Special features of the EPC9 Double and Triple

1. Hardware

The EPC9 Double and Triple contain two or three independent EPC9-amplifiers in one case. All amplifiers share a single ITC-16 AD/DA-acquisition board. The ITC-16 board provides 4 DA-channels which are sufficient to allow simultaneous stimulation of every individual amplifier. The 8 available AD-channels can independently read all voltage- and current-outputs of the individual amplifiers.

One amplifier is always selected as the "active" amplifier. The "active" amplifier is the one which is enabled to receive configuration commands. One can visually identify the "active" amplifier by checking the DIGITAL BUS light. The green LED-light of the "active" amplifier flickers when the digital lines are alive during command transmission. The notion "active amplifier" does not imply that the other amplifiers are not active. They remain fully functional, but not enabled to receive programming commands.

The internal, hardwired DA- and AD-channel assignments for the EPC9 Double and Triple are as follows:

	EPC9 amplifier no.		
	1	2	3
Stim-DA	0	1	2
Vmon-AD	0	2	4
Imon2-AD	1	3	5
Test-DA [1,2]	3	3	3
Mux-AD [1]	7	7	7

Note 1: Mux-AD, Test-DA, and Clipping-lines are connected to the selected "active" EPC9 board only. Stim-DA, Vmon-AD, and Imon2-AD are always accessible for all individual EPC9 boards.

Note 2: The Test-DA can be connected to the stimulus input of all 3 amplifiers. This allows to simultaneously stimulate all 3 amplifiers. Separate scaling factors are available for each individual amplifier.

2. Software

2a. E9Screen

One selects the "active" amplifier in E9Screen with the new "amplifier" button on the top, left side. Only the available amplifiers can be selected in the amplifier list:

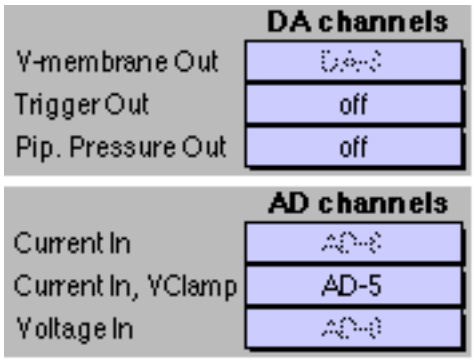
E9Screen updates all parameters to show the state of the selected amplifiers when you switch between amplifiers. The state of each amplifier is independent of any setting of the other amplifiers.

2b. PULSE

PULSE will automatically detect a connected EPC9 Double or Triple, and will pre-configure most settings. Several new fields will be displayed to support the additional capabilities of the EPC9 Double and Triple.

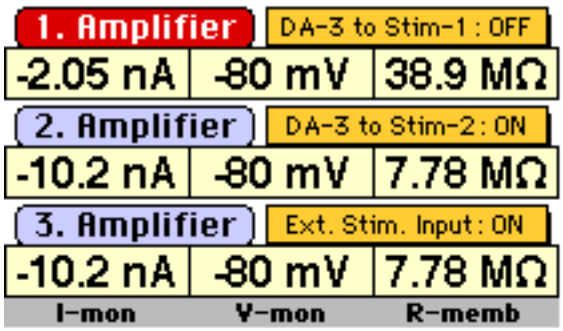
Configuration Window

The fields "V-membrane Out", "Current In", and "Voltage In" cannot be specified, when you are using an EPC9 Double or Triple. These AD- and DA-channels are internally connected and cannot be changed.



Amplifier Window

The following new fields are displayed. The block for the 3. amplifier will be added only, when an EPC9 Triple is connected.



To make a specific EPC9-amplifier the "active" amplifier one must click on one of the "Amplifier" fields. PULSE will immediately update all settings to display the state of the selected amplifier. Thus, amplifier settings can only be changed on the selected "active" amplifier. The other amplifiers will keep their settings until the user makes the respective amplifier the "active" one.

The I-mon and V-mon fields of all amplifiers will be continuously measured and updated, as soon as the respective amplifiers were selected at least once. For example, this allows to keep the 3. amplifier quiet during experiments in which it is not used. If one wants to activate all amplifier from the start on, one can record each amplifier selection as operations in the default "SET-UP" macro.

The "R-memb" field is continuously measured and updated as well. The membrane resistance is computed using two algorithms:

- When a test pulse is applied:
  Membrane resistance is computed using the difference between the pipette current before and during the first test pulse amplitude.
  $R_{memb} = V_{test} / (I_{test} - I_{leak})$
  This algorithm is quite insensitive to problems such as pipette offsets, reversal potential, and liquid junction potential.

- When no test pulse is applied:
  Membrane resistance is computed by using the pipette-current only.
  $R_{memb} = V_{hold} / I_{leak}$
  This algorithm is quite sensitive to many problems such as pipette offsets, reversal potential, and liquid junction potential. In most cases the membrane resistance computed by this second algorithm will differ from that computed by the first algorithm (which is more trustworthy!).

Thus, only the membrane resistance of the "active" amplifier can be computed using the first, better algorithm, and only when the test pulse is applied!

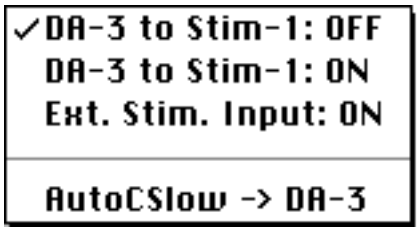
The EPC9 Double and Triple have three ways to stimulate an amplifier:

1. One can send the stimulus directly to the internal Stim-DA of the respective amplifier, e.g., to DA-0 for the 1. amplifier.

2. One can feed the stimulus directly to the internally connected DA-3 and connect the stimulus input of the respective amplifier to DA-3 as well. This allows to simultaneously stimulate multiple amplifiers. Separate scaling factors are available for each individual amplifier.

3. One can use an external stimulator and connect it to the EXTERNAL STIMULUS INPUT. Scaling factors are available for those inputs as well.

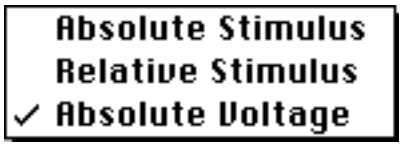
These 3 options can be selected in the pop-up menus "DA-3 to Stim...":



Individual scaling factors can be entered in the "External" field (near the bottom of the Amplifier window), when "DA-3 to Stim-X: ON" and "Ext. Stim. Input: ON" are selected.

This option can be set even when the respective amplifier is not the "active" amplifier. There are two reasons for this exception: first, multiple amplifiers may be simultaneously affected. And second, it has to be possible to set that option by a macro which is executed at the beginning of a particular Pulse Generator stimulus. In that case one does not want to interfere with the selection of the "active" amplifier. The goal is only to connect the required amplifier inputs for that particular experiment without knowing or changing the current selection of the "active" amplifier.

One has to be aware that selecting the second or third option results in the stimulus **to be summed with the holding potential** which is already applied internally. Thus, one has to use the "Absolute Voltage" stimulation type (see picture below), scaling it with the inverse of "Stimulus Scale" (see Configuration Window), i.e. with a factor of 10. The signal inversion according to the selected "mode" (OnCell, WholeCell, etc.) is compensated in hardware and must not be considered for the stimulus.



WARNING:

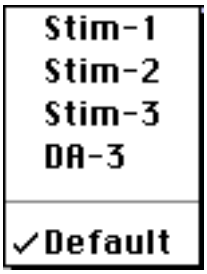
1. The test pulse is sent to all amplifiers with the selection "DA-3 to Stim-X: ON", when the "active" amplifier uses that option!
2. The option "DA-3 to Stim-X: ON" uses DA-channel 3. Therefore, DA-3 will no longer be available for other applications, as soon as one amplifier is set to that option.

The option "AutoCSlow -> DA-3" can be used when compensating CSlow capacitance in cells which are electrically coupled, e.g., when patching cells connected by gap-junctions. Normally, the stimulus used for the AutoCSlow compensation is sent to the Stim-DA of the active amplifier. When the option "AutoCSlow -> DA-3" of the active amplifier is selected, the stimulus is send to all amplifiers which have the option "DA-3 to Stim-X: ON" selected. The purpose is to eliminate the current through, e.g., the gap-junctions, which otherwise would prevent a good "AutoCSlow" compensation.

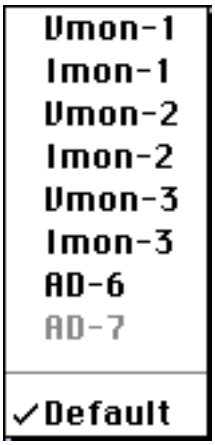
Pulse Generator Window

There are no new fields in this window. The only modification is that the pop-up lists for DA- and AD-selection contain the function names:

Stim-DA pop-up list



Trace-AD pop-up list



The STIM-DA, Vmon-AD, and Imon-AD of the "active" amplifier are used for stimulation and acquisition, when one selects the "default"-entry. Selecting a specific Stim-DA or Trace-AD will cause the stimulation and acquisition using those fixed channels, irrespective of which amplifier is the "active" one.

PULSE will use the known current- and voltage-gains as well as the modes (On-Cell, Whole-Cell, etc.) of the addressed amplifier. E.g., this allows to keep the first amplifier in cell-attached mode with a high current gain, while reading at the same time from the second amplifier in the whole-cell mode with a medium current gain setting.