multichannel systems

MC_Rack Tutorial: Paired Pulse Facilitation (PPF) or Depression (PPD)

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Multi Channel Systems

MCS GmbH

Aspenhaustraße 21

72770 Reutlingen

Germany

Fon +49-71 21-90 92 5 - 0

Fax +49-71 21-90 92 5 -11

info@multichannelsystems.com

www.multichannelsystems.com

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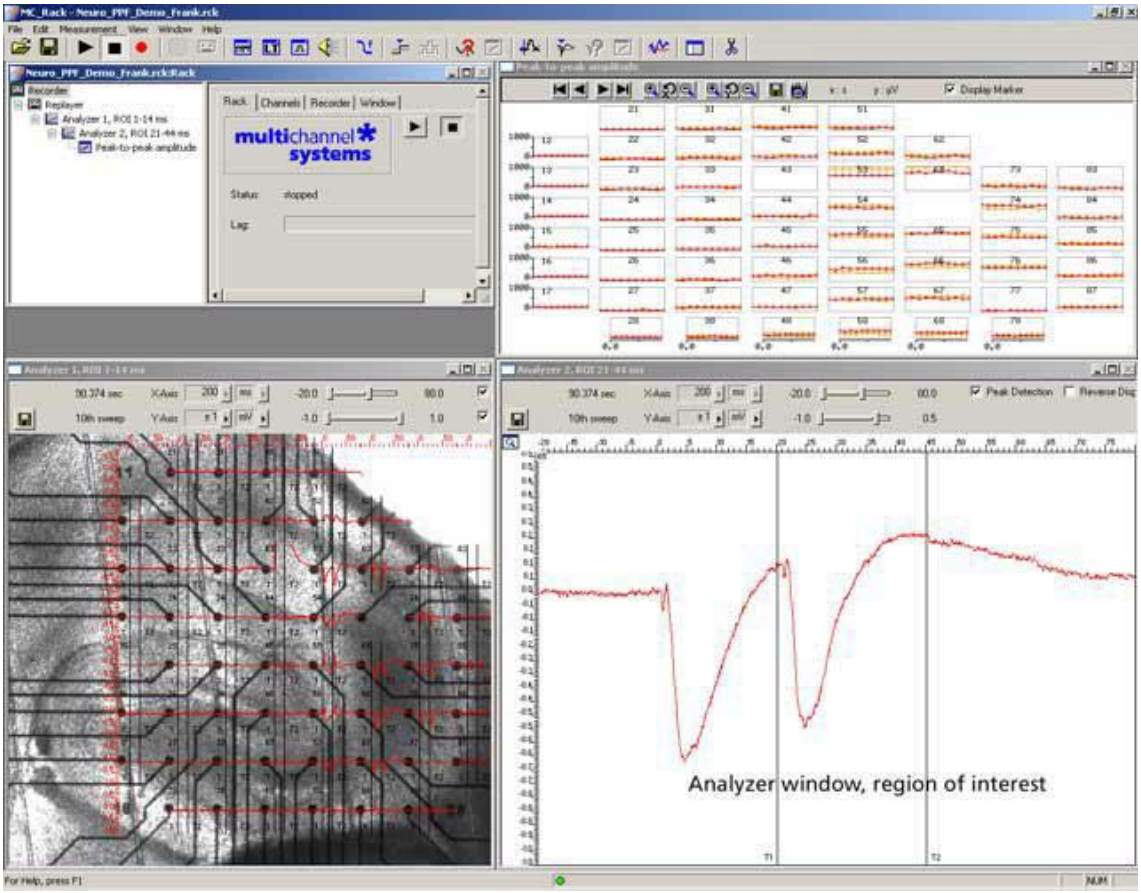
1 Paired Pulse Facilitation (PPF) or Depression (PPD)

1.1 Aim of Experiment



In **Paired Pulse Facilitation** (**PPF**) or **Depression** (**PPD**) experiments, the stimulus is followed by a second stimulus, typically 20 to 80 ms later. The magnitude of the population response to the second stimulus is compared to that of the first one. It is higher in case of a PPF, or lower in case of a PPD.

In MC_Rack, two (or more) regions of interest (ROI) can be defined, one triggered by the first stimulus, the other by the second stimulus, for example. The peak-to-peak amplitudes for both ROIs can be extracted online.

The example data file was recorded from a 300 µm thick **acute hippocampus slice** that was prepared from a 32 days old rat. The slice was stimulated with a single biphasic pulse (2 V amplitude, 100 µs per phase) by one of the embedded MEA-electrodes (No. 64). The second stimulus pulse was applied 20 ms following the first one. Recording was triggered by applying the Sync Out signal of the stimulus generator to one of the analog input channels (A1). Recorded sweeps are 200 ms, with a pre-trigger time of 30 ms, and a post-trigger time of 170 ms.

As the data was acquired with a MEA1060-BC amplifier with blanking circuit, all MEA electrodes were disconnected from the amplifier shortly during stimulation, 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**). This efficiently prevented stimulus artifacts.

Some electrodes recorded PPF and others PPD in this demo data file.

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

Task

Extract the field potential amplitude from two distinct regions of interest 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: Neuro_PPF_Demo.rck
- Data file: PPF_Data.mcd
- Image file: PPF_Image.jpg

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

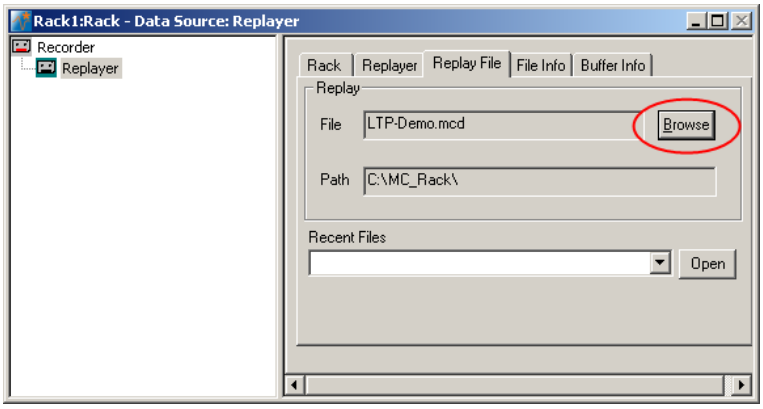
You will learn in this chapter ...

- How to define **basic functions** in your **rack configuration**:
  - Trigger MC_Rack on a TTL pulse (**Trigger Detector**)
  - Extract the peak-to-peak amplitude from two distinct regions of interest (**Analyzer**)
  - Plot the amplitude vs. time (**Parameter 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 "Neuro_PPF_Demo.rck". Click **Open** on the **File** menu to open the rack file.

1. Copy the **complete** MC_Rack **Tutorial** folder from the installation volume into the MC_Rack program 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 **PPF_Data.mcd** into the **Replayer**.



1.3 Triggering MC_Rack on the Stimulus

The analog input channel A1 was used to feed in the TTL signal from the Sync Out channel of the stimulus generator. You can use this TTL pulse to trigger the **Recorder** or any other virtual instruments. (As the trigger data stream was saved together with the data file, you can of course also use this trigger for replaying the data as well, but we recommend to set up a **Trigger Detector** on your own in this tutorial to learn how to do it.)

Note: It is recommended to use the digital input port for feeding in TTL signals. The analog inputs are intended for analog signals, like patch clamp data, for example.

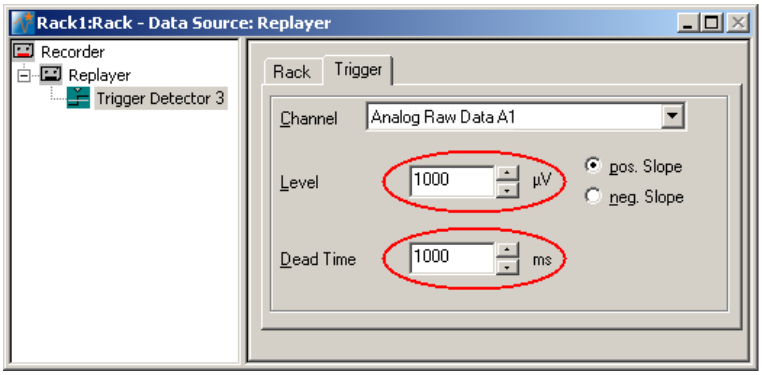
1. Click on the toolbar to add a **Trigger Detector** to the virtual rack. The **Trigger Detector** is automatically named **Trigger Detector 3**, as the replayed data file already contains 3 trigger data streams, so the data stream generated by this new **Trigger Detector** will be **Trigger 4**. You can rename it if you like.

2. In the tree view pane of the virtual rack, select the **Trigger Detector**, and click the **Trigger** page.

3. From the **Channel** list, select the **Analog Raw Data A1** channel, to which the Sync Out channel of the stimulus generator was connected.

4. Enter a threshold. As the TTL signal has a 5 V amplitude, and the input voltage range of the MC_Card was +/- 2048 mV, 1 V should be fine.

5. Enter a dead time that is long enough so that the second stimulus of the PPF protocol does not generate a second trigger event, that is, longer than 20 ms. To be on the safe side, enter 1000 ms, for example.



1.4 Extracting the Peak to Peak Amplitude

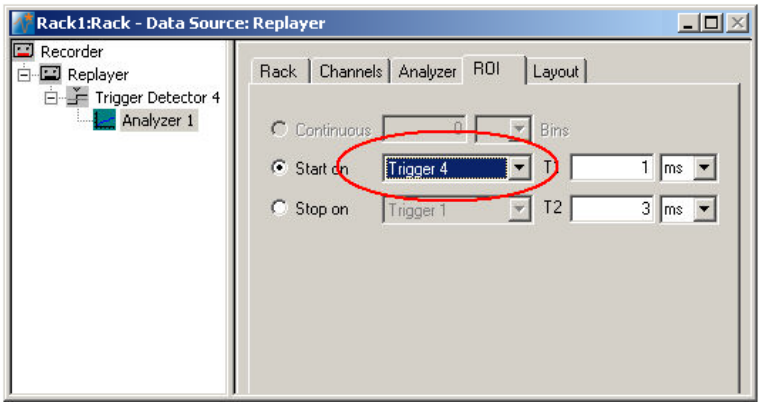
For extracting the peak-to-peak amplitude, you will need an **Analyzer**.

1. In the tree view pane of the virtual rack, select the **Trigger Detector** and click on the toolbar to add an **Analyzer** in **series** with the **Trigger Detector** to the virtual rack. If you would put both instruments in parallel, you would not be able to use the trigger stream generated by the **Trigger Detector** for triggering the **Analyzer**, because 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.

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

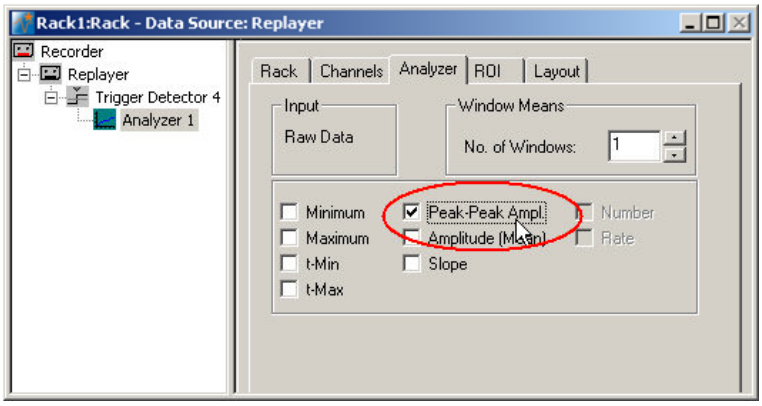
2. Rename the **Analyzer** to **ROI 1** to distinguish it from the second analyzer that we will set up later.

3. In the tree view pane of the virtual rack, select the **Analyzer**, and click the **ROI** tabbed page. The recording trigger **Trigger 1** is already preselected, but you can also select the **Trigger 4** that is generated by the **Trigger Detector 4**.



4. Click the **Channels** tab, and select the **electrodes of interest** (default is all).

5. In the tree view pane of the virtual rack, select the **Analyzer**. Select the **parameters of interest**, **Peak-Peak Ampl.** in this case.



1.5 Defining the Region of Interest

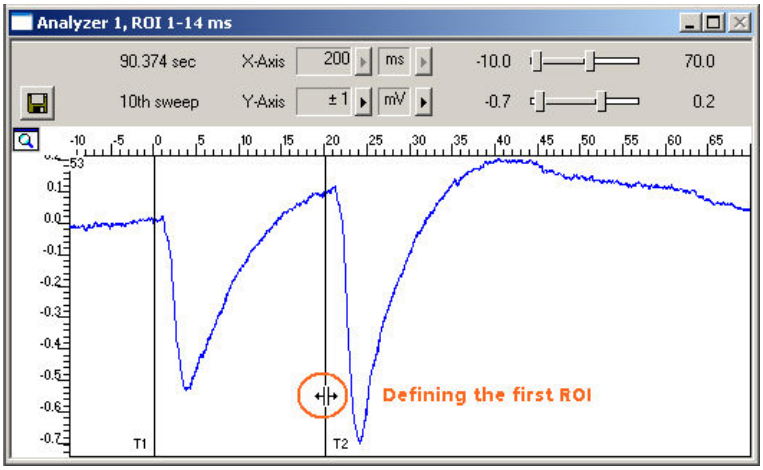
In triggered mode, the **Analyzer** extracts the parameters of interest from a distinct **time window** relative to the **trigger event**, the region of interest (**ROI**). The parameter of interest, the peak-to-peak amplitude in this case, is then extracted from the data trace in the region of interest. 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. 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**.

2. Double-click on any electrode channel with a large response in the **Analyzer** display to **zoom** into the data recorded from this electrode. Use the sliders to adjust the ranges so that you can clearly see the response.

3. Position the bars **T1** and **T2** so that the field potential following the first stimulus is clearly inside the ROI.



1.6 Defining the Second Region of Interest

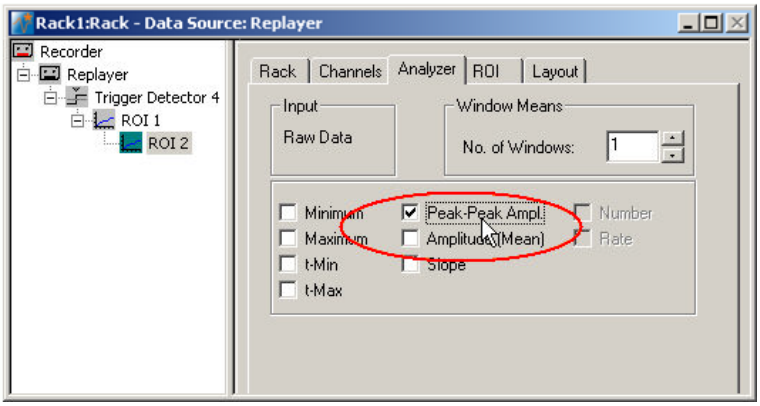
For defining the second region of interest in order to extract the amplitude of the response to the **second** stimulus, you will need to set up a second **Analyzer**. You need to configure the second **Analyzer** similar to the first one, except the distinct ROI.

1. Click on the toolbar to add a second **Analyzer** in **serial** to the first **Analyzer** to the virtual rack. (You will then be able to overlay the parameters extracted from both ROIs in the same **Parameter Display**. If you put the instruments in parallel, you would need two separate **Parameter Displays** for graphing the traces.)

2. Rename the **Analyzer** to **ROI 2** 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 same electrode channels that you have selected for the first **Analyzer**.

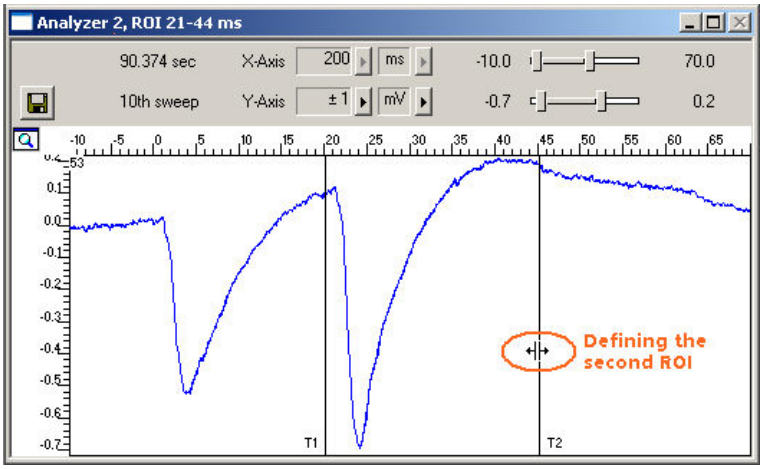
4. Click the **Analyzer** tab and select the parameters you like to extract, again **Peak-to-peak Ampl.** in this case.



5. Click the **ROI** tabbed page. The recording trigger **Trigger 1** is already preselected, but you can also select the **Trigger 4** that is generated by the **Trigger Detector 4**.

6. In the **Analyzer** window, double-click on any electrode with a large response to zoom into the data recorded from this electrode. Use the sliders to adjust the ranges so that you can clearly see the response.

7. Position the bars **T1** and **T2** so that the field potential following the second stimulus is clearly inside the region of interest, or enter the appropriate values (T1 and T2 of the first Analyzer + 20 ms) directly into the **T1** and **T2** text boxes on the **ROI** page.



1.7 Plotting Amplitude versus Time

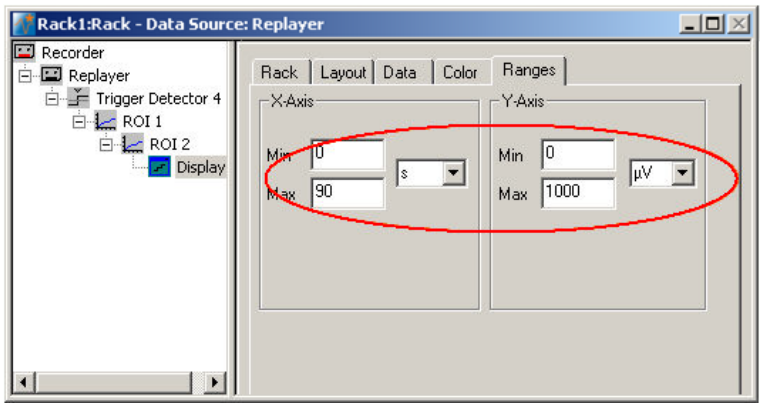
Add a display to graph the Peak-to-Peak plot. As a parameter data stream is very different from raw data, MC_Rack features a specialized display type, the **Parameter Display**, which is optimized for parameter streams.

→ In the tree view pane of the virtual rack, select the second (bottom) **Analyzer** and click on the toolbar to add a **Parameter Display** in **series** with both **Analyzers** to the virtual rack. It would not be possible to add the **Parameter Display** in parallel to the **Analyzers**, 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.

Setting the window extent

The total recording time of the example file is 90 s, as you can see on the **File Info** page of the **Replayer**. Adjust the ranges of the y-axis as well.

1. Click the **Ranges** tab.
2. Enter 90 s for the x-axis range, and 1000 µV for the y-axis.

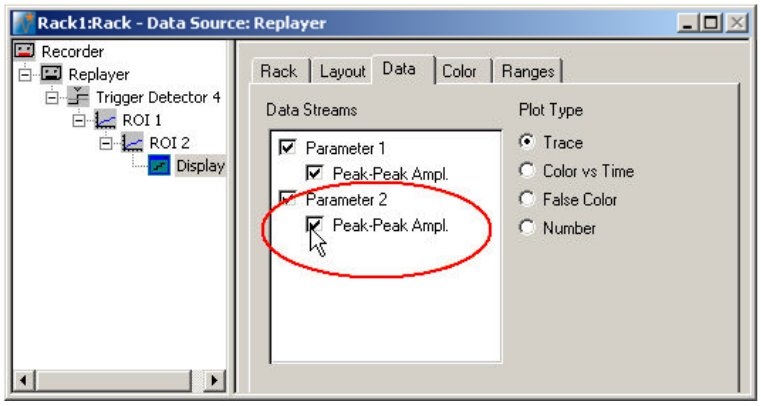


Selecting data streams

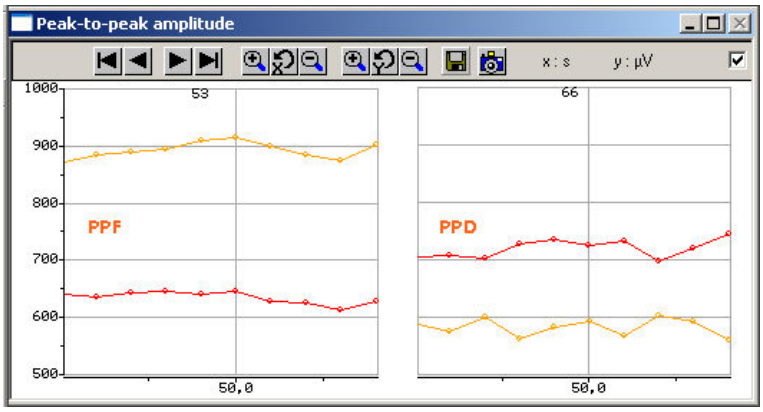
Now, select the data that you like to be displayed.

Note: You can only plot the data streams in the parameter plot that are generated by virtual instruments that are higher in the hierarchy of the rack tree and directly connected with the **Parameter Display**. In this rack configuration, both **Analyzers** (**ROI 1** and **ROI 2**) are **upstream** the **Parameter Display** in the hierarchy (see screen shot below); therefore, both **Parameter 1** and **Parameter 2** streams can be used by the display, that is, are visible on the **Data** page.
If the two **Analyzers** were set up in two separate tree branches (in parallel), you would need two separate **Parameter Displays**, each downstream of each of the **Analyzers**.

1. In the tree view pane of the virtual rack, select the **Display 1**, and select the data streams that you want to plot. You can overlay the extracted amplitudes from both regions of interest, or you can set up two individual **Parameter Displays** for plotting the responses separately.



2. Click **Start** to plot the extracted parameters. The two different data streams are plotted in different colors (red from the first pulse, yellow from the second pulse). You can see a PPF on electrode 53, and a PPD on electrode 66, for example.



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

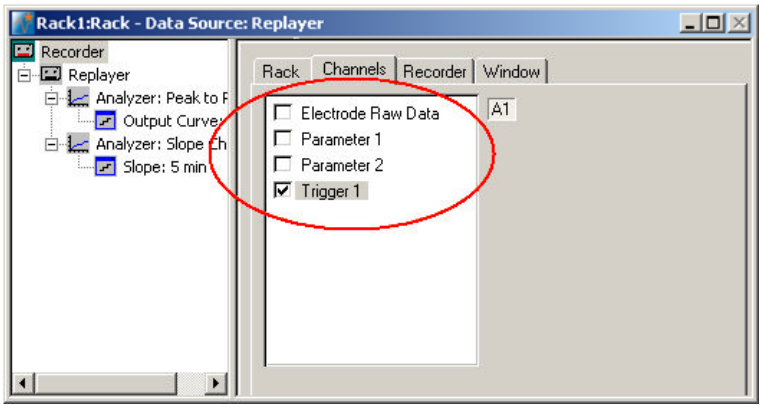
MC_Rack's philosophy is to **strictly separate** the actions of **all** virtual instruments in a rack. That means, that you could **record** to hard disk completely different data streams and channels than you **monitor** on the screen. This has the advantage that you can store exactly the channels you 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**.

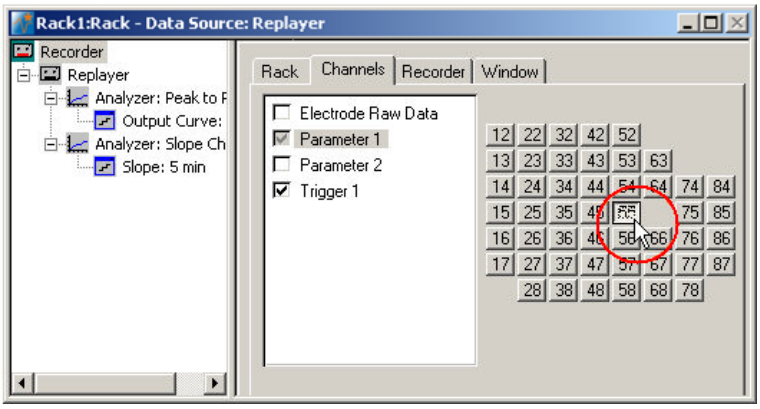
Selecting data streams and channels for recording

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.



2. 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).



3. 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.

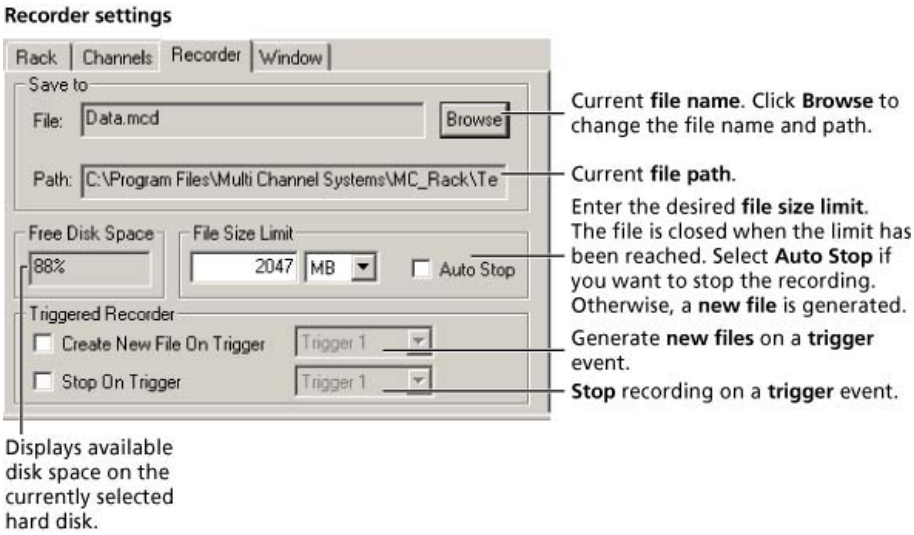
2.3 Creating a Data File

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

1. Click the **Recorder** tab.
2. **Browse** your folders and select a path.
3. Type a file name into the text box.
4. 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**.



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**.

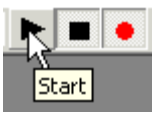
After you added a **data source**, you will see the electrode raw data streams provided by the data source (for example, electrode raw data, analog data, and digital data from the **MC_Card**, or the data streams included in the data file loaded into the **Replayer**). If the rack file contains virtual 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.