Pick one electrode to analyze and zoom in on the signal. Electrodes where both stimulation pathways elicit pop spikes with roughly the same size are optimal, analyze the same electrode for both pathways. In the Raw Data tab, adjust the ROI to include the local maximum before the pop spike and the minimum of the signal. Analyze the complete experiment with the same ROI, and see the results in the Parameter tab. Results can be exported as ASCII data, 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.



6.2.3 Fiber Volleys

Fiber volleys are presynaptic signals picked up directly from the axons. They are sometimes visible as relatively **small negative peak** before an EPSP. They can be used to measure the stability of the presynaptic input. The analysis works the same way as for pop spike amplitude select "Peak to peak amplitude" as parameter and pick an electrode with a fiber volley.

Adjust the ROI only to include the local minimum and maximum of the signal. In the case of fiber volleys, it is not necessary that both stimulation pathways elicit a signal on the same electrode, because the volley serves as a control for the synaptic input for one individual stimulation pathway.



7 Preparation Checklist

This checklist can be printed out and used during the experiment.

- Prepare solutions from stock; freeze ~300 ml ACSF for ice slush.
- Aerate the buffer with carbogen gas for 15 min. Control pH and osmolarity.

Animal preparation

- Instruments: Guillotine or large scissors, small scissors, larger forceps, and spatula.
- Beaker with ice cold ACSF or Sucrose Cutting on ice.
- Chamber with anesthetic and disposal bag.

Brain preparation

- Razor blade (cleaned with Acetone), curved forceps, sharp forceps, scalpel, spoon.
- Super glue and a pipette tip to spread it.
- Petri dish with filter paper in the bottom.
- Petri dish on ice with cold ACSF or Sucrose Cutting Solution.
- Binocular.
- Optional: Agar block.
- Transfer Pipette.
- Mounting platform of the Vibratome.

Vibratome and storage chamber

- Fill outer vibratome chamber with salted ice.
- Fill inner vibratome chamber with crushed ice from ACSF or Sucrose Cutting Solution. Keep a free space in the middle and fill it with cold but liquid solution.
- Add carbogen tube to the vibratome chamber.
- Clean the razor blade with Acetone and fix it in the vibratome.
- Set up the slice storage chamber with ACSF constantly oxygenated with Carbogen next to the vibratome in a water bath at 32 °C.

Cleanup

- After preparation, clean all instruments thoroughly with distilled water, especially if Sucrose Cutting Solution was used. Don't reuse blades for the vibratome, blades for trimming can be reused.
- After the recording, wash all tubing with distilled water and 70 % EtOH and dry them as good as possible. Empty waste bottle of suction pump.
- Remove tissue from the MEA with a water jet and store the MEA in distilled water until next use. Use cleaning with detergent and hydrophilization immediately before the next experiment.

8 Suggested System Configurations

8.1 MEA2100-(2x)60-System-E or MEA2100-120-System-E

The MEA2100-System is the most advanced system MCS can offer today. It is as flexible as the USB-MEA60-System 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 data acquisition 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. Approximately twenty electrode layouts with several additional options are available at the moment. The MEA2100-System 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, perfusion pump PPS2.
Recommended accessories: Slice grids, 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. Multi Boot IFB also usable for other headstages.
Con: Electrodes not accessible for external stimulation devices, no direct access to analog raw data.

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

The USB-MEA60-System is a very flexible system and 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. Approximately 20 electrode layouts with several additional options are available at the moment. 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.

Included accessories: Temperature controller, perfusion heating, floor heating, magnetic perfusion holders, perfusion pump PPS2.
Recommended accessories: Slice grids, 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.

8.3 Suggested Microelectrode Arrays

8.3.1 For MEA2100-(2x)60-System or MEA2100-120-System

For the MEA2100, the same MEAs can be used as for the USB-MEA60-System, 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.

8.3.2 For USB-MEA60-System

MEAs with 30 µm electrodes are most suitable, because stronger stimulation pulses can be applied. 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. 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. They also improve the recording of spontaneous activity.

8.3.3 Ring Options

The standard glass ring is best for running a perfusion and will also allow using the ALA MEA-Insert. A plastic ring should only be used if parallel patch clamp experiments are planned

9 Contact Information

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