HEKA

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Quick-Start-Up User Guide for PATCHMASTER NEXT (v1.0)

HEKA SUPPORT DOCUMENT

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Basic Instructions on How to Use PATCHMASTER NEXT

The original PATCHMASTER software was first introduced by HEKA in 2003. Back then, it was a major step forward from the very popular PULSE software. Modern Patch-Clamp requirements (extensions), user-requested modifications and new computer technologies have been implemented into PATCHMASTER over the years, creating a professional and one of the most advanced Patch-Clamp acquisition and analysis software programs that are available today.

With the new **PATCHMASTER NEXT** software, HEKA continues and further improves this successful program line, while introducing a brand-new user-interface that is specifically designed for modern computer operating systems and therefore easy to use for beginners and HEKA-veterans alike.

Please note, this document focuses on the new features of PATCHMASTER NEXT and does not explain all available buttons or functionalities. A complete PMN manual will be available soon. In the meantime, to find missing information, please consult the original PATCHMASTER manual (2x90.3). Respective page information for the old manual will be marked like this: [page # to #].

To report a problem or a bug, please press "Help" in the "Control" window and select "Report a Bug" (see chapter 5). Send emails to: support@heka.com

Overview

1. What's NEW in PATCHMASTER NEXT

In this section, we sum up most of the novelties of PATCHMASTER NEXT (PMN). More detailed information can be found in each individual window section.
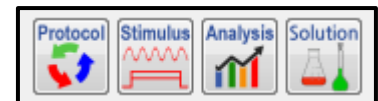
PATCHMASTER NEXT improves productivity and workflow by restructuring the user-interface and introducing new navigational features and modern software handling tools and structures.

- o **User-defined Settings**: easier saving and loading of user-specific settings.
- o **Multi-Window Mode**: every window is freely resizable and moveable (across multiple screens).
- o **Intuitive Navigation & Controls**: **three categories** of action buttons, they have a title & an icon for easy identification by user.
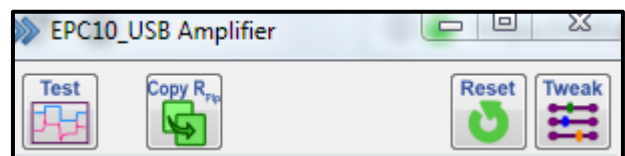   - ▪ **Menu**: (ex.: "Tools" and "Help" in the "Control" window); access to drop-down menu (indicated by little arrow head in corner) that can consist of "On-Off Switch" for many functions and/or options to open other dialog windows.
    - ▪ **One-click**: open specific windows (ex.: "Protocol" in the "Control" window) or execute specific commands or functions.
    - ▪ **Tweak**: allows to customize view & functionality of respective windows.
- o **"Test Pulse" Oscilloscope**: display and controls for the "Test Pulse" are on a separate level, but directly connected to the "Amplifier" window. Press the "Test" button to open it.
- o **Drag & Drop Functionality**: easy creation of stimulation sequences and protocols.
- o **Auto-Complete Function**: Protocol Editor, easy selection of desired macro commands for buttons or fields (drop-down menu).
- o **Right-Mouse-Click Functionality**: copy & print and many other window-specific functionalities and parameters.
- o **Color Coding**: levels of functionality are color coded for easy navigation and causality (Stimulus, Protocol and Analysis Editor).
- o **Helpful User-tips**: improved explanations and tool-tips, appear automatically.
- o **Undo Functionality**: improved undo-functionality, undo stack list for "Protocol Editor".

2. Installation of Hard- and Software Components

Feel free to skip ahead to "Third", if amplifier & dongle have been installed already.

- **First**, you must install the hardware (interface). Please connect your hardware to a USB-cable and plug it into a direct USB-port (2.0 or 3.0) on your computer.

  Successful communication between computer and interface is confirmed, when "HEKA LIH88" shows up in the directory "My Computer" (PC). No need to install special drivers.

  Please note that PATCHMASTER NEXT **only** recognizes "**LIH8+8**" (build-into EPC10_USB amplifiers) or newer HEKA interfaces. EPC800, iTEV90 and other telegraphed manual amplifiers are not supported at this time. Older interfaces (ITC-18, ITC-1600, ITC-16, etc.) do <u>not</u> work either.

- **Second**, you must install a special driver for the software protection key (dongle). Before plugging in the dongle, please download the latest driver from our website at: www.heka.com (Downloads – Dongle) and install it.

  Afterwards, the dongle should be plugged into a direct USB-port (2.0) at the acquisition computer. A red LED light from the dongle indicates full functionality.

- **Third**, PATCHMASTER NEXT is available for download from our website (link).

  If no old HEKA software is installed on the computer, simply run the "PMNext_Installer.exe" file. If older versions of PATCHMASTER NEXT are installed, please rename the respective "HEKA" folders (ex.: HEKA_PMN_old), before running the installer.exe file.

  PMN only runs on PC (Win10, 64-bit). Please see our website for updates.

  - <u>Run Installation as Administrator</u>: Make sure to run the installation procedure with administrator rights enabled.
  - <u>Program Folders</u>: **Two "HEKA" folders** will be created, each one with a "PatchMaster" subfolder inside. The first "HEKA\PatchMaster" folder is in the "Program Files" folder of the c-drive, containing essential program files.
    The 2nd "HEKA\PatchMaster" folder is in the user-account-specific "Documents" location, containing configuration files and other user-specific files.
  - <u>After successful installation</u>: Check the "Launch PatchMaster Next" box and press "Finish" to launch PATCHMASTER NEXT for the first time.
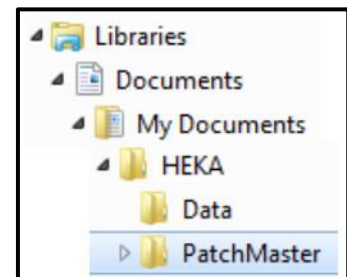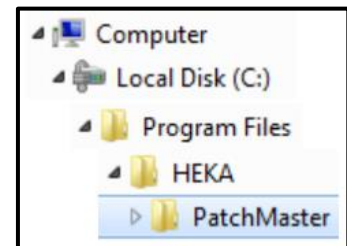
3. First Start of PATCHMASTER NEXT (PMN)

- **Hardware Selection** window: After the initial installation, no "ini-file" (configuration file) has been created yet and therefore this window will pop-up. Allowing you to select from the available list of hardware devices or to "simulate" any amplifier by selecting the "Virtual Amplifier" box.
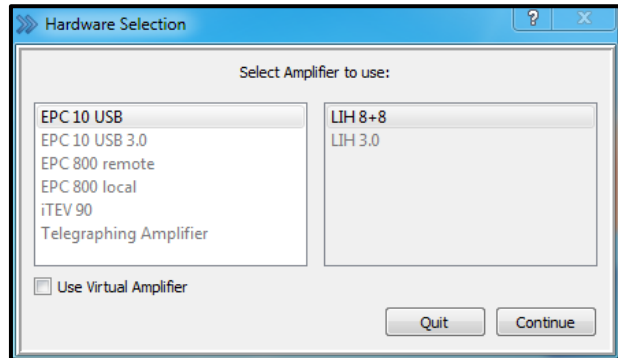
- **Get Data File** window: Allows you to select a quick-start option. Pressing any of those buttons will launch PMN + the respective option.
  - New: Creates a new data file (*.dat).
  - Modify: Opens an existing data file and allows editing of it.
  - Read-only: Opens an existing data file, but does not allow editing.
  - Cancel: Closes this window and continuous with PMN.
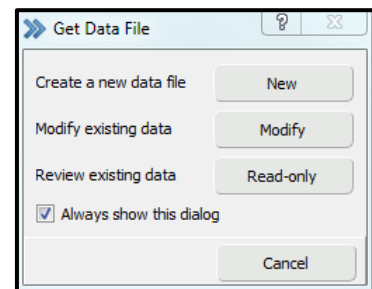
  Uncheck the box to not show this window again at the next start. It can be re-enabled in the "Configuration window" (Files tab – "Ask for Data File").

- **Save Your Configuration File**: There are two ways to save your configuration file.
  - Save using the Configuration window: Open the "Configuration" window and press "Save" to overwrite the currently loaded file or press "Save As" to save your file with a new name.
  - Save when closing PATCHMASTER NEXT: When closing the program, you can press "Save & Exit" to overwrite the configuration file with any new settings and save all support files (pgf, onl and pro). Pressing "Exit" will close PMN without saving your settings. Please note that this "saving" has nothing to do with saving your data-file. Data files are being automatically saved, according to the settings in the "Data Tree – File" dialog.
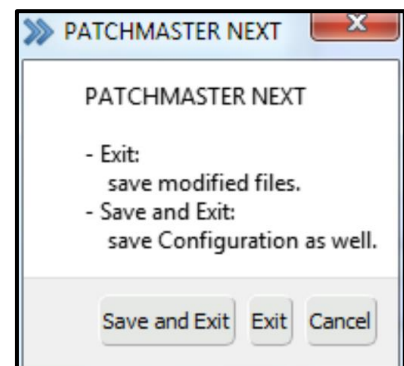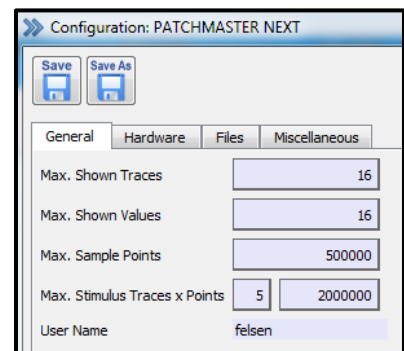
4. Starting PMN, when a Configuration File is Present

We strongly recommend that each user in the lab creates their own configuration file (ini-file) and save it with a special name. If you have your own personal log-in account on the acquisition computer, we suggest to log-into your account and then start PATCHMASTER NEXT (PMN).

- **Settings File Selection**: If an ini-file is present, this window will pop-up when PMN starts, asking the user to select a specific configuration setting. Highlight desired configuration and press "Load" to start PMN.

  - PATCHMASTER NEXT: Default name for the configuration file. Contains default settings, until it is altered by a user.

  - Set Defaults button: Pressing this button resets all configuration parameters to factory default settings and the "Hardware Selection" window pops up.

    For new users, we recommend pressing "Set Defaults" and then follow the instructions given in chapter 3.
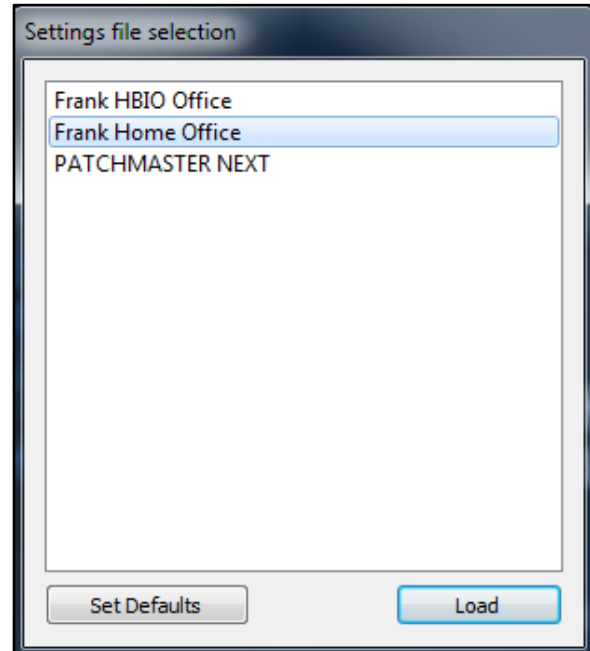
  - "Frank Home Office": Every configuration file (*.ini) that is located inside the "Patchmaster Next" folder will show up in this table. Please note that each user could have multiple files for different projects or applications.

- If no hardware is detected: **"EPC10_USB initialization failed: LIH8+8 error"** warning message pops up.

  - Press "Exit": to close PMN.

  - Press "Run Without Hardware": to start PMN to do analysis only.

  - Press "Repeat": to restart PMN, make sure the appropriate hardware has been switched on first.
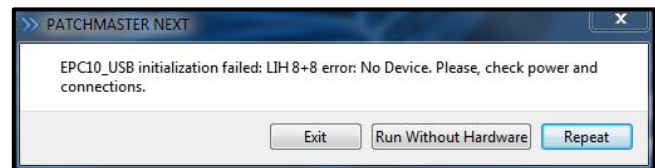
- If no software dongle is detected: **"No appropriate dongle was detected"** warning
message pops up.

  o Press "Demo": to continue in demo-mode.
  No data acquisition or analysis is possible.
  A demo-data file will be automatically
  loaded. Use this mode for pre-purchase
  exploration of PATCHMASTER NEXT.

  o Press "Abort": to close PMN.

  o Troubleshoot: To avoid this warning message, make sure the special dongle
  driver is installed and the appropriate dongle is plugged in (see chapter 2).
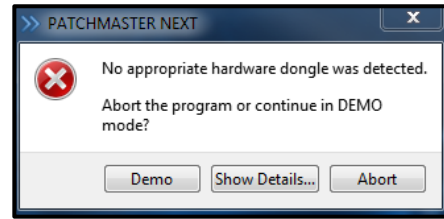
5. Graphical User Interface (GUI)

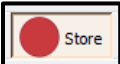The GUI is designed for multiple screen usage. All compartments (windows) can be
freely re-scaled and then be placed across multiple screens, allowing you to create
your own user-specific control platform. Most control boxes or buttons have "right-
mouse-click" functionality that gives you easy access to all respective parameters.

Press the "Win" menu-button (in "Control" window) to open any window.
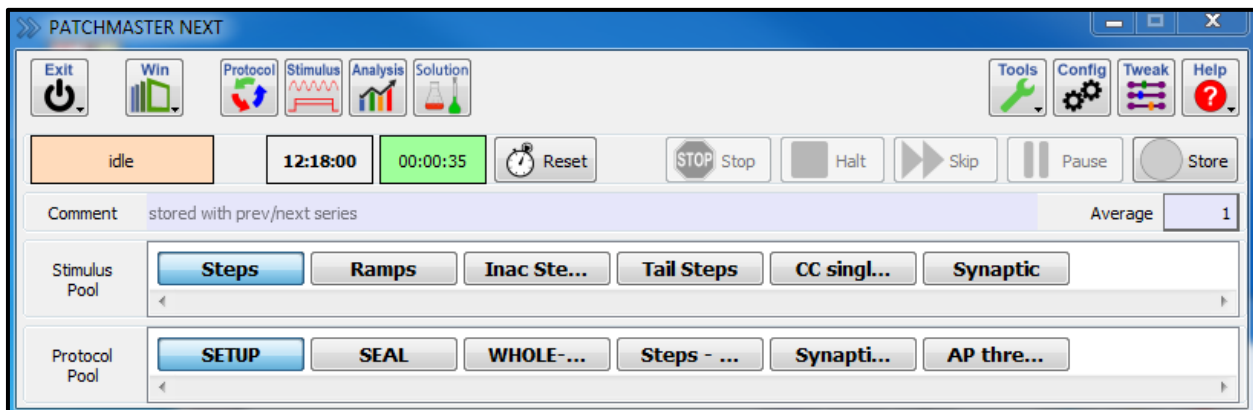
- **Control** window: This is the "main" window, from where you can run your entire experiment. Think of it as the "joystick" that you use to fly the helicopter". Closing this window will end (quit) PATCHMASTER NEXT (PMN).
  - <u>Exit</u> menu: Quit or end PMN. Pressing "Quit" opens the "Close window", allowing you to either:
    - *Save & Exit:* Saves (overwrites) the currently loaded configuration file and gives you the option to save any support files (pgf, onl and pro).
    - *Exit:* Same as above, but configuration file is NOT saved.
    - *Cancel:* Closes the "Close window" and returns to PMN.
  - <u>Win</u> menu: Drop-down window gives access to all windows.
  - <u>Protocol</u> button: opens "Protocol Editor" window.
  - <u>Stimulus</u> button: opens "Stimulus Editor" window.
  - <u>Analysis</u> button: opens "Analysis Editor" window.
  - <u>Solutions</u> button: opens "Solution Editor" window.
  - <u>Tools</u> menu: Gives access to "Shortcut Keys" and "Color Settings".
    - *Shortcut Keys:* Each user can create & save their own settings, which will be automatically loaded with their configuration file. To easily find available commands, type name into "Search field".
    - *Trace/Graph Colors:* Opens "Color Editor" window. Each user can create & save their settings, which will be automatically loaded with their configuration file.
  - <u>Config</u> button: opens "Configuration" window. See below for more details.
  - <u>Tweak</u> toggle-button: allows to alter "View" features.
  - <u>Help</u> menu: access to common "Help" features.
    - Show Tooltips: on/off switch
    - HEKA Support: opens link to HEKA support site
    - HEKA Downloads: opens link to HEKA download site
    - Report a Bug: opens "new email" template (support@heka.com)
    - About PATCHMASTER NEXT: displays "Start-up screen"
  - <u>Recording Status</u> display: shows, if a recording is in progress or not (idle).
  - <u>Time-of-the-day</u> display: shows the time (24-hour) of the computer timer
  - <u>Timer Time </u>display: stopwatch time, runs continuously, set to "zero" when PMN starts or by pressing the "Reset" button.
  - <u>Experiment Control</u> buttons: use these buttons to easily control your experimental procedure. Please make sure the "Store" button is **enabled**, if you want to save your recordings to the open dat-file!

- *Stop* button: stops the currently running sweep immediately. Please make sure the "Save after Stop" box is checked (see "Configuration" window), otherwise the data from the stopped sweep will NOT be saved!
- *Halt* button: stops the currently running series after the sweep has finished. All recordings will be saved to the open dat-file, depending on the "Write-Tree-File" settings (see "Data Tree" window).
- *Skip* button: only for "Protocol" control. Pressing this button will "skip" to the next "protocol event" after the end of the currently recorded sweep (series will be stopped).
- *Pause (Resume)* button: pressing the "Pause" button will stop the currently running series after the sweep is complete. In addition, the display of the button changes to "Resume". Pressing "Resume" will continue the series or protocol.
- *Store* button: when this button is **inactive**, NO recordings will be saved! If you want to save your recordings, please make sure that this button is **active (red)**!

- *Comment* box: text can be entered here, it will be added to the data of the next "Series" and automatically to all future sequences [page 97]
- *Average* box: if a number is added here (other than "1"), the next "Series" will be repeatedly run by that amount and the average response is saved [important additional info on page 98]
- Stimulus Pool area: Every "Stimulation Sequence" (former PGF-template) of the pgf-file that is loaded in the "Stimulus Editor", can be started here with a **double-click** on the respective button. Use the bar underneath to scroll through the entire list of "Stimulation Sequences" that are available.

- Protocol Pool area: Every "Protocol" (script) of the pro-file that is loaded in the "Protocol Editor", can be started here with a **double-click** on the respective button. Use the bar underneath to scroll through the entire list of "Protocols" that are available.
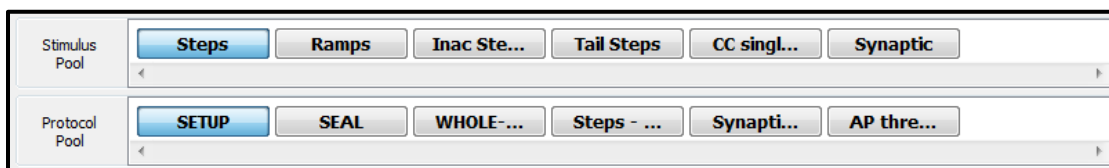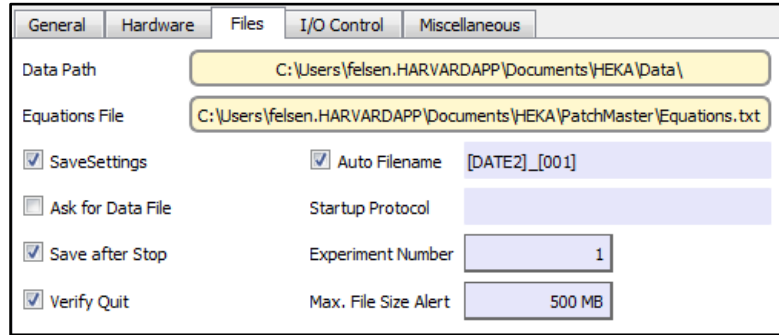
- **Configuration** window: to open this window press "Config" in the "Control" window; press "Save" to save all settings and parameters to the presently open "Configuration-file (ini-file)", we strongly recommend that each user creates their own "ini-file" (see chapter 3 & 4); [page 45 to 70]
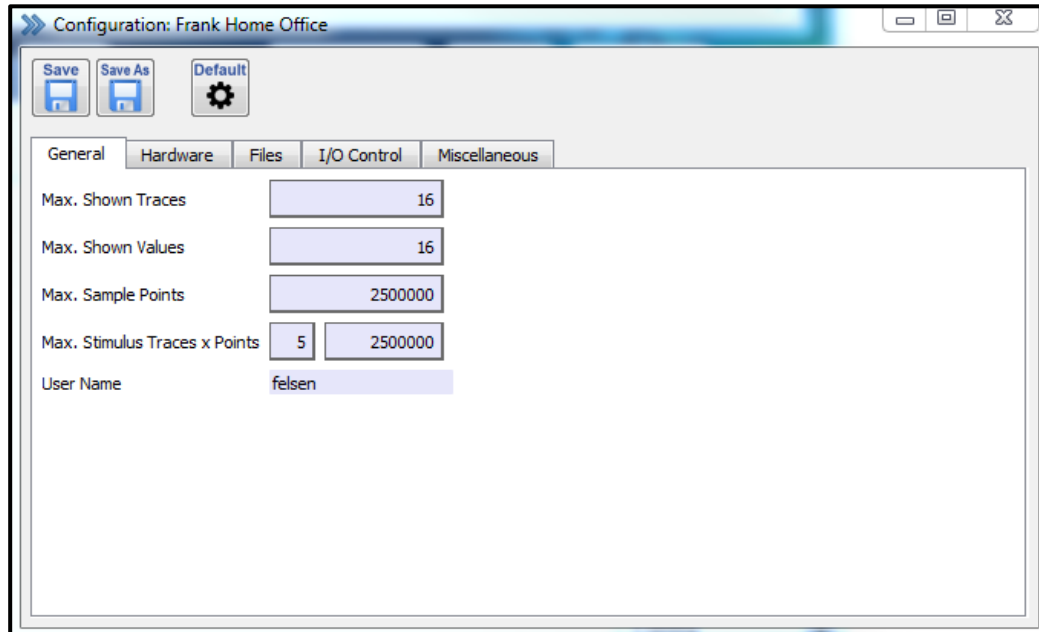
- o Set-file control buttons: (Save, Save As and Default) use these buttons to save and/or reset configuration settings, please note that closing the "Configuration" window will enable the settings, but does **not** save them! The set-file can also be saved, when you close (quit) PMN (see chapter 3, 4).
- o General tab: "Number of Traces" and "Max. Sample Points" can be set here
- o Hardware tab: select and specify connected hardware; enable or disable "Demo Mode"; enable extensions; set (display) scaling factors; set AD-channels for "Current" and "Voltage"; [page 48 to 55]
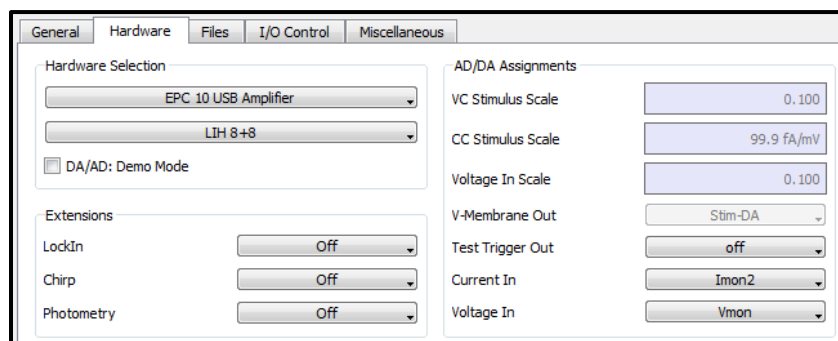
- o Files tab: set path for
  "Data-file" and
  "Equation-file"; set
  other important
  options (auto filename,
  save after stop, etc.);
  [page 56 to 58]

General	Hardware	Files	I/O Control	Miscellaneous

Data Path   C:\Users\felsen.HARVARDAPP\Documents\HEKA\Data\

Equations File   C:\Users\felsen.HARVARDAPP\Documents\HEKA\PatchMaster\Equations.txt

☑ SaveSettings   ☑ Auto Filename   [DATE2]_[001]

☐ Ask for Data File   Startup Protocol

☑ Save after Stop   Experiment Number   1

☑ Verify Quit   Max. File Size Alert   500 MB

- o I/O Control tab: set input/output and scaling options; [page 60 to 65]
- o Miscellaneous tab: "Analysis" and "Test Pulse factor" settings

- **Amplifier** window: using this window, you have full manual control of the amplifier. The window also displays information on current, voltage and resistance values, corresponding to the recording conditions at any given time during the experiment. Using the "Protocol Editor", you can create "protocols" that allow full automatic control of this window.
  Please note that this document does not explain all available buttons or functionalities. For more detailed or missing information, please consult the original PATCHMASTER manual (2x90.2) [page 75 to 88].

  - o Test toggle-button: pressing this button activates a test pulse and opens the corresponding control interface (see picture). Voltage stimulus pulse and current response trace are always both displayed.
   - ▪ *Test Pulse* area: controls for pulse source and settings (see chapter 1)
    - ▪ *Scaling* area: controls to scale pulse and current trace, please note that these settings do NOT affect the actual DA or AD scaling
    - ▪ *Sound* area: on/off switch for sound feature, specific sound values can be set by pressing "Tweak" (Sound/Zap tab)
    - ▪ *ZAP* button: pressing this button executes a single pulse, specific pulse parameters should be set prior with "Tweak" (Sound/Zap tab)

Test Pulse		Scaling	
Pulse	On	I-scale	1.0 x
Mode	single pulse	V-scale	10.0 x
Amplitude	-10.0 mV	I-offset	0.00
Duration	5.0 ms	V-offset	500 m
Input ADC	Imon2		
Overlay	Off		
Sound		Zap	
Sound	Off		Zap

Please note that the "Test Pulse" window does NOT have to be active for the yellow "Auto" buttons to be functional.

o <u>Copy R_{pip}</u> button: copies the presently displayed resistance value to the "Pipette Resistance" parameter field. This information will be saved to the data-file ("Data Parameter" window) [page 87 and 195]

o <u>Reset</u> button: "resets" entire "Amplifier window" back to start-up condition (except for "Offset" values).
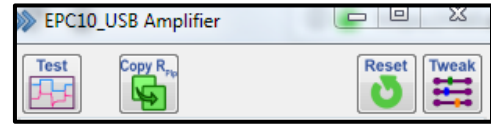
o <u>Tweak</u> toggle-button: opens/closes interface area for user to access additional amplifier-related features, parameters and display options.

- *View* tab: on/off switch for display possibility of all major amplifier functions. Switching a feature "off" only removes it from the display in the "Amplifier" window, it does NOT remove its functionality

- *Hardware* tab: important amplifier-related controls & settings [page 87]
  - ➤ Gentle Switch: on/off switch
  - ➤ Current-Clamp Gain: select scaling factor
  - ➤ V-mon Gain: select "10 x" or "100 x"
  - ➤ Ext. Stim. Input: set stimulus parameters
  - ➤ Low Freq. Voltage Clamp: controls for LFVC

- *Tools* tab: specific utensils for amplifier testing and calibration (parts of former EPC10 main menu [page 39 to 44])

- *Compensation* tab: edit and set parameters for "CFast" and "CSlow" compensation algorithms [page 39 to 41]

- *Sound / Zap* tab: edit parameters for "Sound" and "Zap Pulse" [page 86]
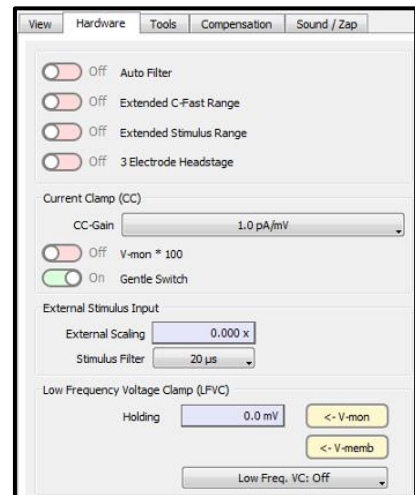
o <u>Readout</u> displays: (light-green background) continuously show current, voltage and resistance values, respective to the present recording config.

- *I-mon* display: Voltage-Clamp -> "I-hold", Current-Clamp -> "I-inject"
- *V-mon* display: Voltage-Clamp -> "V-hold", C-Clamp -> "V-membrane"
- *R-memb* display: Voltage-Clamp -> 3 possible kinds of resistances, depending on state of pipette condition

  - ➤ "open pipette in bath" -> "R-pipette"
  - ➤ "cell-attached patch" -> "R-seal"
  - ➤ "whole-cell-patch" -> "R-membrane"

  Current-Clamp mode ("whole-cell-patch" only) -> "R-membrane"

- o Recording Mode drop-down window: switch between recording modes, PMN always applies the physiological convention, according to the mode setting (downward deflection denotes inward current of cations)
  - *Whole Cell* mode: Voltage-Clamp whole-cell configuration
  - *C-Clamp* mode: Current-Clamp whole-cell configuration
  - *On Cell* mode: Voltage-Clamp cell-attached configuration
  - *In Out* mode: Voltage-Clamp Inside-Out configuration
  - *Out Out* mode: Voltage-Clamp Outside-Out configuration
- o Amplifier Controls section: this section includes all controls for the amplifier; the display of some controls can be switched on or off (Tweak – View-tab); all features can be manually or automatically (Protocol Editor) controlled; a blue outline indicates that the amplifier is in "Voltage Clamp" mode, a green outline indicates "Current Clamp"; for more details on all controls and features see the original PATCHMASTER (2x90.2) manual [page 75 to 88].
- o Predefined Protocol buttons: three buttons (SETUP, SEAL, WHOLE-CELL) are available to easily establish the desired recording configuration. Pressing a button, starts the protocol with that name. Using the "Protocol Editor" you can edit and fine-tune each protocol to your specific application [page 78].
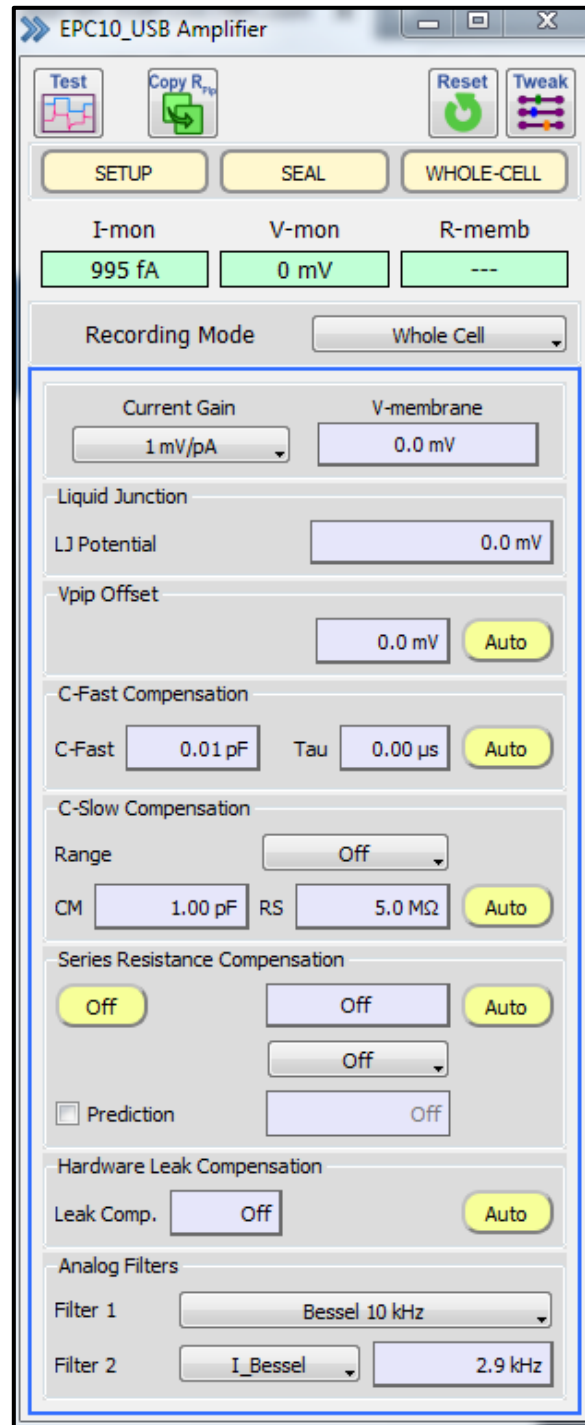
- **Oscilloscope** window: displays live recordings and replayed data traces. Furthermore, you can perform preliminary analysis (measure, scan, etc.) using improved features and display parameters [page 71 to 74].
    - o Osci button: opens/closes drop-down menu (former main menu "Display" tab), allowing you to access all display related features [page 31 to 35].
    - o Cursors, Measure and Scan buttons: allow you to perform offline, preliminary analysis. Right-mouse-click on values (yellow fields) opens **export possibilities** to "Notebook" and "Clipboard". Redesigned to improve usability, while at the same time increasing functionality.
        - ▪ *Cursors* button: on/off switch for cursor-display, newly implemented here, still accessible from analysis methods [page 172, FM manual p.28]
        - ▪ *Measure* and *Scan* buttons: on/off switch, new handling and functionality
    - o Freeze, Wipe and Repaint buttons: controls for display refreshing [page 72]
    - o Traces button: One-click access to "Osci – Trace Properties" window, allows to quickly switch on/off trace display ("Show" box)
    - o Tweak button: on/off switch for "auto-scaling" options [page 73] and "data trace location" box [page 72]
    - o Trace Scaling controls: access to trace scaling and display options [page 73]
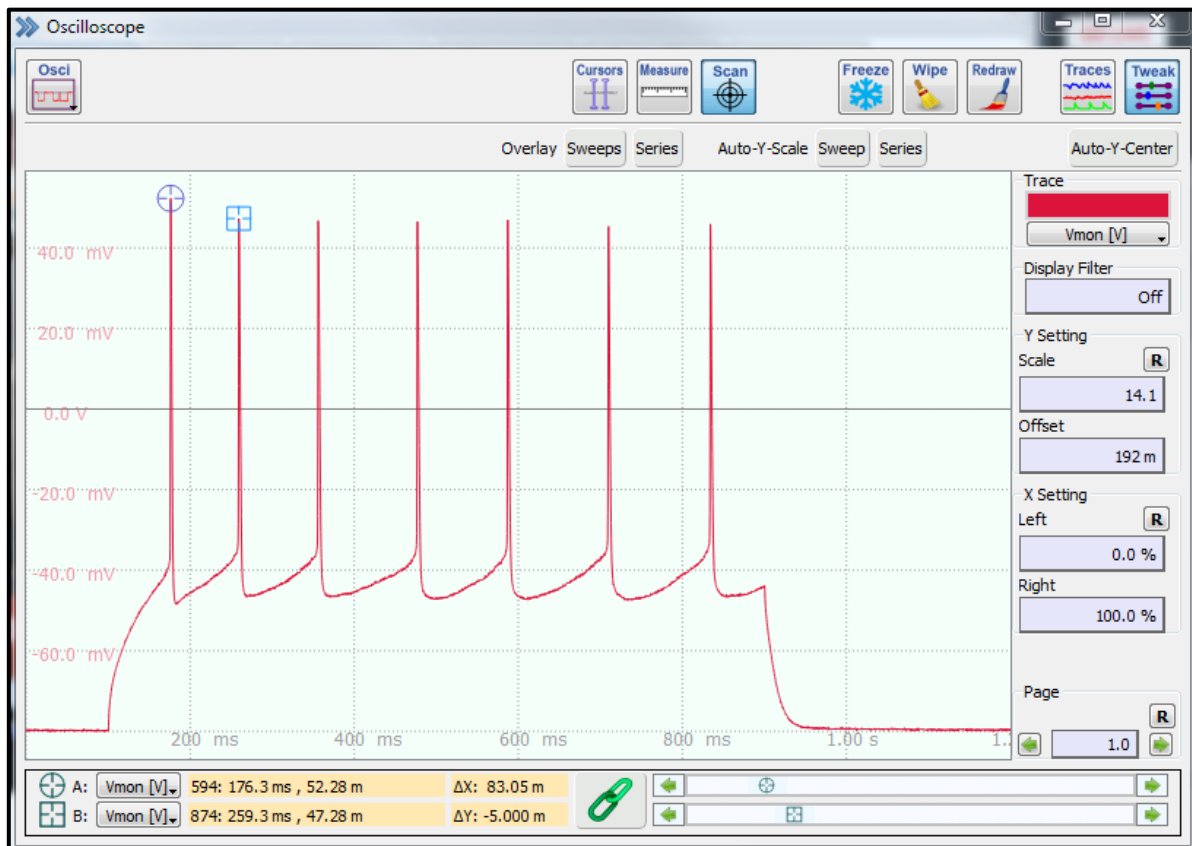
Data trace curtesy of Gilles Martin, U-Mass, Worcester

- **Data Tree** window: re-designed for improved functionality and easy to use data selection, storage and organization; File, Data and Buffer buttons allow for easy access to data related functions; scroll-bar navigation.
  - o File button: gives access to data file related features (open, close, create-new, etc.) and data save options [page 22]
    Please note that modified data files will be automatically saved (overwritten), when PMN or the data file itself is closed (File – Close). There will be **NO warning message**! We strongly recommend to open original data files only in "Read-only" mode. To do data modifications (analysis), we recommend creating and using copies of the original data files.
  - o Merge button: allows to merge two data files into one [page 22]
  - o Data button: gives access to data export/import options (parts of former main menu "Replay" tab) and data analysis selection [page 26 to 31]
  - o Buffer button: gives access to all "Buffer" features and analysis options (former main menu "Buffer" tab) [page 36 to 37]
  - o Lock-symbol: indicates if currently loaded data-file can be modified or not, open data file with "Modify" to enable editing
  - o Tweak toggle-button: enables display of button-field (Replay, Auto, etc.)
    - ▪ *Trace* drop-down window: sets lower level of display (ex.: "Trace" will display data from "Group" to "Trace")
    - ▪ *Colors* toggle-button: switches trace colors on or off
    - ▪ *All other* buttons: adding labels & text, selection for analysis, display options [page 101 to 103]
  - o Data display: the recorded data is categorized into 4 levels [page 101]
    - ▪ *Group (cell)*: we suggest using the "New Group" button (File-menu) to create a new group for each newly patched cell
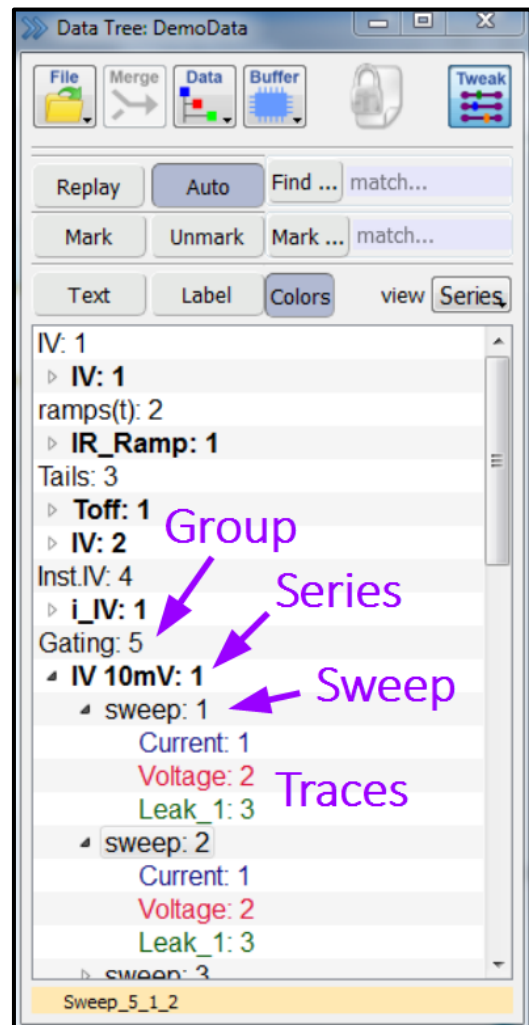
- *Series:* stimulation sequence (steps, ramps, etc.)
- *Sweep*: each "Series" can have multiple iterations
- *Trace(s)*: each "sweep" can record multiple data signals; "Text" can be added to and the "Label" can be edited for "Group" or "Series" levels, whereas "Sweep" and "Trace" levels can only accept "Label editing"
    - Comment line display-only: displays the text that was entered in the "Control" window, this text cannot be edited

Handling of old PATCHMASTER data files

Data files created with PATCHMASTER (2x90.3) can be opened directly in PMN.

Trying to open a dat-file created with older versions will cause the following warning message:
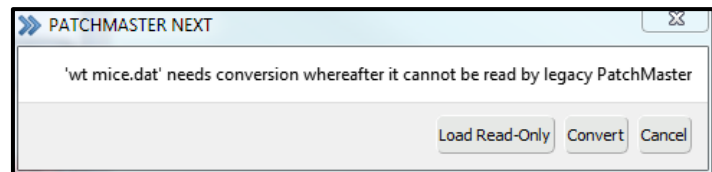

Please note that after converting data files into the new PMN format, they can no longer be opened with the old PM. However, a back-up file with the old format will be automatically saved (*.bak).

- Load Read-only: Allows you to load any dat-file without conversation. No modification (editing or analysis) of the data file is possible
- Convert: Pressing this button will convert the data file into PMN format and it cannot be opened anymore with older (<2x90.2) PATCHMASTER software
- Cancel: closes the warning message without any action being performed

Version 2x90.3 of PATCHMASTER and FITMASTER can open PMN-format data files.

- **Stimulus Editor** window: former "Pulse Generator" window [page 105 – 134]; completely new, intuitive interface to allow easy programming of complex stimulation sequences; functions and features have been sorted to increase usability and work-flow; a "Segment Editing" window allows for clear and understandable editing of segments; press the "Stimulus" button in the "Control" window to open "Stimulus Editor" window.
    - File control buttons: (Load, Merge, Save and Save As) an unlimited number of pgf-files can be created; each file can have over thousand different stimulation sequences; we recommend that each user creates their own pgf-files to avoid accidental editing of stimulation sequences from other users
    - Sequence checking toggle-switch: allows to turn off internal consistency checking routines, if parameter changes might cause intermediate invalid configurations

- o Mode Selection: allows to switch between "editing" and "review-only" mode. "Editing" allows to create and modify stimulation sequences (default). "Review-only" is available after a dat-file is loaded to "review" the stimulation sequence that was used to record the respective data (former "Show – PGF-template" in the "Replay" menu)

- o Stimulus Pool column: display of all stimulation sequences from the currently loaded pgf-file; **single-left-click** to select a sequence
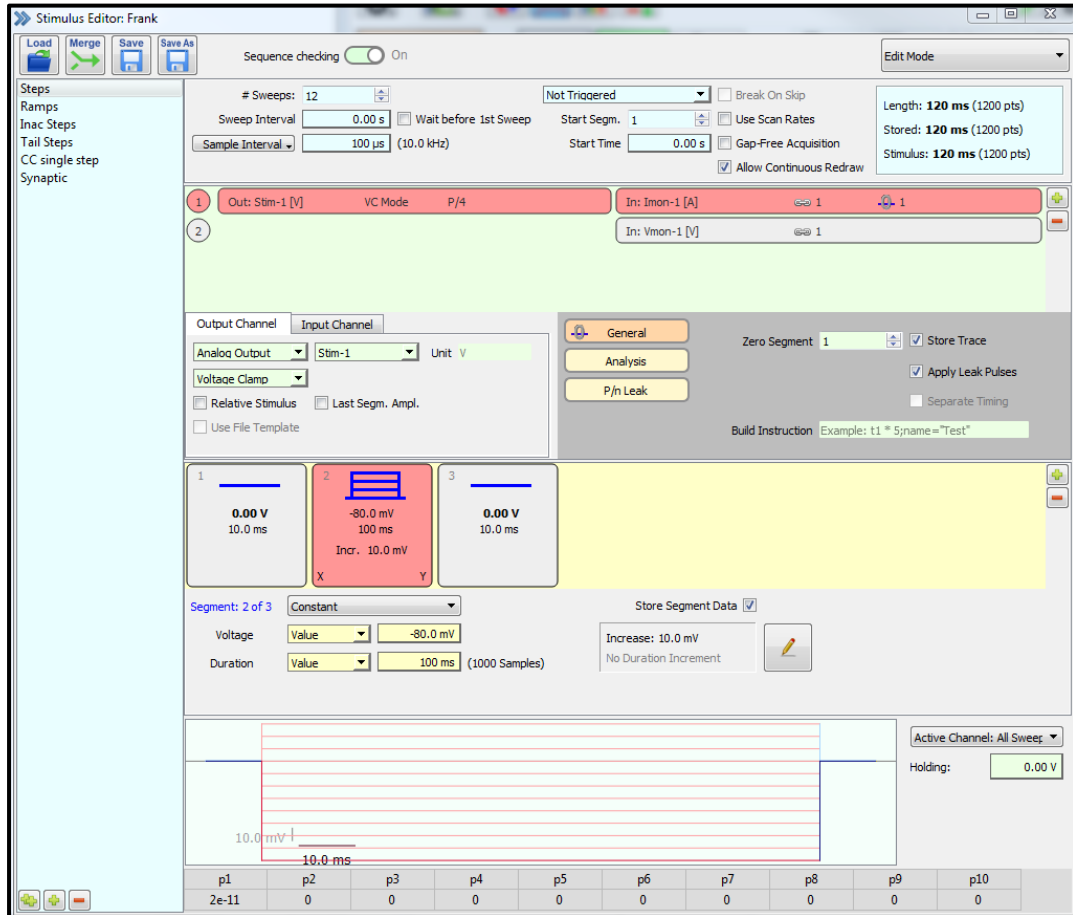
- o Timing settings & display area: here you can set timing parameters (acquisition freq., intervals, start times, etc.) and number of sweeps [page 108 – 109], also displays recorded points and time

- o Stimulation (DA) and Acquisition (AD) channel selection: Use green "plus sign" to open "Select Input and Output" window, make desired selections and press "OK" to add new channels; press red "minus sign" to delete selected row of channels

- o Output and Input Channel tabs: here you can edit either Output (DA) or Input (AD) channel parameters of the selected channel row [page 119 – 121]

- o Additional channel specific parameters: depending on your selection, several more parameters can be set here
  - ▪ *General* button: allows to enable "store" (data will be saved), enable "Leak Pulses" and "Separate Timing" [page 124, 128]
  - ▪ *Analysis* button: here you can enter the link to a specific "Analysis Method" and other relevant analysis information [page 129]
  - ▪ *P/n Leak* button: appears only when "Leak Pulses" is checked in the "General area"; here leak-test-pulses can be specified [page 131 – 133]
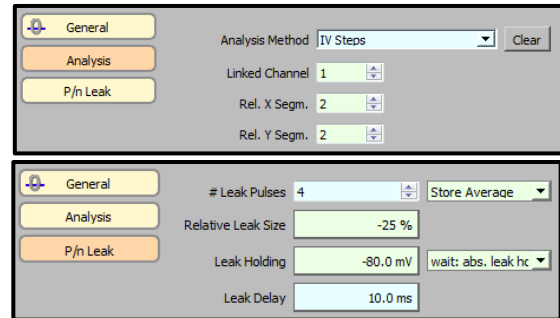
- o Segment area: here you can arrange segment elements to build individual stimulation sequences, press the green "plus sign" to add a segment element and the red "minus sign" to remove the highlighted one; drag & drop elements to reposition order

- o Segment Editing area: edit selected segments; set kind of stimulation, amplitude and duration; press "Edit button" to open "Set Segment Increments" window (see below) [page 125 – 128]

- o Set Segment Increments window: newly designed window to easily set parameters for "amplitude" and "duration" increments, pressing "OK" implements these settings, range information for X- and Y-axis

- o Cartoon of stimulation sequence: drawing of segments [page 130]

- o PGF Parameters entries: can be used to set "Amplitude" and "Duration" values, allows for easy changing of the same parameter in multiple locations [page 134]
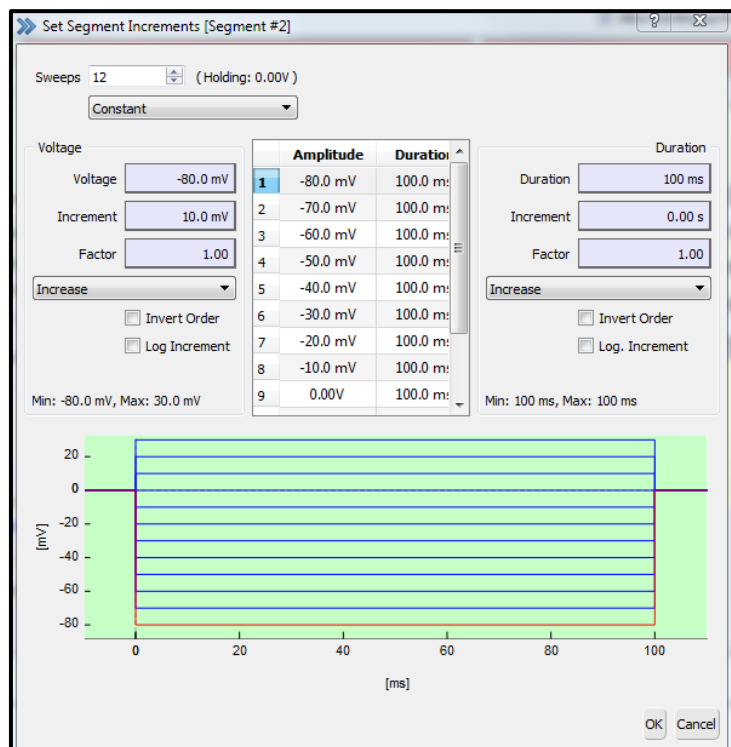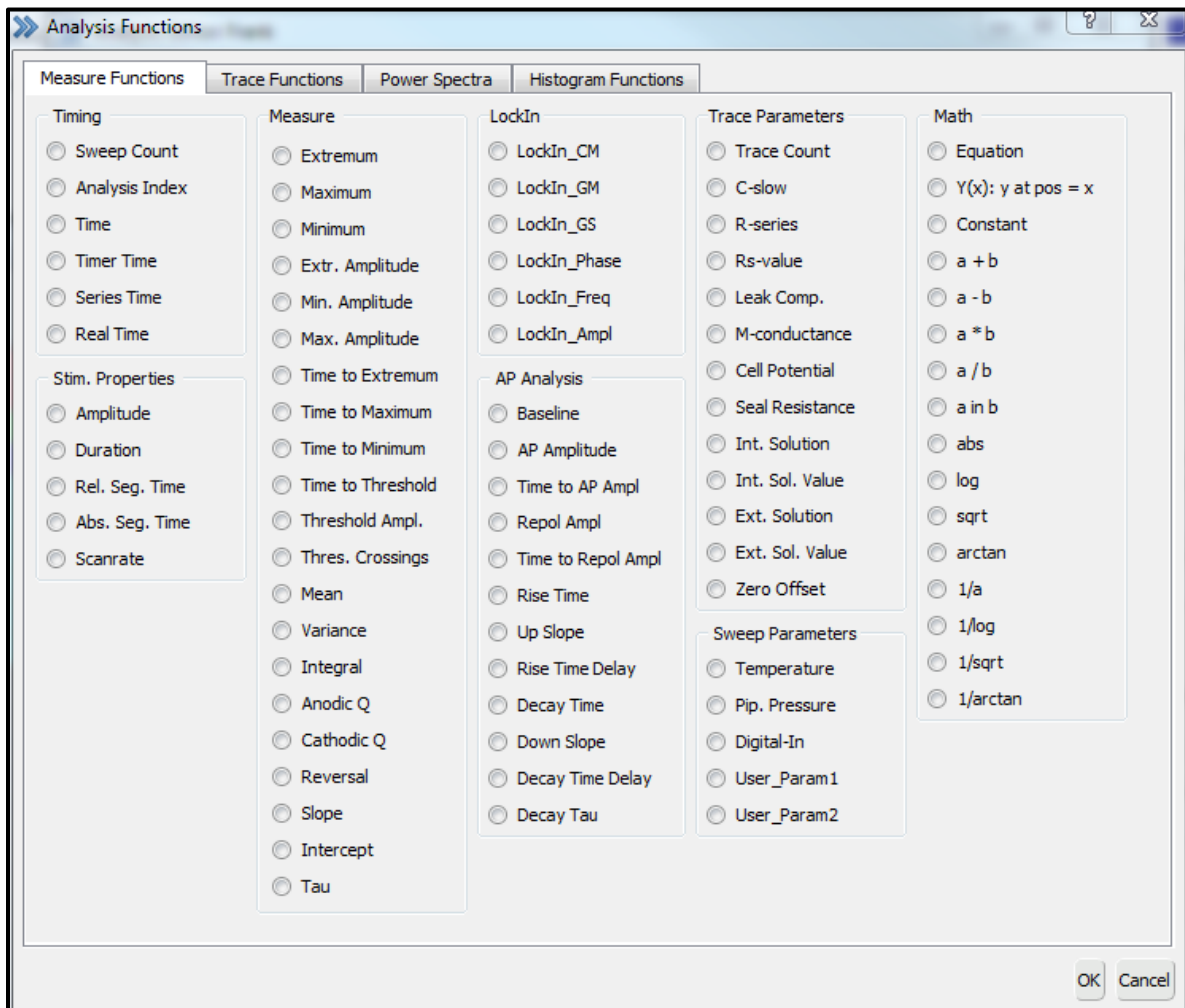
- **Analysis Editor** window: completely new, intuitive interface to allow for easy "Analysis Method" creation; functions and features have been sorted (different "tabs" & right-mouse-click options) to increase usability and work-flow. Can be opened from the "Control" window by pressing the "Analysis" button.
  - o <u>Onl-file control</u> buttons: (Load, Merge, Save and Save As) an unlimited number of onl-files can be created; each file can have thousands of different "Analysis Methods"; we recommend that each user should create their own onl-files to avoid accidental editing of "Analysis Methods" from other users
  - o <u>Main Control</u> drop-down window: enable or disable analysis functionality
    - *Use Selected Method*: the selected (highlighted) analysis method is applied, independent from the link-setting in the "Stimulus Editor"
    - *Automatic Stimulus Control*: enables the link-setting of the "Stimulus Editor" and will execute the respective analysis method
   - *No Analysis*: switch off any analysis functionality
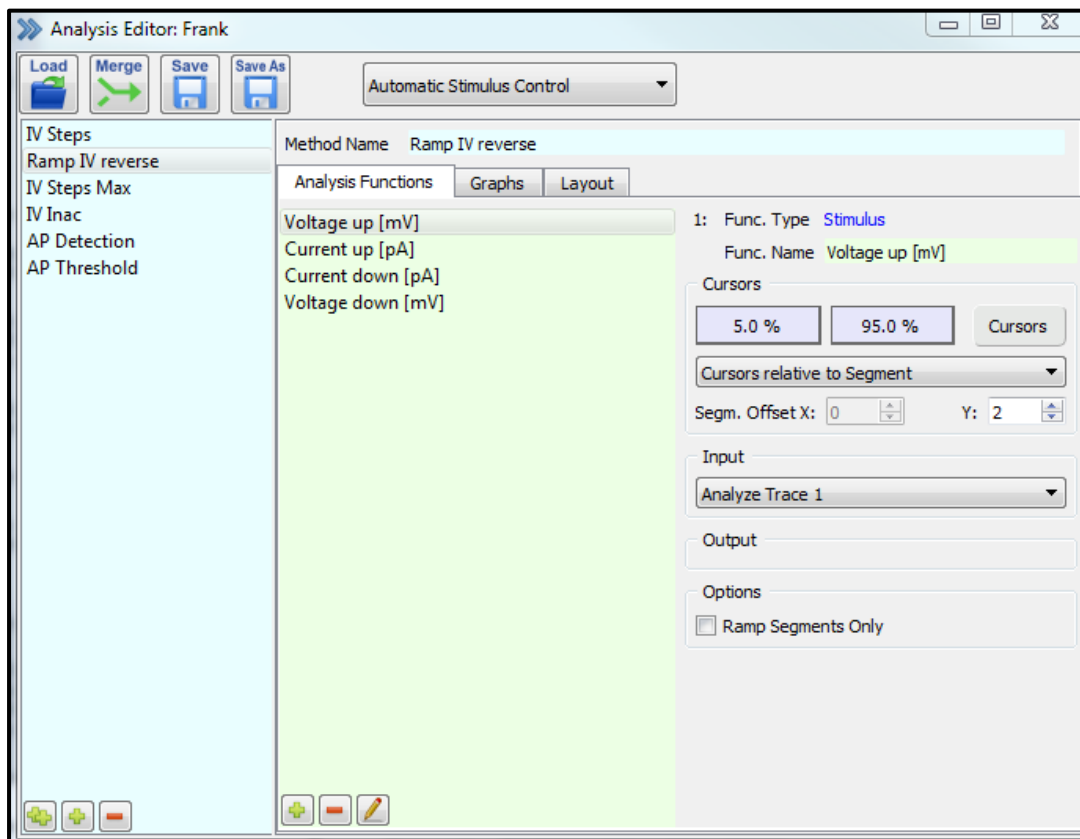
  - o <u>Analysis Method</u> column: display of all "Analysis Methods" from the currently loaded onl-file; **single-left-click** to select a sequence; with the buttons on the bottom, methods can be duplicated, added or deleted

- o <u>Method Name</u> text-field: name of selected "Analysis Method" is displayed and can be edited here
- o <u>Main Tabs</u> functionality: to create and edit an "Analysis Method", you use these three tabs; analysis functionality and graph displays are easily accessed
  - *Analysis Functions*: add, delete and edit (bottom buttons) analysis functions to the selected analysis method [page 171 to 182]
    Press "Add" or **"Edit"** to open the "Analysis Functions" selection window
  - *Graphs*: up to 12 "Graphs" can be created, each one can have up to 4 different plots or curves; **right-click** on "Graph_#" opens additional editing possibilities; scaling and display options are available here
  - *Layout*: set where to display each "Graph_#", two "Graph Layout" windows are available [page 183 to 186]

- **Protocol Editor** window: completely new, intuitive interface to allow for easy "Protocol" creation and editing; GUI features 4 columns, allowing for easy drag & drop placement of "Events" within the "Protocol" column; each "Event (or command)" can be edited in the 4ᵗʰ column; right-mouse-click options available. Can be opened from the "Control" window by pressing the "Protocol" button.
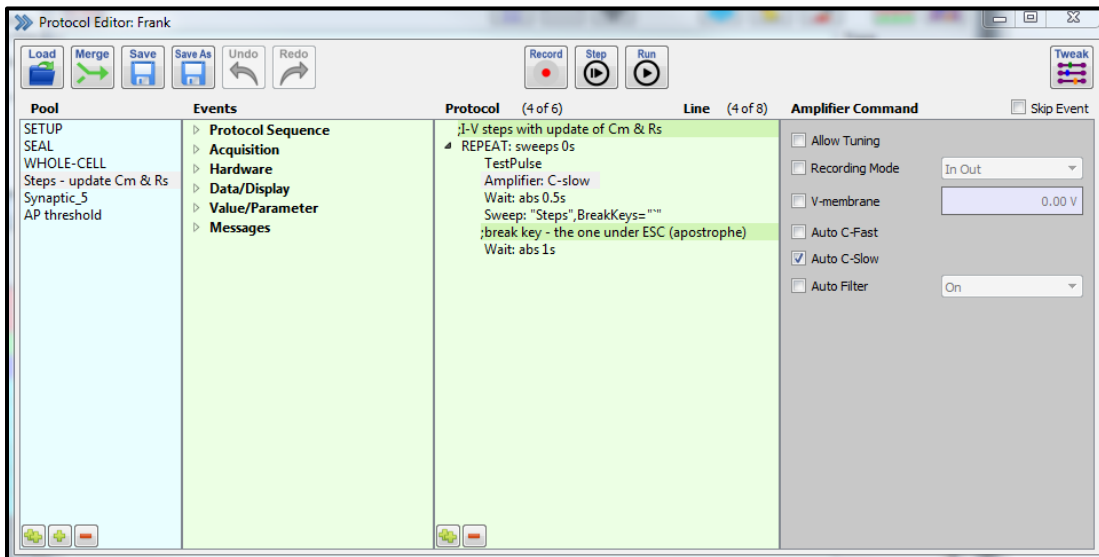

- o <u>Pro-file control</u> buttons: (Load, Merge, Save and Save As) an unlimited number of pro-files can be created; each file can have up-to 99 different "Protocols"; we recommend that each user should create their own onl-files to avoid accidental editing of "Analysis Methods" from other users
- o <u>Undo/Redo</u> buttons: quickly edit last input or use with "Tweak" table
- o <u>Macro Command</u> control-buttons: allow recording & play-back of macro commands [page 136]
- o <u>Tweak</u> button: opens/closes table of previous commands, select to specifically "undo" certain commands
- o <u>Pool</u> column: display of all available protocols in the presently loaded pro-file; with the buttons on the bottom, methods can be duplicated, added or deleted; **single-left-click** to select (right-click options available)
- o <u>Events</u> column: display of all available "Events" or "Commands" that can be used to create a "Protocol Sequence" (3ʳᵈ column) by simply drag & drop to desired location in sequence
- o <u>Protocol</u> column: here you can create specific "Command (Event) Sequences" for the selected "Protocol" (1ˢᵗ column); drag & drop to desired location; **single-left-click** to select a specific "event" or "command" and

display its specific, adjustable parameters in the 4th column; **single-right-click** opens additional options; use bottom buttons to duplicate or delete events, check "skip" box to exclude selected event or command from sequence

- o 4th Column: available parameters depend on selected "Event" or "Command" in 3rd column (Protocol); respective "Event" or "Command" can be modified or further specified here

- **Notebook** window: displays analysis results and warnings or error messages (Log Icon); can be opened from the "Win – drop-down" window in the "Control (Main)" window; text can be saved & exported [page 37 and 201]

  - o Note, Edit, Find drop-down windows: Text editor functionality menus, former main menu "Notebook" tab; possible to save notebook text as txt-file
  - o Science button: toggles between scientific notation and engineering format
  - o Log button: toggles between "Notebook" and "Error-Log"
 - o Trash button: clears (deletes) entire Notebook text
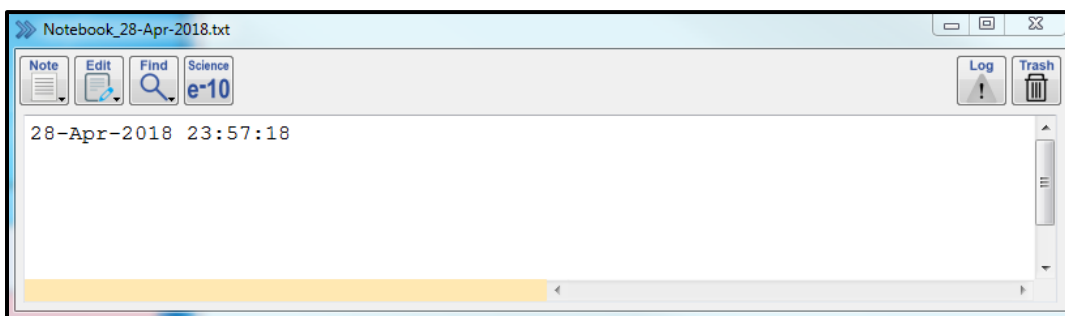
- **Graph Layout** windows: display of analysis results (depends on settings in "Analysis window"), two "Graph Layout" windows can be activated from the "Layout" tab in the "Analysis Editor" window [page 182, functionality]

  o Reset button: re-scalces all graphs to their maximum size and distributes them equally

  o Tweak button: toggles between show/hide of graph selection boxes
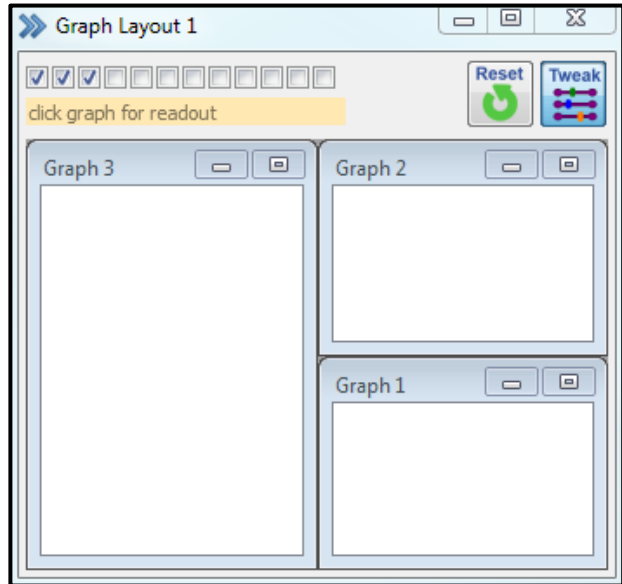
- **Data Parameter** window: newly designed (tab-based categories); all parameters of the experiment are automatically saved to the presently open dat-file [page 189 to 200]

  o Filter button: toggles between "filtered" and "all-tabs" view, to select a parameter to be displayed with the "Filter-view", check the box in front of it
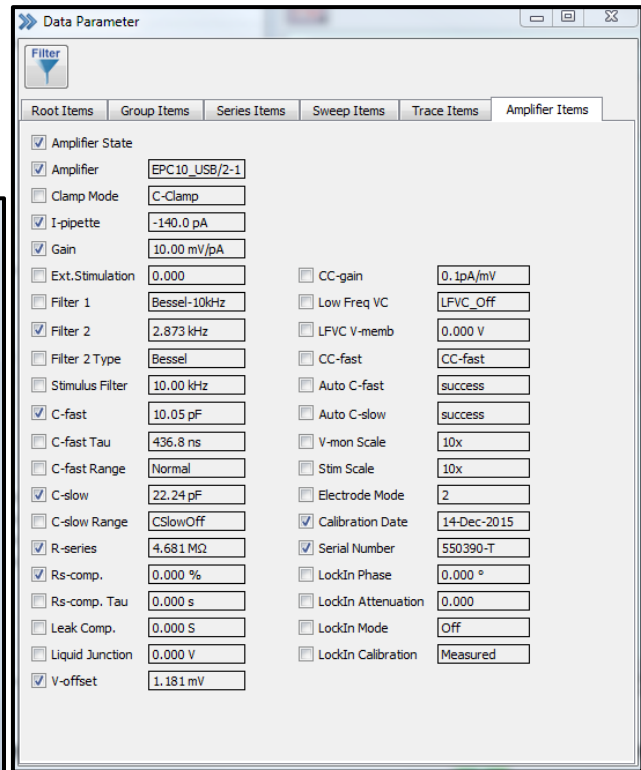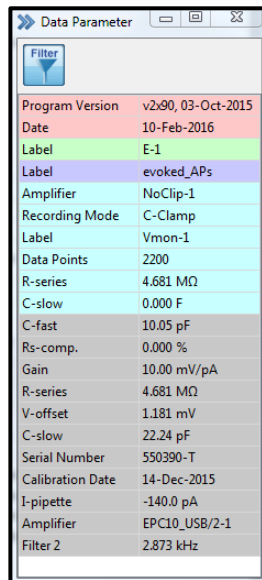

- **All other** windows: to see more details on any other windows (Calculator, LockIn, Photometry, Chirp, etc.), please consult the "old" PATCHMASTER 2x90.2 manual that can be downloaded from our website at: www.heka.com (Downloads – Patchmaster)

6. How to Start a New Experiment

Here we describe how you can start a new experiment in a few easy steps.

- **1st step**: You need to create or open (modify) a data file. In the "Data Tree" window, click "File" and select the desired action (New or Modify); *we recommend creating a new data-file for every new experiment-day.*

- **2nd step**: This step is optional! Rename "label" (ex. "MC-10") and/or attach "text" to the newly created "Group" level. **Right-click** to access additional options.

- **3rd step**: Establish desired recording configuration (see chapter 7).

- **4th step**: Open or create respective stimulation sequence (see chapter 8), run experiment using "Control (Main)" window buttons. Make sure that "Store" button is enabled, if you want to save the data to the hard drive.

- **Next cell**: to keep the data organized, *we would suggest pressing "File – New Group" to create a new group level for the next cell that will be recorded.* Again, feel free to rename the label and/or add text to this new group level.
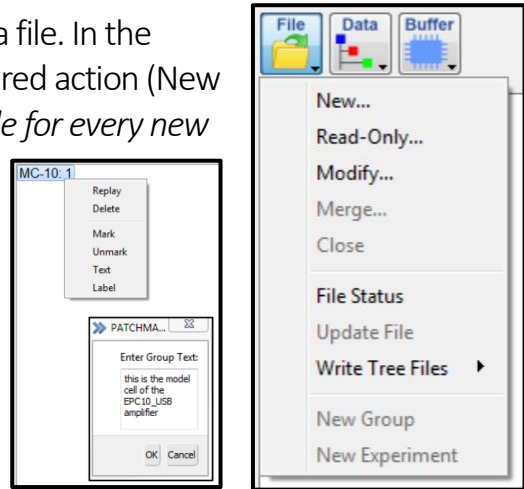
7. Establish Recording Configuration

After opening or creating a dat-file, the next step is to establish a recording configuration with your target cell. To demonstrate this procedure, we use an EPC10_USB-1 (single) amplifier with the model cell (MC-10) connected to the headstage (probe). Here we simulate the whole-cell recording condition.

You can control the amplifier **manually (a)** or **fully-automatic (b)** by using the light-yellow "Main Protocol" buttons [page 78].

a) Manual Amplifier Control: Controls of the amplifier can be accessed always from the "Amplifier" window (see chapter 5).

- Press the "Reset" button to make sure that the status of the amplifier window is in start-up conditions (values & switches reset, except "Offset values").

- Set the "Recording Mode" selection to the desired final recording configuration. This is an important setting, because PMN observes the physiological convention depending on the "Recording Mode" setting [page 79].

- To monitor the conditions at the pipette tip, please enable the Test Pulse by pressing the "Test" button. Please feel free to adjust the settings (chapter 5, [page 80]) to your liking. Please note, the test pulse does NOT have to be on for any "Auto" or "Main Protocol" buttons to be functional.

- Apply positive pressure to the patch pipette and lower it into the bath solution (we simulate this condition by setting the MC-10 to "10 M". Under visual control, move the pipette tip closer to the target cell, but make sure it does not touch any tissue yet!

- Enter the appropriate liquid-liquid-junction (LJ) potential value in the box and press the "Vpip Offset" Auto-button [page 81]. For practice purposes, we enter "5 mV".

- Press the "Copy Rpip" button to write the presently displayed "R-memb" value to the "Pip Resistance" parameter (Data Parameter window – Trace Items).

I-mon	V-mon	R-memb
-3.08 pA	-5 mV	1.67 GΩ

Recording Mode — Whole Cell

Current Gain	V-membrane
1 mV/pA	-5.0 mV

Liquid Junction
LJ Potential — 5.0 mV

Vpip Offset — 5.0 mV — Auto

- Under visual control, lower the tip towards the target cell. Observe when it touches the cell membrane (dip forming), immediately release the positive pressure and apply slight negative pressure. Observe how the amplitude of the current response from the Test Pulse gets smaller.

- When the current amplitude is sufficiently closed (flat line), set "V-membrane" to "-60 mV" (or any other expected resting membrane potential). Observe how the overall current response trace moves down and away from the zero line. Please note that "R-memb" now (cell-attached-patch conditions) displays the "Seal Resistance".

- Try to improve the "Seal Resistance" by applying increasing negative pressure. Observe the movement of the overall current trace towards the zero line. We would suggest to try and achieve a "Seal Resistance" of at least 1 – 10 G-ohm.

- We simulate the seal conditions or cell-attached-patch (CAP) by setting the model cell switch to the middle position.

- Optional: Feel free to adjust gain and/or scale settings to improve the visibility of the current response trace. When increasing the gain setting, please make sure the amplifier does not saturate (clipping). Clipping is indicated by a red display background of "I-mon" (Voltage-Clamp mode).

- To compensate the pipette capacitance, please press "Auto-C-Fast". The respective values are displayed in the "C-Fast Compensation" area and in addition, they are saved to the appropriate parameters at the "Data Parameter" window (Amplifier items – C-fast).
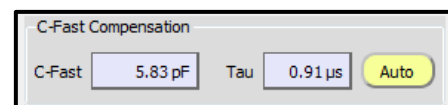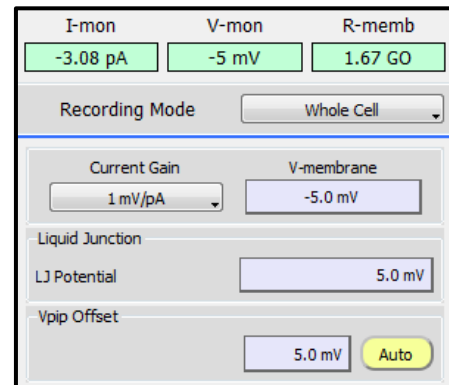
C-Fast Compensation
| C-Fast | 5.83 pF | Tau | 0.91 µs | Auto |

- Now try to establish the "whole-cell recording" configuration by opening the membrane patch underneath the pipette tip. Observe the current response trace, while applying "pounding" negative pressure pulses. Appearance of the "cell capacitance transient" current indicates successful electrical access to the interior of the cell (please note, the larger the transient amplitude, the smaller the access resistance).
We simulate the "whole-cell" recording condition by setting the MC-10 to "0.5 G".

- To neutralize the current that flows across the cell capacitance and cell input resistance, please press "Auto-C-Slow". The respective values are displayed in the "C-Slow Compensation" area and in addition, they are saved to the appropriate parameters at the "Data Parameter" window (Amplifier items – C-slow). Every time you press "Auto-C-Slow", these values will be updated in the "Data Parameter" window as well.
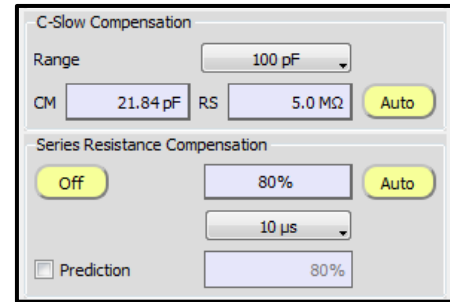
Please note, the "Range" (max. cell size) value is set automatically to "1000 pF" [p. 82].

- If you want to do voltage-gated (voltage-activated) experiments, you need to also compensate for the "voltage-drop error" caused by the access (serial) resistance. Please press the "Auto-Rs" button. This will automatically set a save level of percentage of error correction and set the level of feedback compensation to "10 μs" [page 83].

- Set the percentage to a higher number and press the "Rs-Auto" button again to further improve the Rs-error-compensation. To avoid "oscillations", we recommend to not exceed a setting of 80%. These values are saved as well in "Data Parameter" window.

b) Automatic Amplifier Control: Using the "Amplifier Protocol" Auto-buttons, the "whole-cell recording" configuration can be established with a few easy "clicks". Pressing a "Main Protocol" button will start its respective protocol.
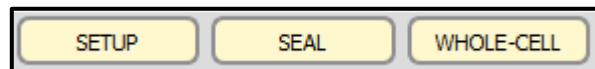

- Apply positive pressure to the patch pipette and lower it into the bath solution (we simulate this condition by setting the MC-10 to "10 M"). Under visual control, move the pipette tip closer to the target cell, but make sure it does not touch any tissue yet!

- Press the "Test" button to open the "Test Pulse" display.

- Enter the appropriate liquid-liquid-junction (LJ) potential value in the "LJ Potential" box [page 81]. For practice purposes, we enter "5 mV".

- Press the "SETUP" Auto-button. This will execute the "SETUP" protocol.

- Under visual control, lower the tip towards the target cell. Observe when it touches the cell membrane (dip forming), immediately release the positive pressure and apply slight negative pressure. Observe the closing amplitude of the current response trace of the test pulse.

- When the current amplitude is sufficiently closed (flat line), set "V-membrane" to "-60 mV" (or any other expected resting membrane potential). Observe how the overall current response trace moves down and away from the zero line. Please note that "R-memb" now displays the "Seal Resistance".

- Try to improve the "Seal Resistance" by applying increasing negative pressure. Observe the movement of the overall current trace towards the zero line. We would suggest to try and achieve a "Seal Resistance" of at least 1 – 10 G-ohm.

- Here we simulate the seal conditions or cell-attached-patch (CAP) by setting the model cell switch to the middle position.

- Press the "SEAL" Auto-button. This executes the "SEAL" protocol, which compensates the pipette capacitance currents. The "C-Fast" values are written to the respective data parameters.

- Now try to establish the "whole-cell recording" configuration by opening the membrane patch underneath the pipette tip. Observe the current response trace, while applying "pounding" negative pressure pulses. Appearance of the "cell capacitance transient" current indicates successful electrical access to the interior of the cell (please note, the larger the transient amplitude, the smaller the access resistance).
  We simulate the "whole-cell" recording condition by setting the MC-10 to "0.5 G".

- Press the "WHOLE-CELL" Auto-button. This executes the "WHOLE-CELL" protocol, which neutralizes the cell capacitance and input resistance.

- If you want to do voltage-gated (voltage-activated) experiments, you need to also compensate for the "voltage-drop error" of the access (serial) resistance. Please press the "Auto-Rs" button. This will automatically set a save level of percentage of error correction and set the level of feedback compensation to "10 µs" [page 83].

- Set the percentage to a higher number and press the "Rs-Auto" button again to further improve the Rs-error-compensation. To avoid "oscillations", we recommend to not exceed a setting of 80%.
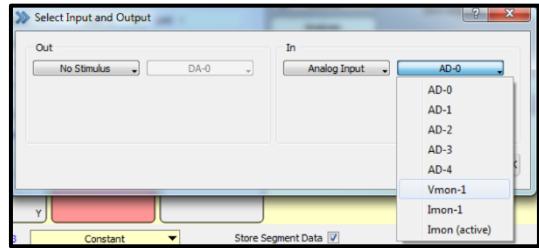
8. Create Stimulation Sequences

The newly designed "Stimulus Editor" allows for simple, intuitive creation of complex stimulation sequences. Here we demonstrate this procedure by creating three stimulation sequences from scratch.
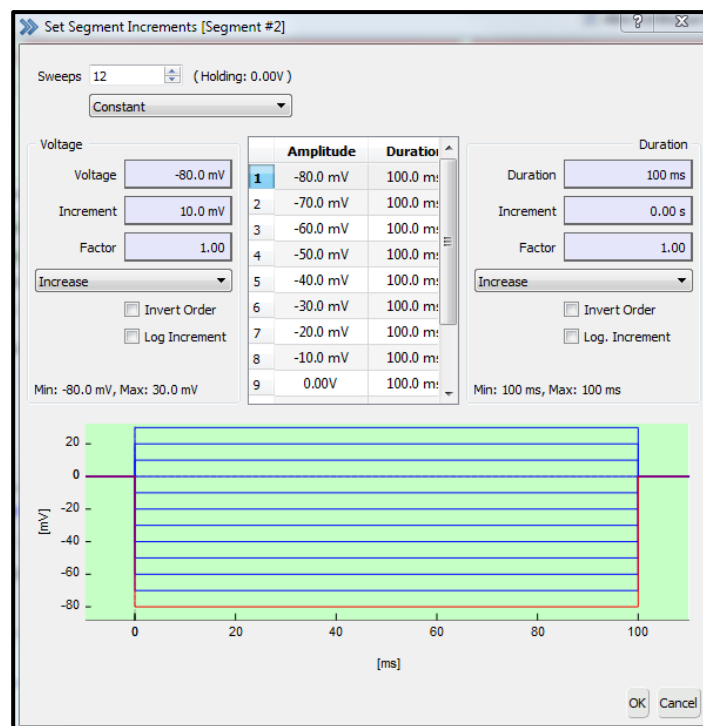
- **Voltage-Clamp Step Sequence**: this simple test sequence can be executed with or without "Leak-Subtraction" pulses. Please note that the current response traces to the leak pulses can be stored as average or individually [page 124]. If leak-traces are saved, the raw data traces (non-leak-subtracted) are always saved as well. This allows you to display your data either raw or leak-subtracted [page 31, "Subtract Leak Traces"]. If leak-traces are NOT saved (set "Store None"), then no raw traces are saved, ONLY the online leak-subtracted ones.

  - o Open the "Stimulus Editor" by pressing the "Stimulus" button in the "Control" window or by pressing "F8".
  - o (1) Press "single-cross" button to create a new sequence ("double-cross" to duplicate one, "minus" for deletion of the selected sequence).
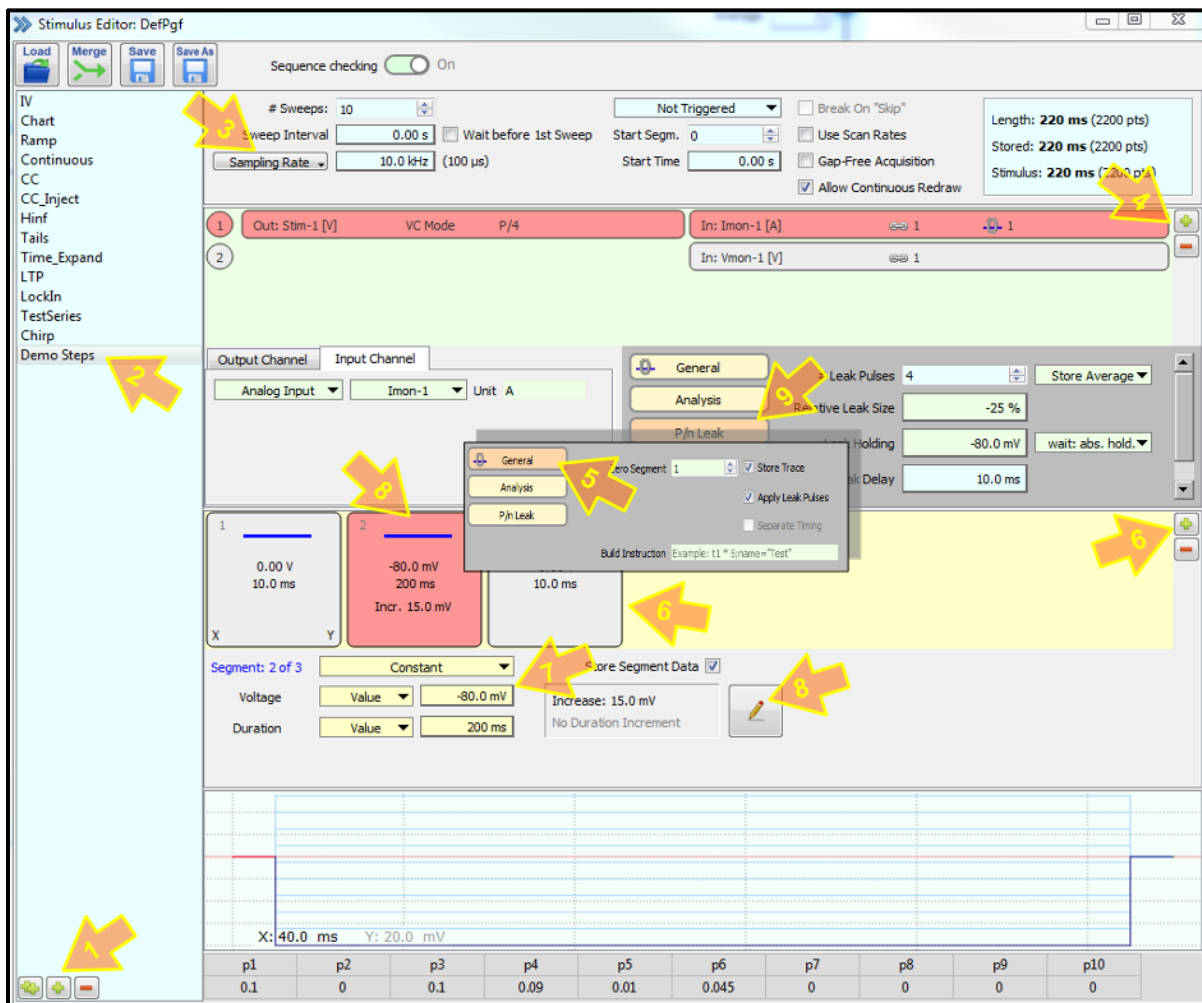
- o (2) Right-click on new sequence to edit its name.
- o (3) Select "Sample Rate", enter "10k" to set the acquisition freq. to 10 kHz.
- o (4) Add Input & Output Channels, use the "Select Input and Output" window to specify channels, here we select "Vmon-1" to record the stimulation trace as well.

- o (5) Select "General" and check "Store Trace" to make sure that the data are being saved to the hard drive (see also chapter 5, "Control" window). Set "Zero Segment" to "1" to enable all functionalities that are associated with this parameter. Enable "Leak Pulse" functionality by checking the "Apply Leak Pulses" box.
- o (6) Add, delete and move around (change order of) segments.
- o (7) Select specific segment and alter its general settings (kind, voltage, duration)
- o (8) To adjust sweep increments, select the desired segment and then press the "Edit" button to open the "Set Segment Increments" window. Please note that numbers entered here will overwrite the respective parameters in the main "Stimulus Editor" window (ex.: 10 sweeps,-80 mV, 200 ms).
- o (9) Finally, click on "P/n Leak" button to enter the specifics for the P/N leak subtraction algorithm [page 124, 131 − 133]. Setting a negative "Relative Leak Size" will generate leak pulses with opposite direction to the actual test pulse.

- o **Current-Clamp Step Sequence**: please note, to run a voltage-clamp or current-clamp stimulation sequence, the <u>corresponding amplifier needs to be set to the respective recording mode (see "Amplifier" window)</u>.
    - o (1) Create a new sequence by duplicating the "CC" stimulation sequence. Select the sequence and press the "double-cross" button. Feel free to rename the newly created sequence.
    - o (2) Select "Sample Rate", enter "10k" to set the acquisition freq. to 10 kHz.
    - o (3) Add Input and/or Output Channels, use the "Select Input and Output" window to specify channels, here we select "Imon-1" to record the stimulation trace as well.
    - o In the "Output Channel" section, make sure to select "Current-Clamp" mode.
    - o (4) Select "General" and check "Store Trace" to make sure that the data are being saved to the hard drive (see also chapter 5, "Control" window). Set "Zero Segment" to "1" to enable all functionalities that are associated with this parameter.
    - o (5) Add, delete and move around (change order of) segments.
    - o (6) Select specific segment and alter its general settings (kind, voltage, duration)
    - o (7) To adjust sweep increments, select the desired segment and then press the "Edit" button to open the "Set Segment Increments" window. Please note that numbers entered here will overwrite the respective parameters in the main "Stimulus Editor" window (ex.: 8 sweeps,-20 mV, 100 ms).
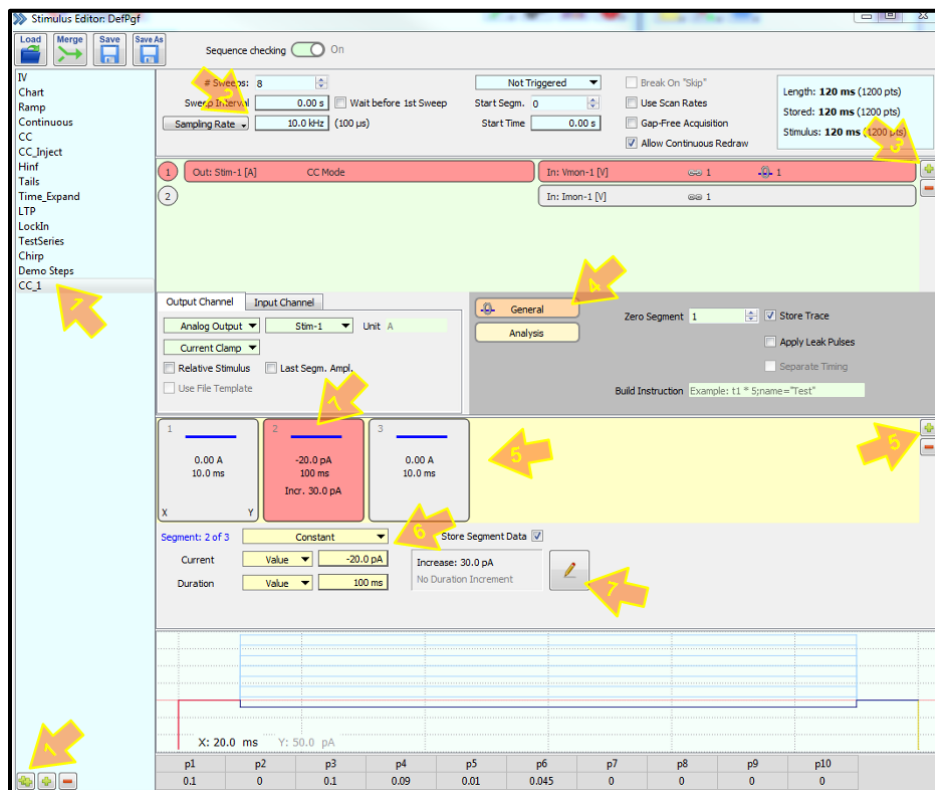
- **Voltage-Clamp Single-Channel Sequence**: here we create a long-time recording (120 s) (single-channel or synaptic). Please note that every segment class (other than "Continuous") requires PMN to reserve an appropriate amount of computer memory for the requested amount of sample points, because the sum of all data points from those "fixed-length" segments need to be displayed in the oscilloscope window (100% page-size). The "Continuous" segment class does not contribute to the reservation amount of sample points, because it uses the "same" page-size for display that is set by all prior "fixed-length" segments. Please note, the "Continuous" segment needs to be therefore always the last one in the order of segments.

  - (1) Create a new sequence (press "single-cross" button). Feel free to rename the newly created stimulation sequence.

  - (2) Select "Sample Rate", enter "50k" to set the acquisition freq. to 50 kHz.

  - (3) Select "General" and check "Store Trace" to make sure that the data are being saved to the hard drive (see also chapter 5, "Control" window).

  - In the "Output Channel" section, make sure to select "Voltage-Clamp" mode.

  - (4) Add, delete and move around (change order of) segments. Please make sure that the "Continuous" segment is the last one in the order of segments.

  - (5) Select specific segment and then change its general settings (kind, voltage, duration)

Don't forget to save the "pgf-file" as a new file. Press "Save As" and give it a user-specific name.
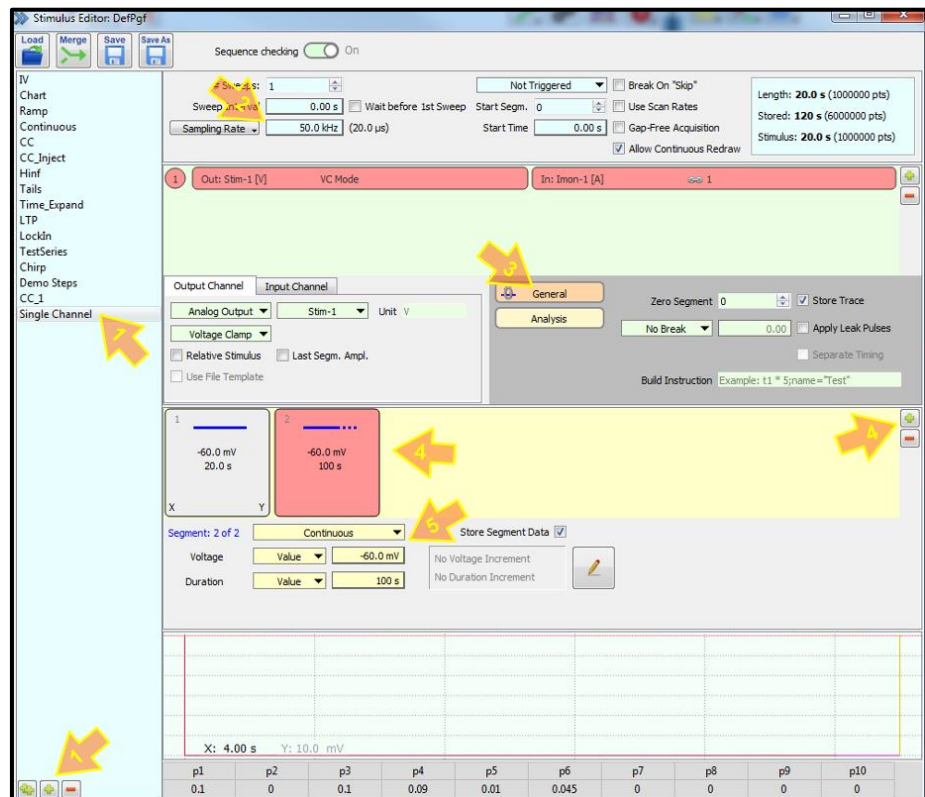
9. Conduct Analysis

Using the "Analysis Editor" more than thousand different analysis methods can be created. Each "Analysis Method" can have over thousand different analysis functions that can all be executed simultaneously. Data analysis can be performed offline or online, using the same analysis methods.

Here we can only give you a small glimpse into the many analysis possibilities of PMN. Please feel free to explore!
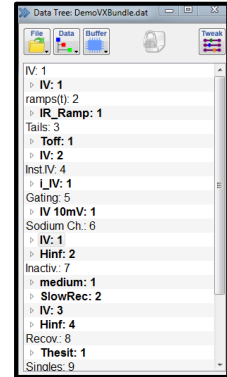
- **Create IV-curve**: in this section, we create an IV-curve from a voltage-activated sodium current from scratch. Please open (read-only) the demo data file (DemoData.dat).

  - In the "Oscilloscope" window, click on the "Oscilloscope" button and check:
    - Subtract Leak Traces
    - Subtract Zero Offset
    - Overlay Traces & Sweeps
    - Auto Y-scale
    - Labeling: Grid + Values
  - Double-click on "IV:1" of "Sodium Ch: 6" to display the series.

  - (1) Create a new analysis method. Feel free to rename the newly created method (ex.: IV demo). The selected method is displayed in the "Method Name" field. Set "Analysis Control" to "Use Selected Method".
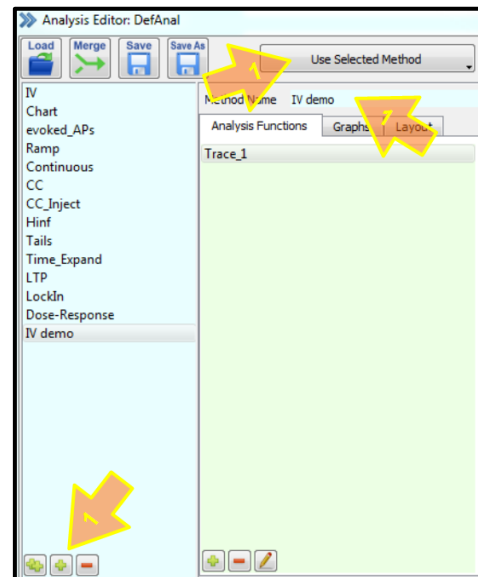
  - (2) Right-click on standard function (Trace_1) to open the "Analysis Functions" window and select "Amplitude" (Measure Functions – Stim. Properties).

  - (3) Name of selected function is displayed in the "Func. Name" field, its functionality is displayed in the "Func. Type" field (ex.: Amplitude).

  - (4) Input drop-down window: select the source of the desired data (ex.: Trace 1).

  - (5) Check this box, if you would like to display the analysis results in the notebook for observation or later export.
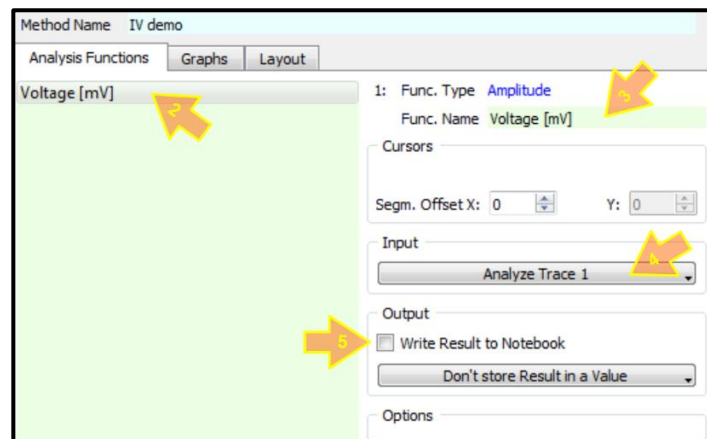
o (6) To add a new analysis function, press the "single-cross" button. This opens the "Analysis Functions" window, please select "Extremum" (Measure). This function delivers the maximum or minimum of the data trace in the specified region. Feel free to rename it.

o (7) The "Cursors" section is used to fine-tune what specific part of the data you would like to use for the respective analysis function. Indicate, if you want the cursors to be bound to a specific segment or the entire trace. Press "Show" to display the cursors in the "Oscilloscope" window. Cursors can be moved in several ways (drag & drop, enter numbers or right-click-hold scrolling).

o (8) Input drop-down window: select the source of the desired data (ex.: Trace 1).
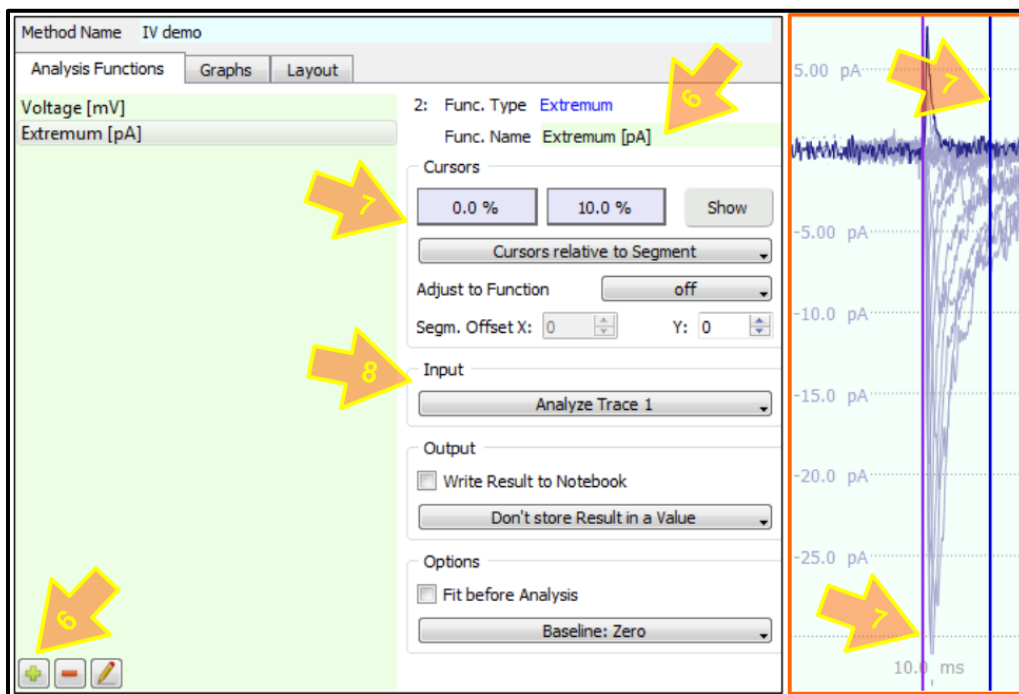
o (9) Press "Graphs" tab to open the interface for the graph settings.

o (10) Select "Graph_1" and check "show Graph". Please note, up to 12 graphs can be created, each one with 4 different entries.

o (11) Check the 1st "Graph Entry", click on the drop-down windows to select analysis results for Y-axis (Extremum [pA]) versus X-axis (Voltage [mV]) and click on the color bar to set the desired display properties.

o (12) Finally, right-click on "Graph_1" to open a scaling dialog. Here you can set X and Y-axis scaling as well as open the "Graph Properties" window.
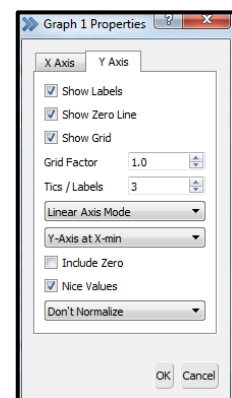
PMN offers two windows to display graphs (see chapter 5). Select the "Layout" tab to choose where to display the graphs. Open a "Graph Layout" window from the "Win" button in the "Control" window. The "Tweak" button in the "Graph Layout" window allows also to select which graph to display.
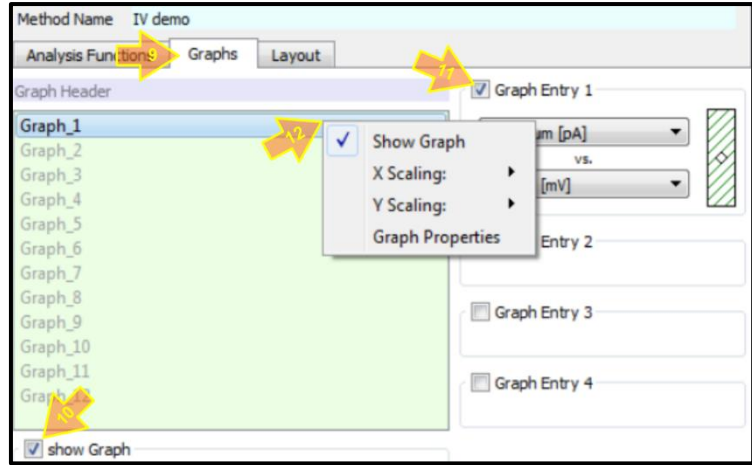

o Now replay the data series (IV: 1) and the IV-curve is being drawn in the "graph Layout" window.
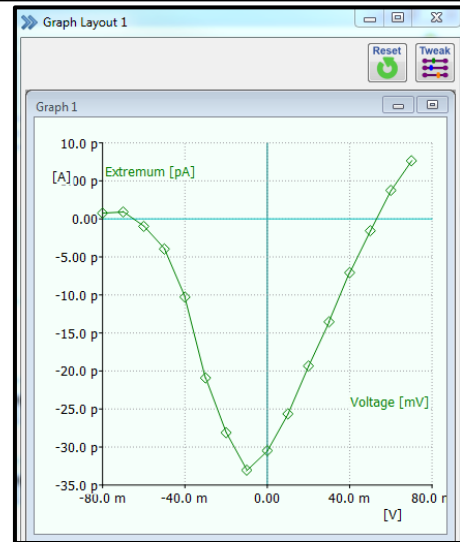

- **IV-curve from double-ramp:** In following example, we separate fast-inactivating sodium currents from long-lasting calcium currents. We applied a double-ramp stimulation sequence (see picture). On the upswing, both currents (sodium and calcium) are present, whereas on the downswing only the long-lasting calcium current remains. Potassium currents have been pharmacologically blocked.
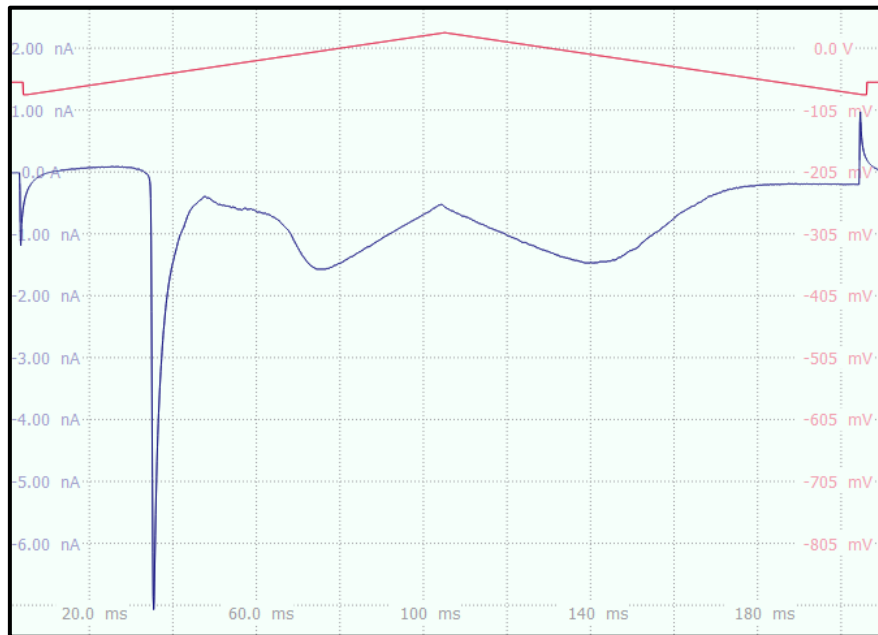
- o (1) The analysis method "Ramp IV reverse" has four analysis functions to create two IV-curves that are displayed in one graph.
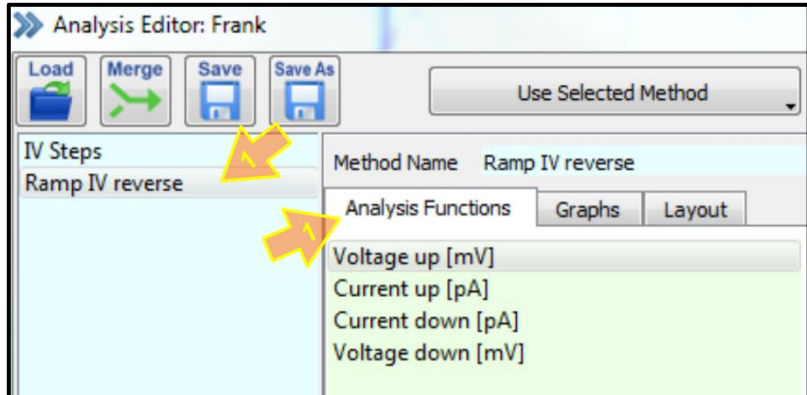
- o (2) Voltage values for the X-axis were calculated with the "Stimulus" function. Cursors were set to "5 %" and "95 %".
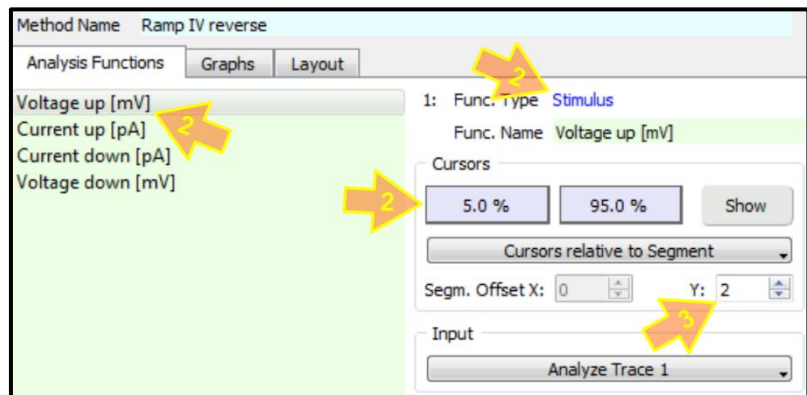
- o (3) Therefore, the "Segm. Offset" for both, "Voltage up" and "Current up" functions had to be set to "2" to make sure the cursors were at the correct segment. Click "Show" to display the cursors.

- o (4) Current values for the Y-axis were calculated with the "Trace" function. Cursors were set to "5 %" and "95 %".
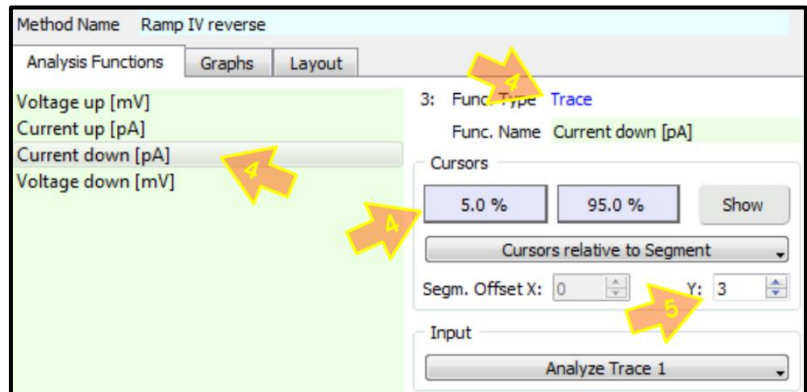
- o (5) The "Segm. Offset" had to be set to "3" for both, "Voltage down" and "Current down" functions, so that the cursors are defined for the downward segment.

- o (6) Pressing "Graph" tab opens the interface to adjust graph settings. Like before, we select "Graph_1" and check "show Graph" to enable the graph display.
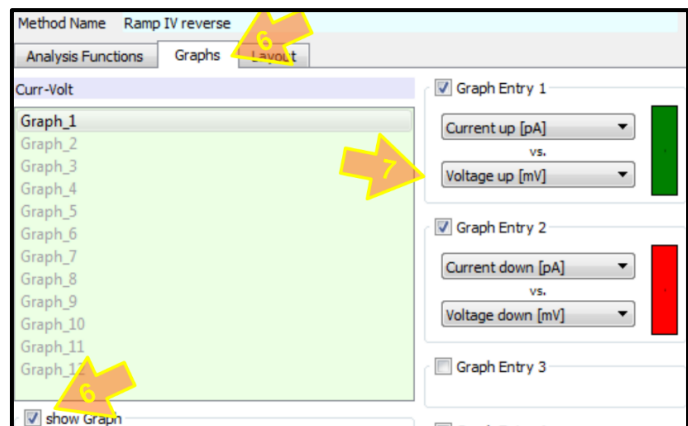
o   (7) Checking the 1st and 2nd entries and using the drop-down windows to select the appropriate X and Y functions.

The "Graph Layout 1" shows the IV-curve of the ramp current response trace. The green trace represents the upward ramp response and the red trace shows the downward part of the double-ramp.
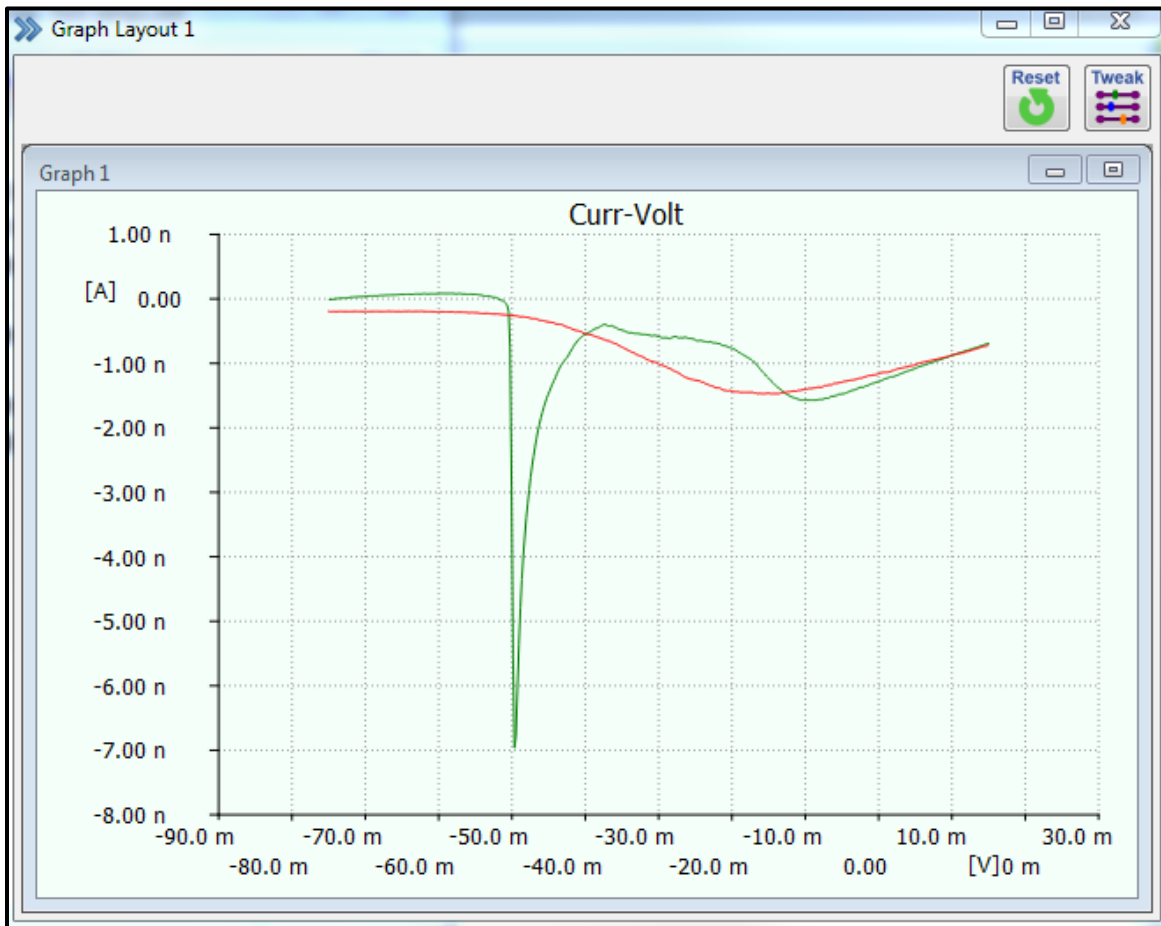

- **Online Analysis procedure**: Like we mentioned in the beginning of this chapter, it is possible to run any analysis method automatically, while data are being recorded (online). You must "link" the stimulus sequence to the respective analysis method and in addition, set the "Analysis Editor" to "Automatic Stimulus Control". To see how this feature works, please feel free to follow the instructions in the next paragraph.

    ○ <u>IV-step & IV-curve online procedure</u>: please create a new data file and make sure all default support files are loaded (pgf, onl, pro).

- Using the model cell (MC-10), establish a simulated whole-cell recording condition (see chapter 7). Don't forget to set the holding potential to "-60 mV", before executing any stimulation sequence.
- Feel free to test the "IV" sequence by starting it from the "Control" window.
- In the "Oscilloscope" window, press "Auto-Y-Scale" (Tweak-button) to improve the display of the currents.
- Apply a digital filter of "800 Hz" using the "Digital Filter" box in the "Oscilloscope" window (enter value or right-click-hold scroll), afterwards press "Redraw" to display all sweeps.
- Optional: Feel free to "switch off" the "Voltage traces" from the display (Trace Properties – uncheck "show" for 2nd trace). Please remember that you need to check this box again, if you would like to "see" these traces again!
- Open the "Stimulus Editor" to check the settings for the analysis link. Click on "Analysis" and make sure the analysis method "IV" is selected from the drop-down window. In addition, both relevant segments need to be set to "2". Close the "Stimulus Editor" window again.
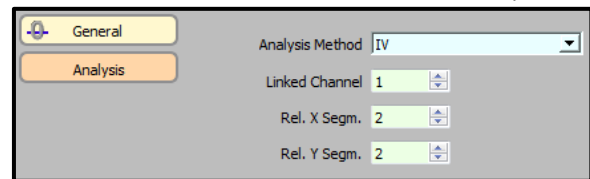

- Open the "Analysis Editor" to check the settings of the "IV" analysis method. Please note that executing this analysis method will run four analysis functions on the respective current response traces. Selecting each function will present you with further information on the respective function type, cursor usage, input and output settings.
- Click "Graphs" tab to find information on graph settings.
- Click "Layout" tab to see, where each graph will be displayed (Graph Layout windows). Please close the "Analysis Editor" again.
- Open "Graph Layout 1" window and place & re-size it to your liking. Please remember that you can drag & drop it to a second screen, if necessary.
- Now run the "IV" sequence again, by starting it from the "Control" window and observe how the IV-curves are being generated, while the data is being recorded.
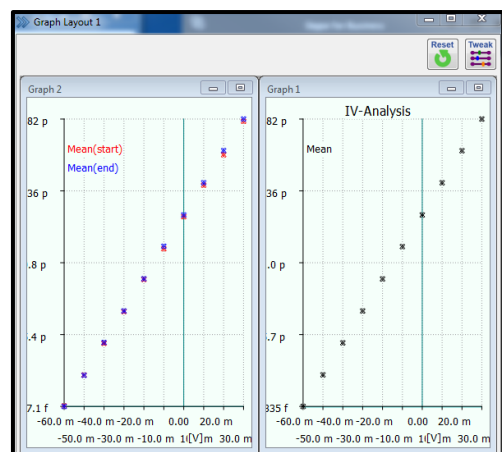
10. How to Use the "Protocol Editor"

The "Protocol Editor" is a very powerful feature of PMN. It allows you to fully automate entire experiments by writing simple protocols, using the new, intuitive "Protocol Editor" interface (see chapter 5). You can create complex experimental arrangements by combining stimulus sequences with other operations (amplifier control, change settings, etc.) and/or adding analysis functionality (causality loops). This allows to create a fully automatic, interactive data acquisition process. While recording data (trace amplitudes, kinetics, etc.), stimulation input could be altered online, depending on protocols (IF – THEN loops).

- **Synaptic Recording with intermittent check of Recording Quality**: here we created a protocol that records synaptic events for a duration of 5 minutes. Every minute the protocol checks the cell capacitance, access resistance and holding current and automatically saves those values to the recorded data trace for later offline analysis.
  - First, we created a stimulus sequence (Synaptic) that records 5 sweeps of synaptic events at a holding potential of-60 mV for 60 seconds, for a total of 5 minutes.
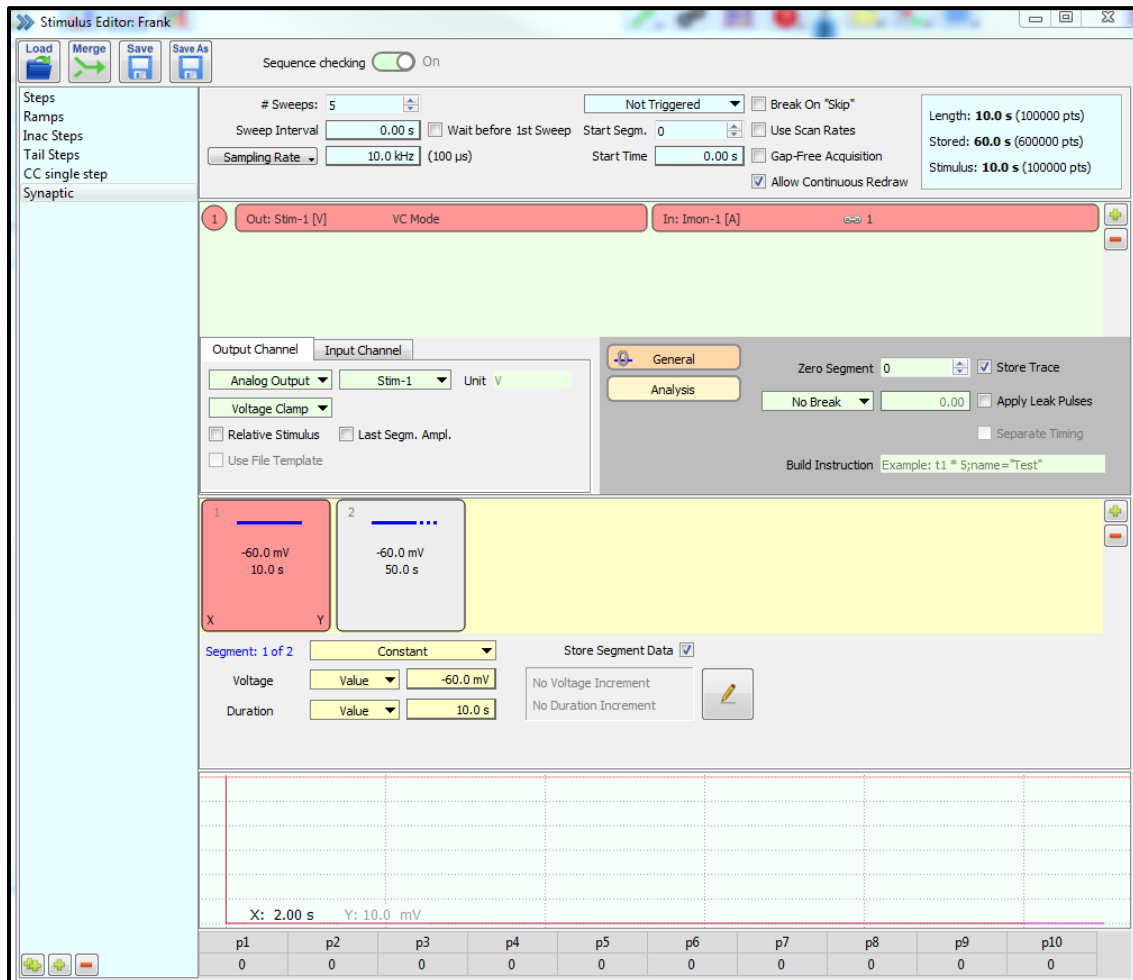
- o Then we created the following protocol: "Synaptic_5" (5 minutes)
  - Open the "Protocol Editor" (press "F9").
  - (1) Create a new protocol and rename it to "Synaptic_5".
  - (2) Drag & drop "Acquire Each Sweep" to "Protocol" column.
  - (3) Click on "Sequence" and select "Synaptic" from the drop-down menu.
 - (4) Click on "little arrow" to display "REPEAT contend"
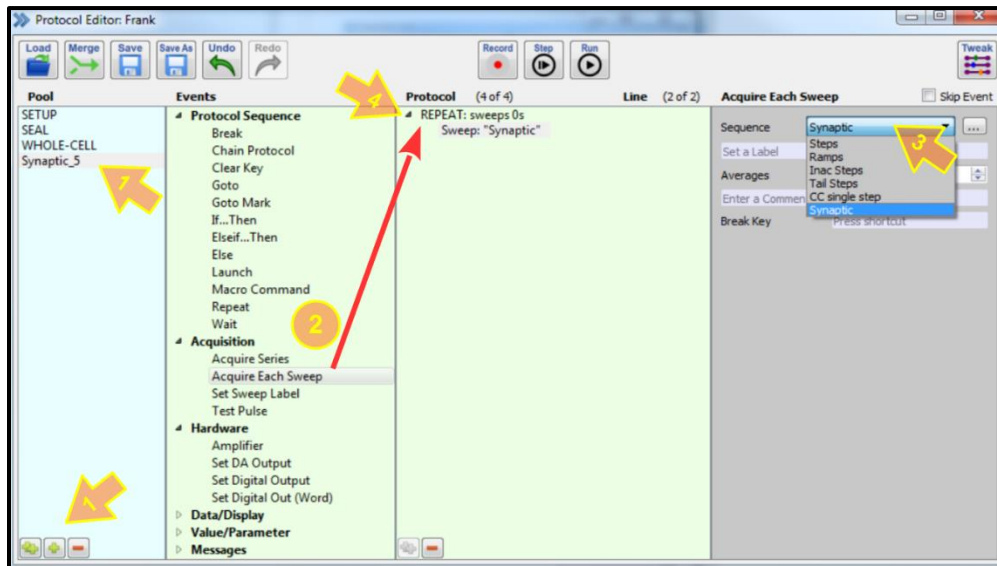
- (5) Drag & drop "Test Pulse" to the "protocol" column and move it into 2ⁿᵈ position. This command will execute one test pulse to update the value for the "R-memb" parameter. In the whole-cell recording configuration, this would be the input resistance (membrane resistance, Rm) of the cell.
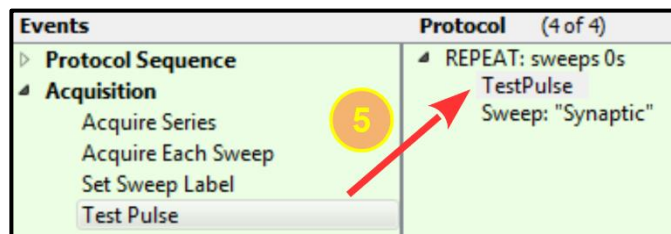
- (6) Drag & drop the "Amplifier" command to the position indicated in the picture below. Check "Auto-C-Slow", which will "press the Auto-C-Slow" button in the "Amplifier" window and thereby update the respective parameter values for cell capacitance and serial resistance.
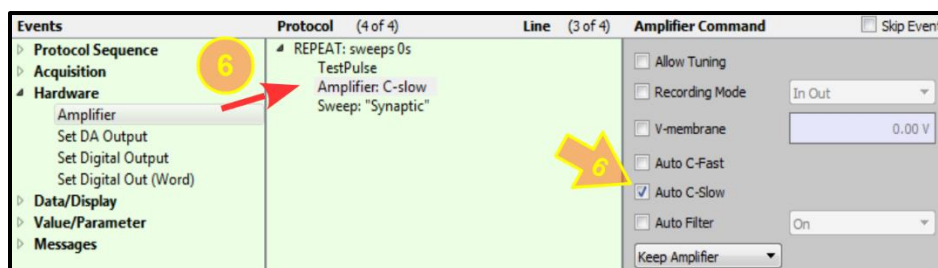
- (7) Drag & drop the "Wait" command in the position indicated in the picture below. "Wait Type" is set to "Absolute Wait" and the "Duration" to "500 ms". This command will cause the protocol to stop for 500 ms, thereby allowing the "Auto-C-Fast" algorithm to safely finish, before starting the next sweep.
- Before closing the "Protocol Editor", don't forget to save your "pro-file".

- To start this protocol, double-click the "Synaptic_5" button in the "Control" window.
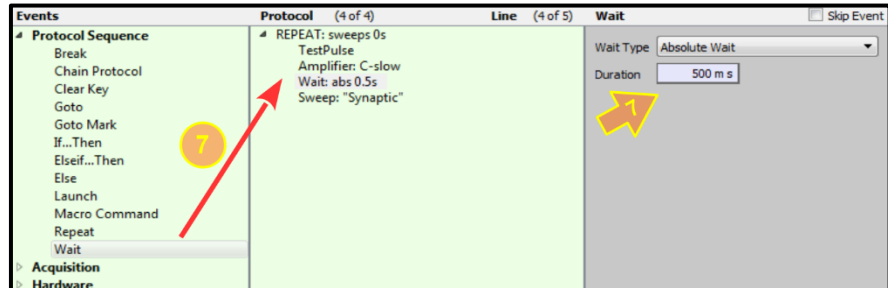
Concluding Remarks

PATCHMASTER NEXT is a professional Patch-Clamp acquisition and analysis software. It is the most advanced, most complex and most sophisticated software available today. Due to its new Graphical User Interface, it is easy to use by beginners and HEKA-veterans alike.

Its 3-in-1 functionality (Amplifier Control, Data Acquisition and Online Data Analysis) allows to create fully automatic, interactive, feedback-controlled experimental procedures.

Please feel free to contact your HEKA Support Team, if you have any HEKA-specific or general Patch-Clamp questions.

Best regards from your HEKA Research & Development, Sales and Support Teams!

We wish you exciting and successful experiments!

Support

If you have any questions, suggestions, or comments, please contact the HEKA Support Team. The best way would be to send an e-mail (support@heka.com) with as much information as possible that describes and specifies your inquiry or problem:

- Your contact information
- The program name: e.g. PATCHMASTER, POTMASTER or CHARTMASTER software
- The program version number: e.g. v2.65, v2.73.1, v2x90.2
- Your acquisition hardware: e.g. EPC10_USB, EPC800 + LIH8+8 or PG340_USB
- The serial number and version of your amplifier: e.g. EPC9 single, version "920552 D"
- Your operating system and its version: e.g. Mac-OS 10.8, Windows-7 64-bit
- Your type of computer: e.g. Intel Core i3 3.2 GHz with 2 GB RAM
- The questions, problems, or suggestions you have
- Under which conditions and how often the problem occurs

We will reply to your email as soon as possible, usually within 24-48 hours.

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