

MC_Rack Tutorial: Input / Output I/O Relationship



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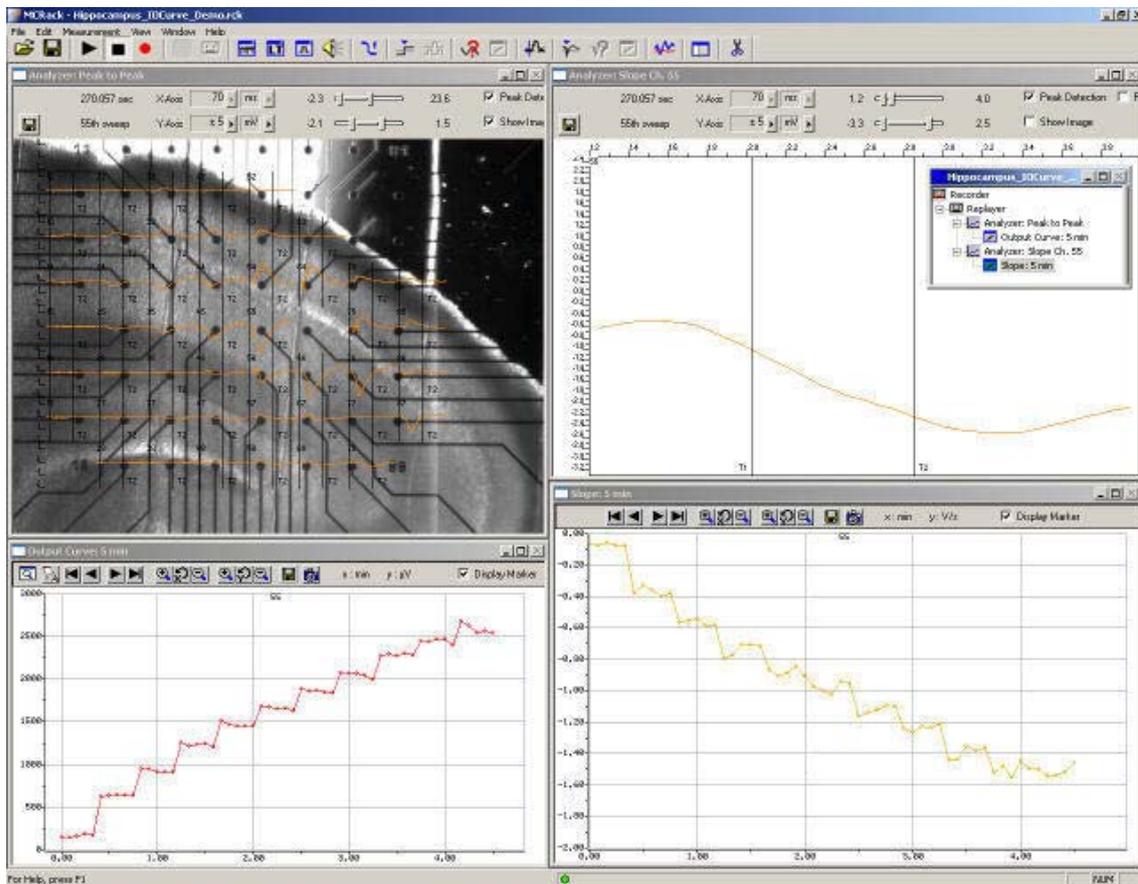
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Table of Contents

| | | |
|----------|---|-----------|
| 1 | Input / Output (I/O) Curve | 5 |
| 1.1 | Aim of Experiment | 5 |
| 1.2 | Preparations | 6 |
| 1.3 | Extracting the Peak to Peak Amplitude | 7 |
| 1.4 | Aligning the Electrode Pattern to the Slice | 7 |
| 1.5 | Deselecting Unused Electrodes | 9 |
| 1.6 | Defining the Region of Interest | 10 |
| 1.7 | Plotting the Intensity of the Biological Response | 11 |
| 1.8 | Extracting the Slope | 12 |
| 1.9 | Customizing Displays | 13 |
| 1.10 | Plotting a Slope Curve | 14 |
| 2 | Saving the Rack and Saving Data | 15 |

1 Input / Output (I/O) Curve

1.1 Aim of Experiment



This example file was recorded from an **acute hippocampal slice** on a microelectrode array (MEA) with 64 channels with the aim to analyze the **input / output (I/O) relationship** of the biological response to the electrical stimulation. The **magnitude** of the **synaptic response amplitude** is relevant in this experiment. It can be assessed based on the **minimum** or **peak-to-peak amplitude** of the field excitatory postsynaptic potential (fEPSP) or on the **slope** of the initial response decay. The latter has the advantage that it is a more linear measure of the EPSP strength when population spikes mask the fEPSP. It is, however, more sensitive to noise and more difficult to extract with propagating waves of activity.

The biological sample was 300 μm thick and stemmed from a 22 days old rat. The tissue was stimulated with one of the substrate integrated electrodes (# 65). A monopolar electrical stimulation with biphasic voltage pulses (duration of 100 μs per phase) with amplitudes from 500 to 3000 mV, in 250 mV steps, was applied. Individual voltage steps were repeated 5 times with a delay of 5 s, that is, 5 x 500 mV, then 5 x 750 mV, then 5 x 1000 mV, and so on. The Sync Out signal of the STG (stimulus generator) was used to trigger the recording sweeps, with a pre-trigger of 10 ms and a time window of 70 ms.

This example shows a recording with the new MEA amplifier with blanking circuit (MEA1060-BC), featuring a superior stimulus artifact suppression. The Sync Out signal of the STG also triggered the MEA amplifier. The electrodes were transiently, in this case for **540 μs** (300 μs Sync Out pulse of the STG, plus intrinsic **Wait** of 40 μs , plus user-defined **Wait** of **200 μs**) separated from the amplifier stage during stimulation. Stimulus artifacts could be efficiently prevented on all recording channels.

The demo data used in this part of the tutorial was kindly provided by Dr. Frank Hofmann, University of Heidelberg, Germany.

MC_Rack Tutorial: MEA Application Examples

Task

Analyze the **input / output (I/O) relationship** of the biological response to the electrical stimulation by an offline analysis; graph, print and save the results.

Note: In this chapter, you will set up an offline analysis rack (for the demo data file) step by step. You can set up a virtual rack for online recordings and analysis likewise, simply add the **MC_Card** as the data source to the rack instead of the **Replayer**.

- Rack file: Hippocampus_IOCurve_Demo.rck
- Data file: I-Ocurve_Demo.mcd
- Image file: Hippocampus_IOCurve.jpg

Please see also the MEA Application Notes for more information on the preparation techniques and experiments.

You will learn in this chapter ...

- How to define **basic functions** in your **rack configuration**:
 - Select electrodes of interest / deselect unused electrodes.
 - Analyze field potentials, for example, extract peak-to-peak amplitude and the slope in parallel (**Analyzer**)
 - Plot the extracted parameters vs. time (**Parameter Display**)
- How to **customize** the rack:
 - Modify the display layout (all display types)
- More **details**:
 - Align the data traces to a slice picture (**Analyzer**, **Data**, or **Averager** display)

1.2 Preparations

We recommend that you take some time for rebuilding the virtual rack for this application step by step in this tutorial, but if you prefer having a look at the completed rack or if you get stuck during the tutorial, you can also open the rack file "Hippocampus_IOCurve_Demo.rck". Click **Open** on the **File** menu to open the rack file.

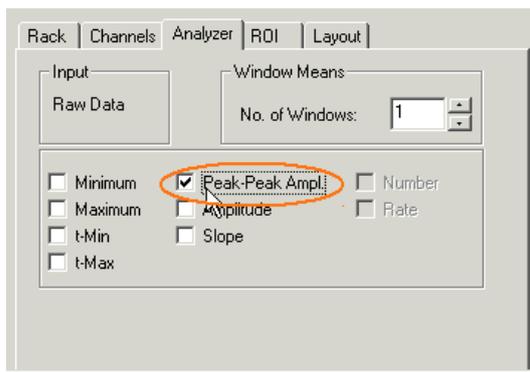
1. Copy the **complete** MC_Rack **Tutorial** folder into the MC_Rack directory with the following path "c:\Program Files\Multi Channel Systems\MC_Rack\"
2. Start MC_Rack or click **New** on the **File** menu to generate a new virtual rack file configuration.
3. Click  on the toolbar to add a **Replayer** to your virtual rack.
4. In the tree view pane of the virtual rack, select the **Replayer** and click the **Replay File** tab. Click the **Browse** button and browse to the **Offline** subfolder of the **Tutorial** folder, and load the data file **I-Ocurve_Demo.mcd** into the **Replayer**.

1.3 Extracting the Peak to Peak Amplitude

1. For extracting the peak-to-peak amplitude and the slope, we will need an **Analyzer**.
Click  on the toolbar to add an **Analyzer** to the virtual rack.

The **triggered Analyzer** display with the **region of interest** appears because the data was recorded as triggered sweeps.

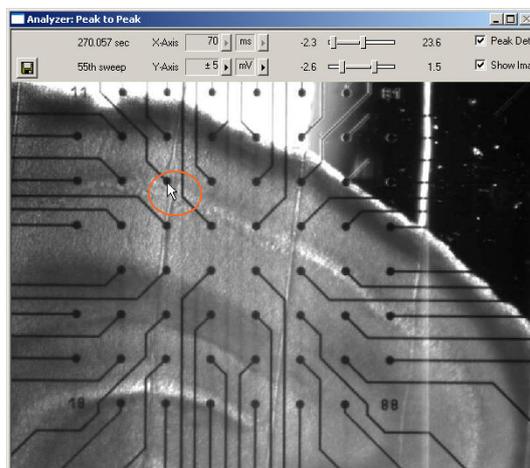
2. Rename the **Analyzer** to **Peak to Peak** to distinguish it from the slope analyzer that we will set up later.
3. Select the **Analyzer** in the tree view pane. Select the parameters you like to extract, **Peak-Peak Ampl.** in this case.



1.4 Aligning the Electrode Pattern to the Slice

You can load a picture (from a digital camera, for example) of the acute slice as a background picture of the display and align the traces to the electrode positions.

1. On the **File** menu, click **Load Image**.
2. Browse your folders and open the **Tutorial** folder on the installation volume.
3. Select the **Hippocampus_IOCurve.jpg** file and click **Open**.
4. On the **Analyzer** window, click **Show Image**. You can now see the slice picture.
5. Now, you map the channels to the electrodes in the picture. Hold down the **SHIFT key**, point to any electrode in the picture, for example, No. 33, and **double-click**.



A text box opens.

MC_Rack Tutorial: MEA Application Examples

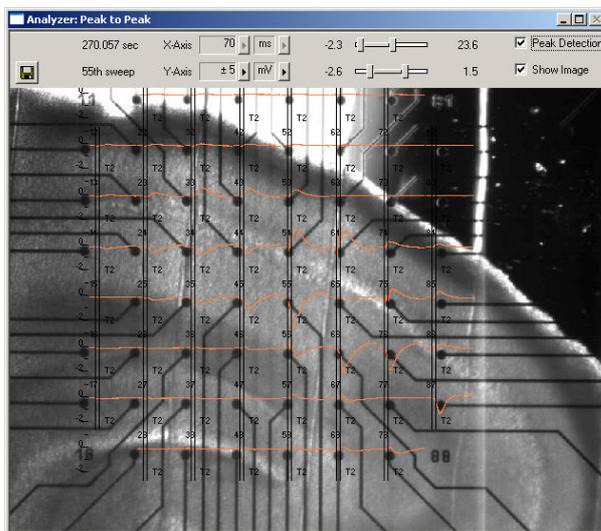
6. Type in the number of the electrode according to the MEA layout, for example 33.



7. Hold down the **CTRL** key, point to any electrode in **another** row, for example the bottom right, and **double-click**.
8. Type in the number of the electrode according to the MEA layout, for example 78. The electrode numbers appear on top of the picture in the typical 8x8 grid.
9. Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the replaying of the data.

Each virtual instrument in the rack configuration starts to process the channels and data streams that were assigned to it. For example, data is graphed in the displays, and so on. You can adjust the replaying **speed** on the **Replayer** tabbed page of the **Replayer**.

The data traces appear on top of the picture.



Note: If you have made a mistake, simply repeat the assignment from step 58.

Hint: If you record data with a background picture in your rack, the file path of the picture is saved together with the data, that is, it is opened automatically when you replay the file in a rack that has a display with the **Show Image** option selected. (File paths are absolute, that means MC_Rack cannot find picture files if they have been moved to another folder or directory. You will be informed by an error message.)

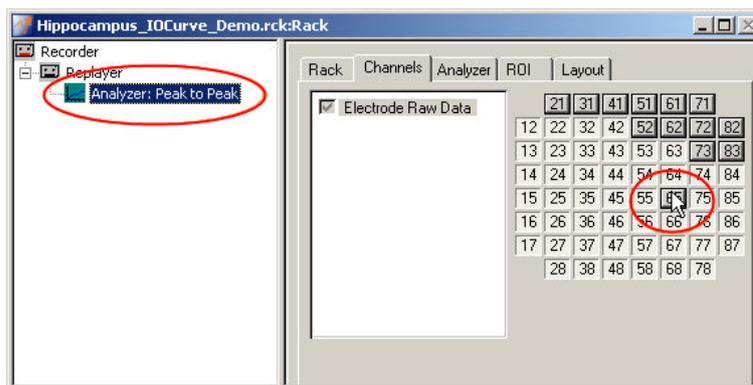
1.5 Deselecting Unused Electrodes

One of the main advantages of MC_Rack is the flexible data management. You can now **deselect** any **unused electrodes**, for example, the electrodes that are not covered by the slice or the stimulating electrode, in the **Analyzer**, so that the peak to peak amplitude is extracted only from the selected electrodes.

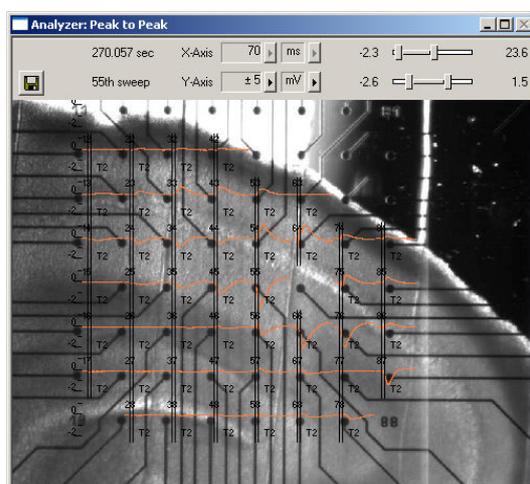
Hint: With a **MEA Switch**, you could use unused channels on the data acquisition board for recording from an additional amplifier.

Note: The **Recorder** that writes data to the hard disk is an independent virtual instrument, that is, you have to select the channel numbers that you want to save in the generated data file, for each data stream separately, on the **Channels** tabbed page of the **Recorder**.

1. In the tree view pane of the virtual rack, select the **Analyzer**, and click the **Channels** tab.
2. Click the data stream **Electrode Raw Data**.
On the right half of the **Channels** page, the channels appear in the MEA-layout. You can select the check box next to the stream name to select all channels, or click single buttons to pick single channels. The check box next to the stream name appears shaded if some, but not all channels have been selected.
3. Deselect the stimulating electrode 65, and all unused electrodes such as 21, 31, 32, 33, 34, 35, 36, 37, 52, 62, 72, 82, 73, and 83, by clicking the corresponding channel buttons. **Selected** channel buttons appear **pressed in**.



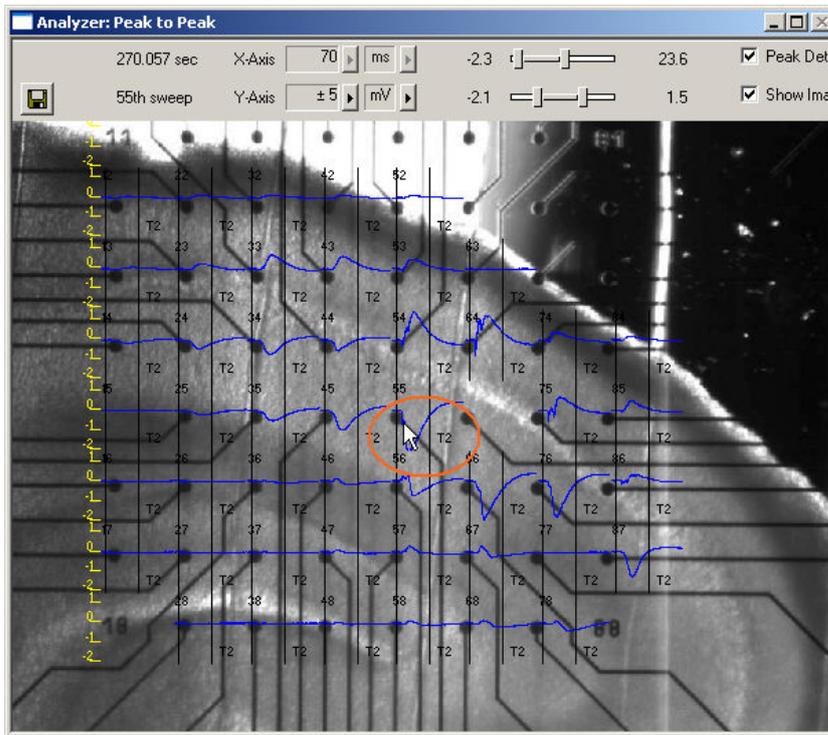
If you start the rack again, only the traces from selected electrode are displayed and only the peak to peak amplitudes from selected electrode channels are extracted.



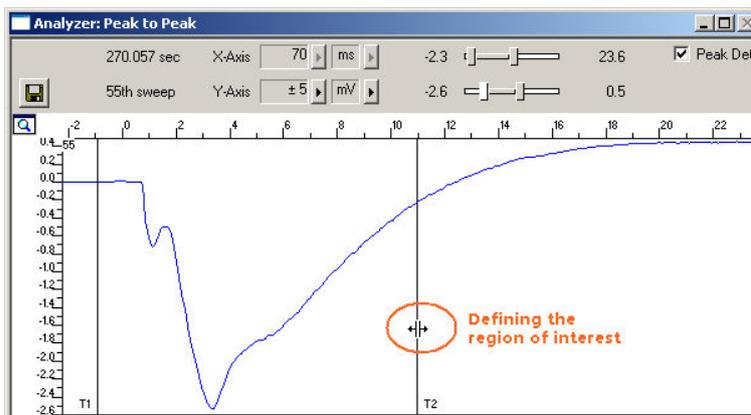
1.6 Defining the Region of Interest

You have to define a time range relative to the trigger event as the region of interest (**ROI**). The parameter of interest, the peak-to-peak amplitude in this case, is then extracted from the data trace in the region of interest. You can define the region of interest with the two vertical bars in the **Analyzer** display or by editing the **T1** and **T2** boxes on the **ROI** tabbed page of the **Analyzer**.

1. Double-click on any electrode with a large response to zoom into the data recorded from this electrode.



2. Position the bars so that the field potential is clearly inside the region of interest. As the demo data was recorded with a MEA1060-BC amplifier with blanking circuit, there is no stimulus artifact that you would need to exclude from the analysis.



1.7 Plotting the Intensity of the Biological Response

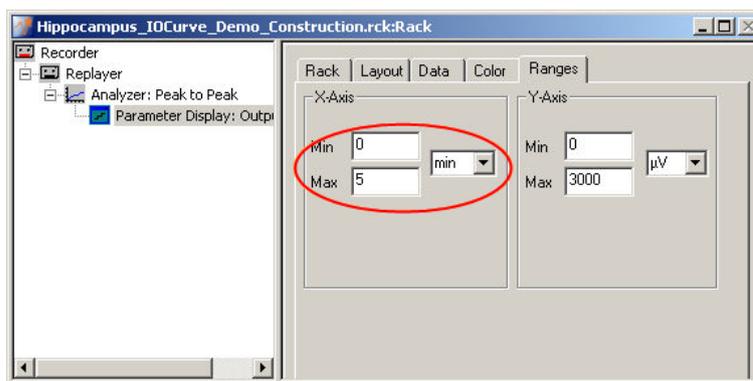
As a measurement for the response to the stimulus, we will plot the **peak-peak amplitude** and the **slope** versus time. We will start with the peak-peak-amplitude. For graphing the extracted data, we will need a **Parameter Display**.

- In the tree view pane of the virtual rack, select the **Analyzer** and click  on the toolbar to add a **Parameter Display** in **series** with the **Analyzer** to the virtual rack. (It would not be possible to add the **Parameter Display** in parallel to the **Analyzer**, as the replayed data file does not contain any parameter streams as input streams for the display, and virtual instruments can only use the output streams of other virtual instruments that are upstream in the virtual rack tree). You can rename it so that you can identify it later in the virtual rack.

Setting the window extent

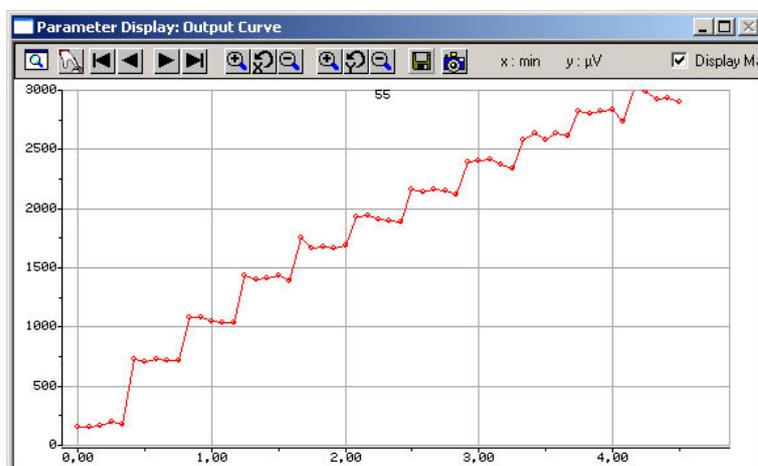
As this demo data file has about 4 min recording time (see **File Info** page of the **Replayer**), 5 min will be fine for the x-axis range.

1. In the tree view pane of the virtual rack, select the **Parameter Display**, and click the **Ranges** tab.
2. Enter 5 min for the x-axis range, and adjust the y-axis to the maximum response, for example, 3000 μV .



1. Click **Start**  to generate the plots. You can select the option **Display Marker** to mark each extracted data point in the plot.

The output is nicely increasing with the increasing stimulus amplitude.

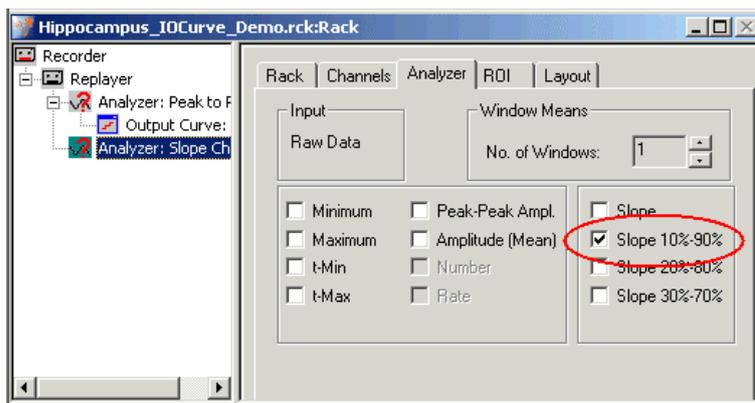


1.8 Extracting the Slope

In triggered mode, the **Analyzer** extracts the parameters of interest from a distinct **time window** relative to the **trigger event**, the region of interest (**ROI**). You could use the same **Analyzer** for extracting both the peak-to-peak amplitude and the slope, but as two distinct regions of interest are required for extracting the two parameters, we will need to set up a second **Analyzer**.

1. Click  on the toolbar to add a second **Analyzer** to the virtual rack.
2. Rename the **Analyzer** to **Slope** to distinguish it from the first analyzer.
3. In the tree view pane of the virtual rack, select the **Analyzer**, and click the **Channels** tab. Select the electrode channels that you would like analyze, for example, channel 55.
4. Click the **Analyzer** tab and select the parameters you like to extract, slope in this case. You have the choice between four options: **Slope** is the slope of a straight line fitted through all data points in the ROI. When choosing **Slope 10% / 90%**, only the data points in the range of 10% / 90% of the peak-peak amplitude (stretching from minimum to maximum) in the region of interest is considered for fitting the line. This is generally more useful as the exact timing of the regions of interest may differ slightly from channel to channel and it might be difficult to position the ROI to fit all channels of interest.

Select checkbox slope 10% / 90%.



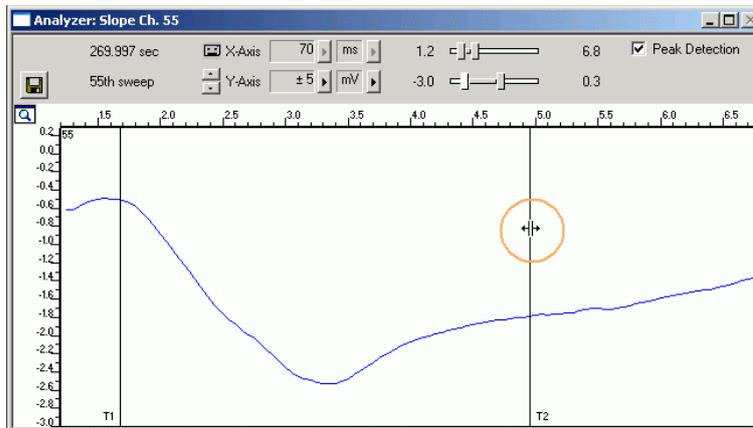
Defining the ROI

For extracting the slope, you need to frame ideally only the linear part of the response decay with the ROI cursors.

1. Zoom into a single channel and use the sliders to adjust the ranges so that you can clearly see the linear part of the response decay.



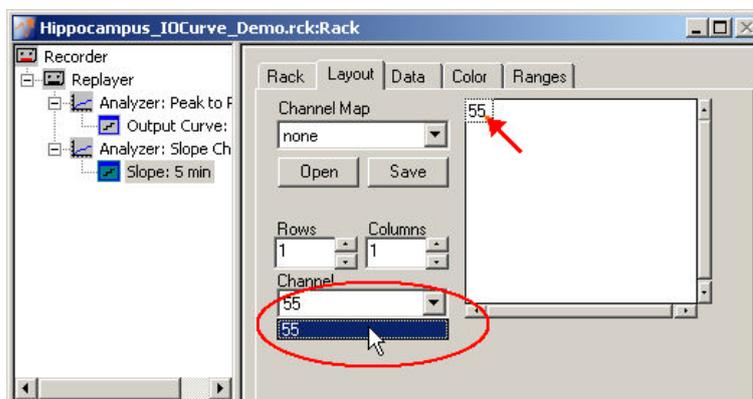
- Define the region of interest with the vertical bars **T1** and **T2**. When choosing the **Slope 10 % / 90 %** option, it is not required to position T1 and T2 exactly, as 10 percent, that is, the non-linear part of the response, is not regarded. The slope is then computed from a linear fit regression line (Least Square Algorithm) in the ROI.



1.9 Customizing Displays

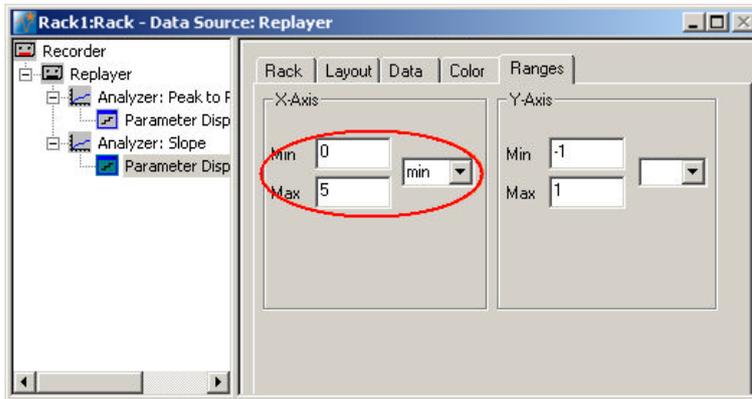
As it may not be useful or possible to extract the slope from all channels, you can set up your custom layout for the **Analyzer** or the **Parameter** display (this is true for all displays), to save space on the screen. You may be interested only in one or two representative channels. (Remember: You can save the parameters of all channels in the **Recorder** to review it later or to analyze it further in your custom evaluation software.)

- Click the **Layout** tab.
The default layout is loaded. In the **Rows** and **Columns** boxes, you can now modify the layout.
- Type or select the number of **Rows**, for example 1, and the number of **Columns**, for example 1. On the right, a custom layout with a single channel appears.
- Assign the appropriate channel numbers to the empty slots. Click the empty slot to select it. The active slot is highlighted by a dotted line.
- Select the channel of interest, for example, 55, for the active slot from the **Channel** list, or type **55**. The **Parameter Display** is updated accordingly. Likewise, you can set up any custom configuration for all display types.



1.10 Plotting a Slope Curve

1. On the toolbar, click  to add a second **Parameter Display** following the second **Analyzer** to the virtual rack configuration. (If it follows the first **Analyzer** in the tree view, it will not be possible to assign the output data stream of the second **Analyzer**, that is, the slope, to this **Parameter Display**.) You can rename it so that you will identify it later in the virtual rack.
2. Adjust the x-axis range to the total recording time of about 5 minutes.



3. Start the rack to generate the plot.

The slope becomes more negative, that is, the decay time decreases, with an increasing stimulus amplitude.



2 Saving the Rack and Saving Data

2.1 Saving a Rack

Save the virtual rack configuration if you like to keep it for future use, for example for an offline analysis of identical data recorded in another experiment.

1. On the **File** menu, click **Save As**.
2. The **Save As** dialog box opens.
3. Browse your folders and select a path.
4. Enter a file name and confirm by clicking **Save**.

The file extension for the rack files is **.rck**.

2.2 Selecting Data Streams for Recording

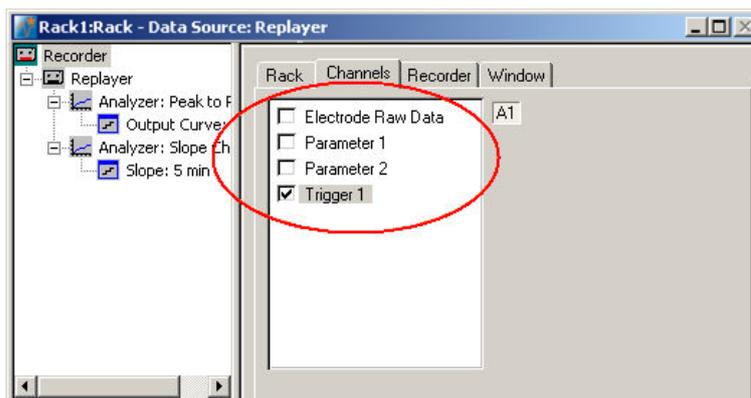
MC_Rack's philosophy is to **strictly separate** the actions of **all** virtual instruments in a rack. That means, that you could **record** to hard disk completely different data streams and channels than you **monitor** on the screen. This has the advantage that you can store exactly the channels you are interested in, but it also has the slight disadvantage that all virtual instruments have to be set up **separately**. Please be especially careful when configuring the **Recorder**, to avoid data loss.

When you have finished setting up the rack, you can select the data streams and channels that you want to save to the data file specified in the **Recorder**.

Selecting data streams and channels for recording

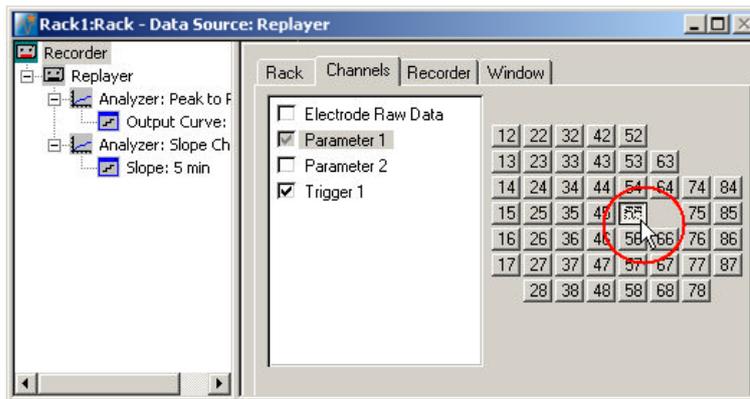
The fate of each single channel is **independent** from other channels. You can pick exactly the channels you like to save from all generated data streams. For example, you can decide to save only **one** channel of **raw data**, but the **peak-to-peak amplitude** results of **all**, or of a **specific selection** of channels.

1. Select the **Recorder** in the virtual rack tree view pane and then click the **Channels** tabbed page. On the white pane on the left of the **Channels** page, you see the data streams that are available with your rack configuration. When replaying data, you are generally more interested in the parameter data streams, but you can rerecord the raw data as well, in case that you want to save the raw data and the extracted parameters to the same data file.



MC_Rack Tutorial: MEA Application Examples

- Click the data stream that you are interested in, generally the parameter streams. The available electrode channels appear in a button array on the right side. Parameter stream 1 is generated by the first analyzer in the rack (here: for extracting the peak-peak amplitude), parameter 2 is generated by the second analyzer (here: for extracting the slope).



- You can now either select all channels by clicking the check box next to the data stream name, or you can pick single channels by clicking the corresponding buttons. For more information, please see "Channel Selection" in the MC_Rack Features section. Only data from the selected channels will be saved to the hard disk.

2.3 Creating a Data File

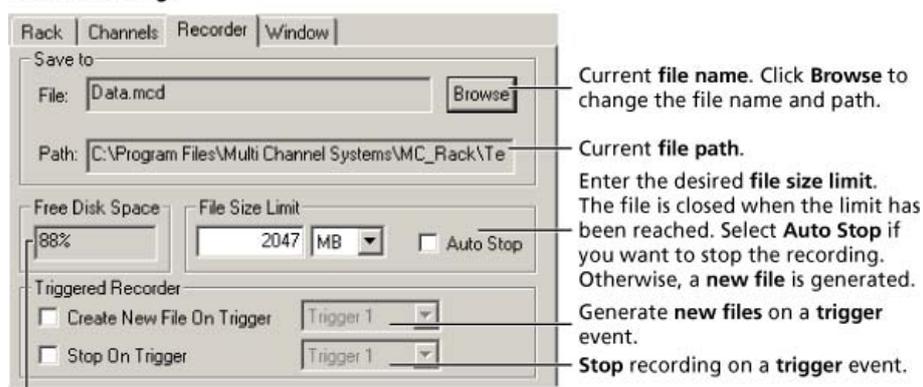
If you want to write the parameters that you will extract in an offline analysis to the hard disk, you have to specify the file name and path in the **Recorder**. (It is not possible to change or overwrite existing data files in MC_Rack, for example, adding the spike rate to an existing data file, but you can record the raw data together with the spike rate stream to the same file.)

Choosing the file name and path

- Click the **Recorder** tab.
- Browse** your folders and select a path.
- Type a file name into the text box.
- Confirm by clicking **Save**.

The data file is then generated automatically when you start MC_Rack in **recording** mode. The file extension for the data files is ***.mcd**.

Recorder settings



Displays available disk space on the currently selected hard disk.

File size limit

When the maximum file size specified by the user has been reached, a new file is generated automatically. The file name is extended by four digits, counting up, for example LTP-Parameters0001.mcd, LTP-Parameters0002.mcd, and so on.

If you rather prefer that the recording is completely stopped when a file has reached the maximum size, please select the option **Auto Stop**.

For information on more options, please see "Generating Data Files" in the MC_Rack Features section.

Selecting data streams and channels

As has been said before, the fate of each single channel is **independent** from other channels. You can pick exactly the channels you like to save from all generated data streams. For example, you can decide to save only **one** channel of **raw data**, but the **peak-to-peak amplitude** results of **all**, or of a **specific selection** of channels.

→ Click the **Channels** tab.

As long as the rack is still empty, you see an empty box. There are no channels available at this point, because you have not chosen a data source yet (**MC_Card** for online data acquisition or **Replayer** for replaying data files). Without a data source, there are no data streams available for recording. Remember later, when you have completed the rack, to assign the channels that you like to save to the data file to the **Recorder**.

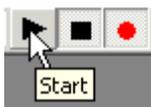
After you added a **data source**, you will see the electrode raw data streams provided by the data source (for example, electrode raw data, analog data, and digital data from the **MC_Card**, or the data streams included in the data file loaded into the **Replayer**). If the rack file contains virtual instruments that **generate** data streams such as Spikes from a **Spike Sorter** or Parameter streams from an **Analyzer**, these data streams will be available for recording as well.

2.4 Starting Data Acquisition and Recording

Now that you have completed the virtual rack, you are ready to start the rack.

→ Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the data acquisition. Each virtual instrument in your rack starts to process the channels and data streams that were assigned to it.

→ Click first **Record**  and then **Start**  to write data to the hard disk. The data from the electrodes selected in the **Recorder** is saved to the file and location specified in the **Recorder**.



→ Click **Stop**  to stop the data acquisition.



Warning: **Only** data of the channels and data streams that were **selected** in the **Recorder** are saved in your data file when you start a recording. Data is **only** saved to the hard disk when the red **Record** button is pressed **in**. Make always sure that you have selected all channels of interest, and that the **Record** button is active before starting an experiment to avoid data loss.