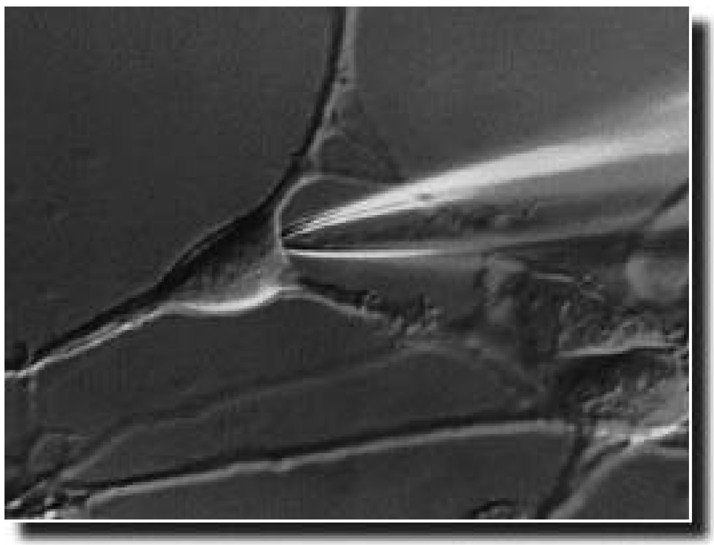
8.5 / 1.10
PULSESIM Manual



HEKA Elektronik Dr. Schulze GmbH

Wiesenstraße 71 • D-67466 Lambrecht • Germany
Tel: +49 (0) 6325 95530 • Fax: +49 (0) 6325 955350
Web Site: http://www.heka.com
E-mail: sales@heka.com • support@heka.com

Table of Contents

1. Introduction

Installation

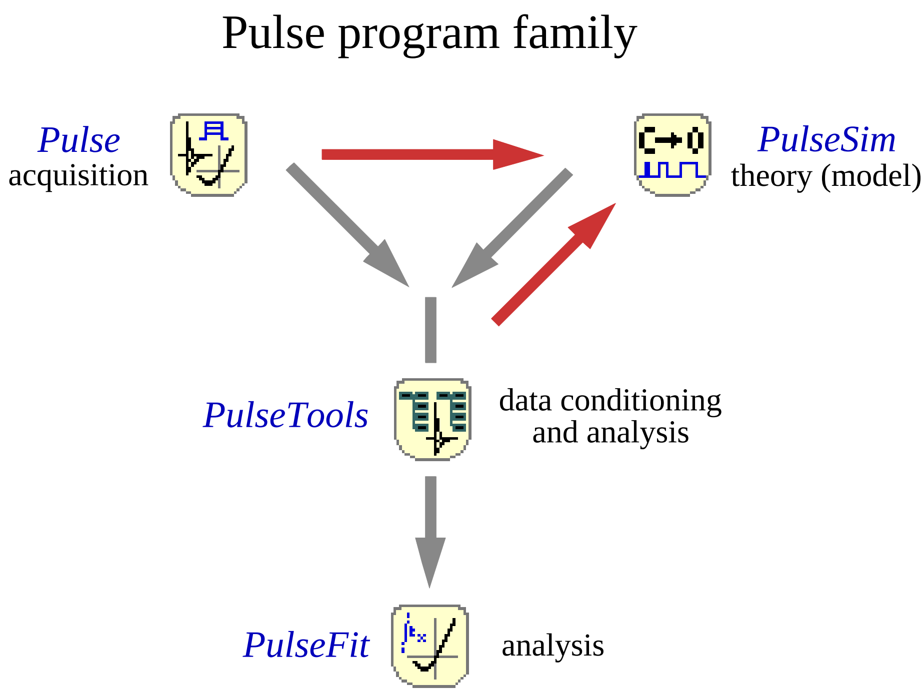
The installation procedure for software and hardware is described in the separate **“Installation_8x5” manual**.

Program Scope

The *PulseSim* program is a program for modeling and simulation of *Pulse* data based on kinetic schemes or discrete Markov models. It was developed by Rüdiger Steffan and Stefan H. Heinemann. For help with earlier versions, stimulating input, program library support, and ß-testing we would like to thank Hubert Affolter, Christian Hennesthal, Ken McCormack, Andreas Neef, Angela Siebert, and Fred Sigworth.

Purpose of PulseSim

Experimental data of ion channel function is acquired with the program *Pulse* and is basically analyzed with the program *PulseFit*. In the following figure, the flow of data for these operations is indicated by arrows.

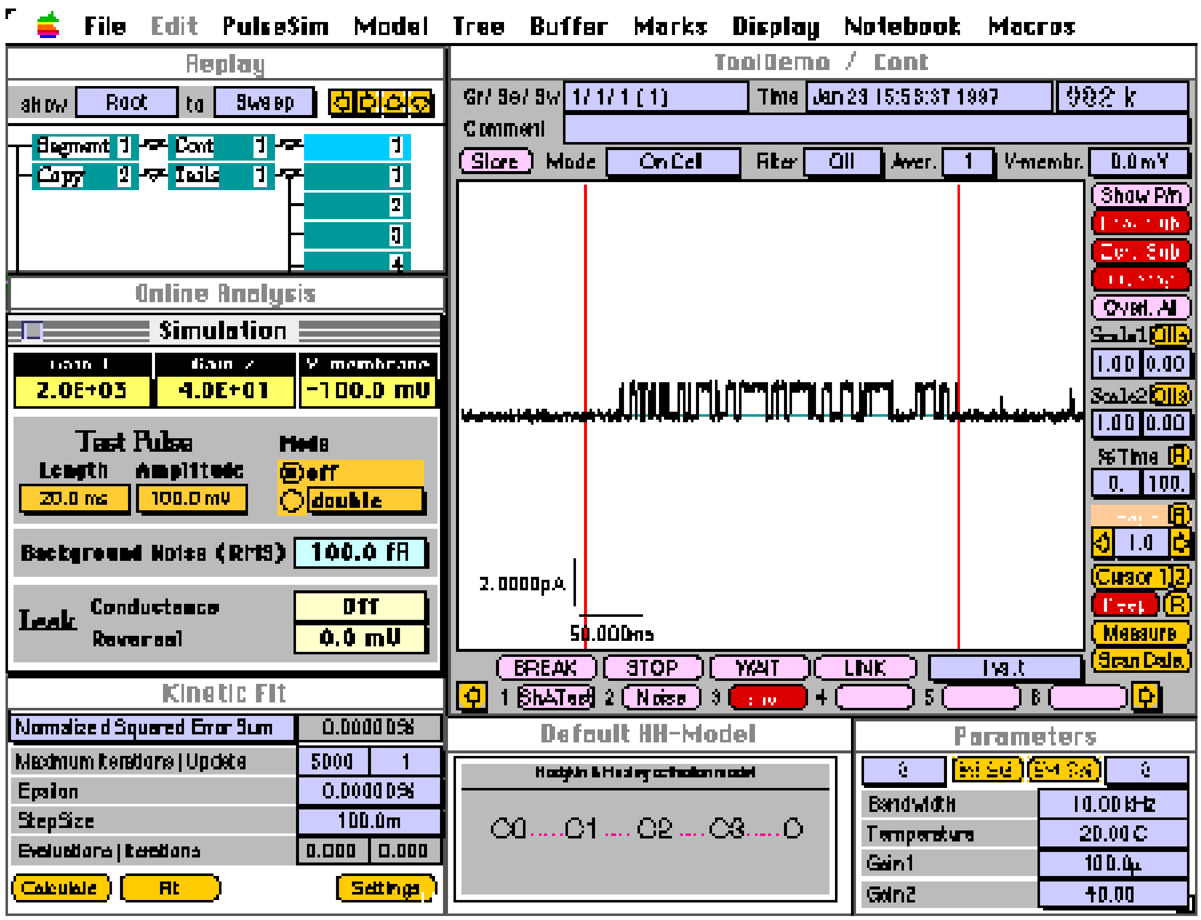


The *Pulse* program family also contains the program *PulseTools*, which is a mandatory “tool” for data conditioning and / or pre-analysis of data such as off-line leak correction, for example. *PulseSim* is an additional program for both, data acquisition, i.e. data generation (simulation), and analysis (modeling). In contrast to *PulseFit*, it makes use of kinetic state models and is therefore based on the interpretation of ion channel function as a stochastic process (Markov process).

Markov models are valuable tools for the investigation of the structure and function of voltage-dependent ion channels (see Colquhoun and Hawkes, 1995; Sigworth, 1993). Therefore, the main purpose of *PulseSim* is the mathematical description of *Pulse* data based on detailed kinetic schemes (Markov models) rather than more general mathematical functions as used in *PulseFit*. In addition, *PulseSim* serves as a tool for the generation of synthetic data sets. Such simulated data sets are stored in the *Pulse* data format and can be analyzed exactly like “real” data (see *PulseSim Manual*, *Chapter 2 - Files and Menus*). *PulseSim* can therefore also be used for the development of novel analysis procedures as well as for teaching purposes. The interaction between data acquisition, analysis and simulation, the basic program concept, and a brief summary of the theory of Markov models are described in more detail in Steffan et al., 1998. An overview of the basic functions as well as the user interface of *PulseSim* is given in the next section.

Program overview

Similarly to *PulseFit* and *PulseTools*, *PulseSim* was developed in the *PowerMod* environment and makes use of the modular concept of *Pulse*. Therefore, most of the features of *Pulse* are also available in *PulseSim*.



Menus that differ from *Pulse* are the *PulseSim* drop-down menu and the *Model* drop-down menu. The *PulseSim* drop-down menu mainly contains the commands to switch between dialogs, whereas the *Model* drop-down menu contains the commands for loading, modifying, and saving specified kinetic schemes ("models").

Additional dialogs contain the *Simulation* window, the design of which is similar to an amplifier dialog in *Pulse*, and the *Kinetic Fit* window, which has quite similar functions as the *Sweep Fit* dialog in *PulseFit*. The graphic window below the oscilloscope window visualizes the currently loaded model; the window name corresponds to the name of the model file.

For clarity reasons, the notebook window is not shown in the picture above. As an initial example, the demo data file of PulseTools “ToolDemo” is loaded and a sweep, which contains single-channel data generated with *PulseSim* is displayed in the oscilloscope window.

Making new models

When *PulseSim* is started, a default model is automatically loaded, which corresponds to the Hodgkin-Huxley formalism (see picture above). Alternatively, a build-in two states model can also be loaded through the *Model* drop-down menu (see *PulseSim Manual, Chapter 2 - Files and Menus*). In order to create new models, one would start from one of these two default models. The new model can then be stored in a separate model file and can serve as input for further model extensions.

Access to the model file with respect to other *Pulse* files as well as the basic format of the model file is briefly described in the following chapter *Files and Menus*. In the subsequent chapter *Dialogs* it is shown how a model file can be modified by using model editor dialogs of *PulseSim*.

About this manual

The main purpose of this manual is to describe the user-interface, i.e. the entries of the drop-down menus and the usage of the dialogs of *PulseSim*. Several examples of application of *PulseSim* can be found in Steffan et al., 1998. A brief tutorial how a very simple first simulation with *PulseSim* could look like is given in the chapter *The First Simulation* of this manual.

As a result of the modular concept of *PulseSim*, many features of the program corresponds to *Pulse&PulseFit*. Moreover, the design of additional drop-down menus and dialog windows is also very similar. This manual should therefore always be used together with the *Pulse&PulseFit* manual.

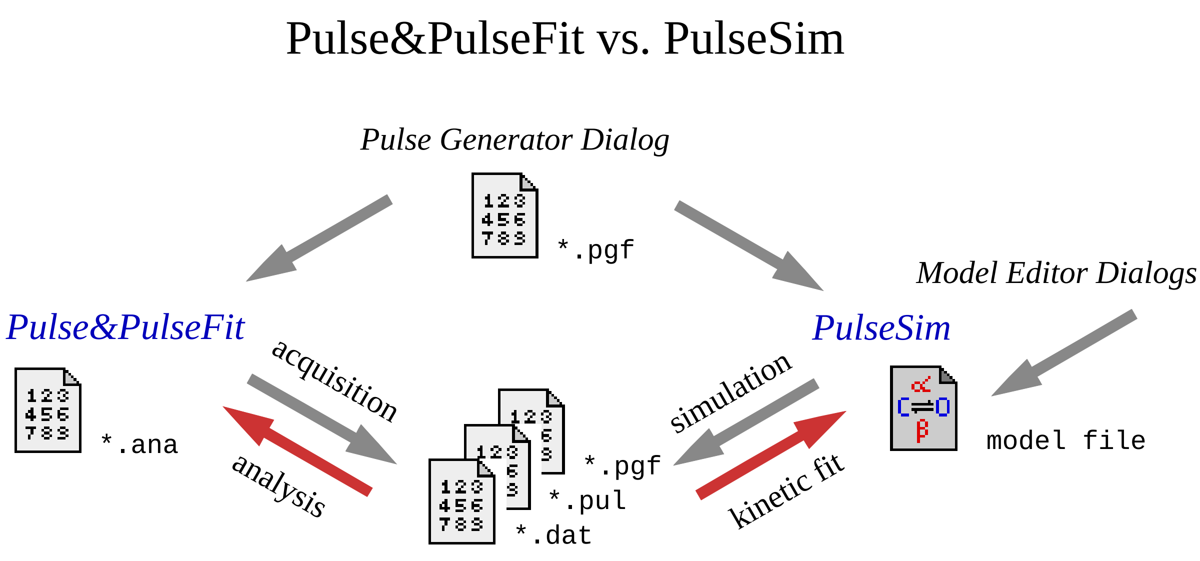
2. Files and Menus

Files

Overview

An overview of the file handling of *PulseSim* in comparison to that of *Pulse&PulseFit* is given in the picture below.

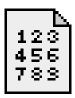
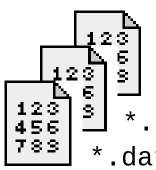
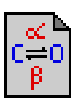
The functions of *PulseSim* are based on the specification of a kinetic state diagram, which can be stored in a separate model file. All other files are identical to those used in *Pulse*. Similarly to the acquisition program *Pulse*, *PulseSim* can generate voltage-clamp data where a pgf-file (voltage segments) as well as the model description (the "channels") serve as inputs. The data are stored in *Pulse* data format, which consists of the three files *.pgf, *.pul, and *.dat (see *Pulse* manual). *PulseSim* can also fit the parameter of the model to the acquired data. In this case, the pgf-file of the data file triplet serves as input. The "optimized" model is stored in a separate model file. Beside the investigation of structure and function of specific ion channels, the optimized model can be used for more realistic experiment simulations.



*Note: The analysis program PulseFit fits the parameter of a mathematical function to the acquired data and also stores the parameters in a separate file *.ana, which has a tree structure.*

File Access

In the following table, the files of *PulseSim* are listed. In the right column it is described how the corresponding file is generated and through which dialog the user has access to this file.

 *.pgf	The pulse generator file (pgf) is generated by the *Pulse Generator*, which can be launched by all the programs of the *Pulse* family. It has a tree structure and serves as a pool of segment-oriented sequences for pulse stimulation.
 *.pgf *.pul *.dat	*Pulse* data files basically consist of three files. The sequences that were used for stimulation are stored in the pgf file. It therefore contains a subset of the sequences of the pool pgf file. Raw data are stored in a binary file (*.dat) and can be accessed by an additional tree-oriented file (*.pul). The structure of this file is visualized in the *Replay* window.
 model file	The model file is a file specific for *PulseSim* and contains the specification of a state model. *Model Editor* dialogs to modify this file are described in the chapter *Dialogs* of this manual. Since the model file has plain text format, it can be modified using any text editor. However, the structure, such as text order or spacing, must not be altered.

Model File

A model file consists of four sections, Parameters, Variables, States, and Transitions:

Parameters are predefined entries, which are used for model calculations. Most of them can be determined experimentally (see below) and may be fixed during model optimization (number of channels, reversal potential, voltage clamp risetime, bandwidth, delay).

Variables are used to assign values to text identifiers of formulae which describe specific properties of the model and which are parsed and interpreted on-line.

The definition of **States** comprises a state label, the single-channel conductance, the total charge movement with respect to state zero, and coordinates used for the graphical display of the kinetic model (see *Program Overview* above).

Transitions comprise information about the charge transfer, the symmetry of the energy barrier, a voltage offset, and the rate; all entries are expressed as text string formulas to be parsed.

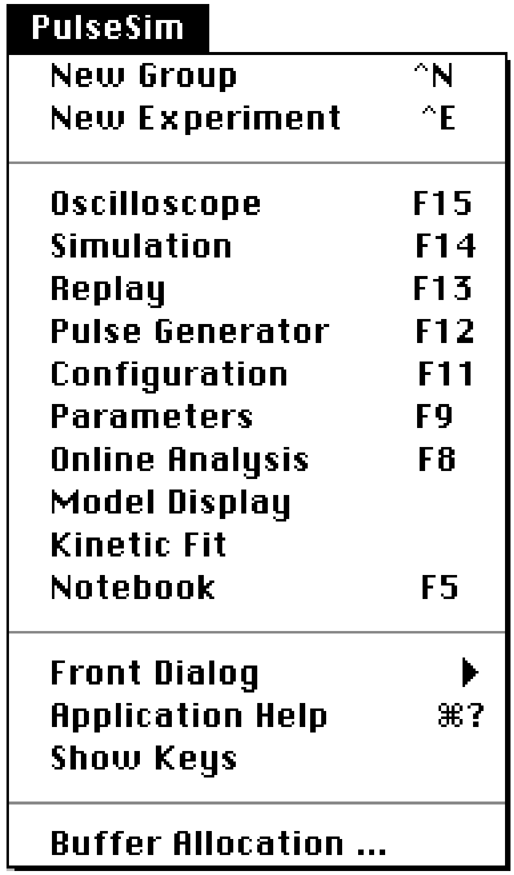
Each parameter or variable has an extra flag specifying whether or not it is to be varied during data fits.

The file of the simple two states model shown on the right and as it is also depicted in the icon of a model file is listed below. This model is used in the chapter *Dialogs* of this manual where the model editor dialogs are described.

$$C \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} O$$

```
Two states activation model



Parameters: No-Name-Value-Unit-FitFlag
  12
   0  Channels    5.000E+01            0
   1  Erev       -8.000E-02  V         0
   2  Tau 1       0.000E+00  s         0
   3  Tau 2       0.000E+00  s         0
   4  Amp 2       0.000E+00  %         0
   5  Filter      0.000E+00  Hz        0
   6  Delay       0.000E+00  s         0
   7  Temp        2.500E+01  °C        0
   8  - Block    -2.000E-02  V         0
   9  - Slope     1.000E-02  V         0
  10  + Block     5.000E-02  V         0
  11  + Slope     2.000E-01  V         0

Variables: No-Name-Value-FitFlag
   6
   1  extra       0.000E+00    0
   2  alpha       3.000E+02    0
   3  beta        3.000E+02    0
   4  q           2.300E+00    0
   5  s           5.000E-01    0
   6  sigma       2.000E+01    0

States: No-x-y-Conductance-CondFormula-Charge-ChargeFormula-Label
   2
   1   1   1   0.000E+00             0.000E+00                                    C
   2   2   1   2.000E-11  sigma      2.300E+00  q                                 O

Transitions: No-Charge-ChargeForm-Symetry-SymetryForm-Rate-RateForm-Offset-OffsetForm
   1    0.00            0.00            0.00E+00                                         0.00          @
   2    2.30  q         0.50  s         3.00E+02  alpha                                  0.00          @
   3   -2.30  -q        0.50  1.0-s     3.00E+02  beta                                   0.00          @
   4    0.00            0.00            0.00E+00                                         0.00          @
```

PulseSim Menu



Simulation: Switch to the *Simulation* window, which is comparable to an amplifier window in *Pulse*. With this dialog window, the simulation of time course responses based on pgf-pool sequences is controlled (see *PulseSim Manual, Chapter 4 - Dialogs*).

Model Display: Switch to the dialog window, which is entitled according to the name of the model file currently loaded. Within this dialog window, the model is displayed as a simplified kinetic scheme.

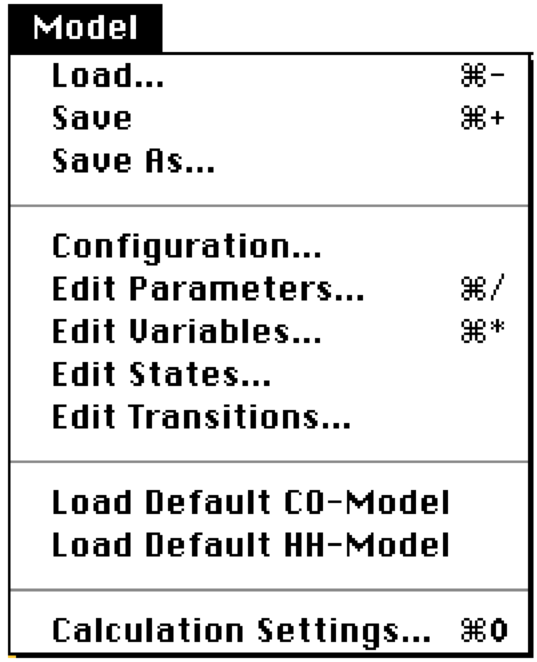
Kinetic Fit: Switch to the *Kinetic Fit* window. Current responses can be calculated based on the pgf(s) of a selected item or of several marked items in the *Replay* window. The selected model parameters can be fit to the corresponding stored raw data (see *PulseSim Manual, Chapter 4 - Dialogs*).

Buffer Allocation...: Specify the memory that can be allocated by *PulseSim*. This setting is stored in the "Preferences" folder ("PulseSim Settings" file).

All remaining items correspond to the entries in the *Pulse* menu and are therefore not described here (see *Pulse Manual, Chapter 3 - Menus*).

Model Menu

Model File Commands



Load...: A file selector is called to select a model file to be loaded.

Save: The currently loaded model file is stored by using the same file name (title of the *Model Display* dialog) and directory. An already existing model file is automatically replaced. If a build-in model is active, the corresponding default model name is used as file name (see below).

Save As...: Saves the active model where a file selector is called to specify file name and directory. The user will be asked if an already existing file shall be replaced or not.

Load Default CO-Model: The build-in two states model is loaded (see *PulseSim Manual, Appendix*).

Load Default HH-Model: The build-in five states model, which corresponds to the Hodgkin-Huxley n^4-formalism, is loaded (see *PulseSim Manual, Appendix*).

Model Editor Commands

Configuration...: Open a dialog for general model specification.

Edit Parameters...: Open a dialog for editing model “Parameters”.

Edit Variables...: Open a dialog for editing model “Variables”.

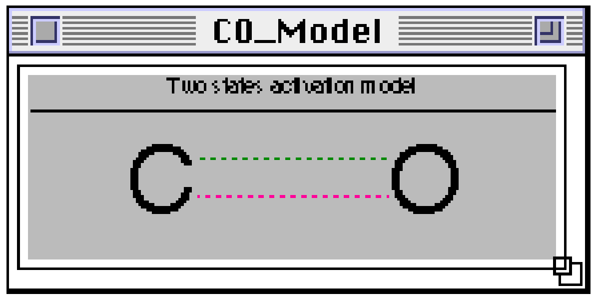
Edit States...: Open a dialog for editing model “States”.

Edit Transitions...: Open a dialog for editing model “Transitions”.

Calculation Settings...: Open a dialog for specifying additional calculation parameters.

3. Dialogs

Model Window



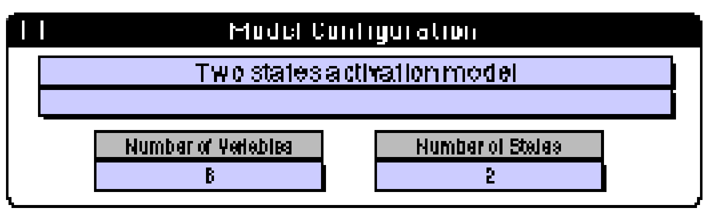
Graphic window which displays the currently loaded model as a simplified kinetic scheme. The window name corresponds to the file name of the model; the first comment line of the model file is displayed at the top. Forward transitions are indicated by green, backward transitions by red dashed lines if the transitions are specified properly. Therefore, the model window is a valuable tool during model design.

Model Editor

The different sections of the model file are edited with four different dialogs, which are called from the *Model* drop-down menu: Parameters, Variables, States, and Transitions. In the *Model Configuration* dialog the size of the model is specified.

Model Configuration

The two items at the top are comment lines. The first line can be specified by the user whereas in the second line various information on results of data fits are stored on-line (see *PulseSim Manual, Chapter 4 - Dialogs*).

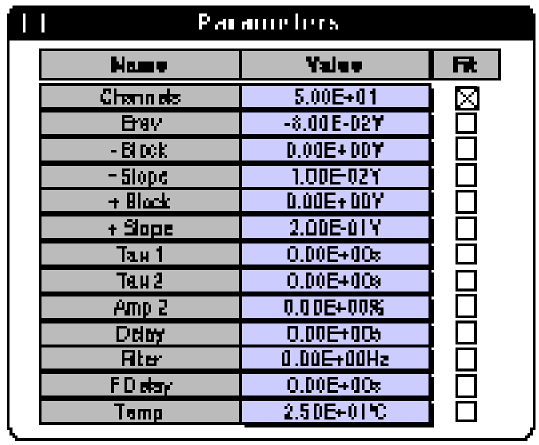


Number of Variables: Maximum number of variables usable as text identifiers in formula strings.

Number of States: Maximum number of states the specified model can have.

Parameters

The pre-defined names of the parameters are listed in the left column, the corresponding user-defined values in the middle column. If the item of the right column “Fit” is checked, the corresponding parameter is varied during a data fit.



Channels: Number of active channels.

Erev: Reversal potential.

- Block, - Slope, + Block, + Slope: Parameters of a voltage-dependent single-channel block decreasing (-) or increasing (+) with voltage. “- Block” and “+ Block” are the half maximum voltages (for definition see *PulseFit Manual, Chapter 5 - Series Fit*). If “- Slope” or “+ Slope” is zero, the corresponding block is inactive and ignored during calculation.

Tau 1, Tau 2, Amp 2: The rising phase of voltage steps are described by the sum of two exponential functions with time constants “Tau 1” and “Tau 2”. “Amp 2” is the amplitude fraction of the second component in %.

Delay: Model-independent delay time of the current with respect to the voltage.

Filter: Cut-off frequency of a Gaussian low-pass filter.

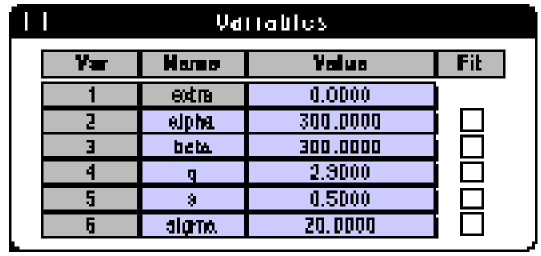
F Delay: Delay time of a Gaussian low-pass filter.

Temp: Temperature in °C.

Variables

The user-defined variable names and values are listed. The indices are shown in the leftmost column “Var”. If the item of the rightmost column “Fit” is selected, the corresponding variable is varied during a data fit.

extra: Pre-defined variable which equals the ExtraLongReal value stored in a group record (see *Pulse Manual*) of the data file. “extra” is updated on-line according to the group of the currently processed sweep.



States

The definition of a each state is listed. The item in the leftmost column is used by the program for different state-dependent functions such as the display of the time course of the net-probability of the selected states, for example.

State: State index.

Label: Text symbol that is used to identify a state in the transition dialog and for model visualization in the *Model Display* window (see above).

Conductance: The state conductance is specified by a text formula. If the formula string is empty, the conductance is zero. The result of the interpreted formula is shown in the near left item. Unspecified conductance (e.g. empty formula string) is indicated by a dash.

Charge [e0]: Total number of charge movement of a channels during activation at equilibrium. The charge is specified in units of the elementary charge e0 by a text formula. The result of the interpreted formula is shown in the near left item. Unspecified charge movement (e.g. empty formula string) is indicated by a dash.

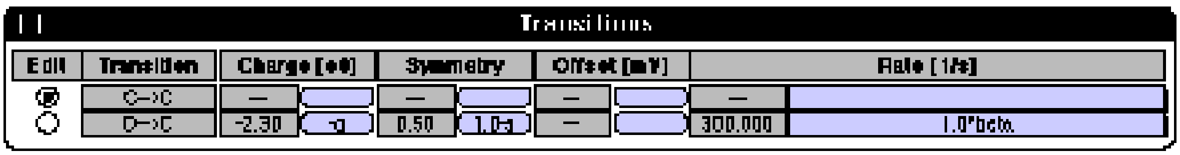
Transitions

Voltage-dependent transition rates k_{ij} between two arbitrary states i and j are calculated according to Eyring’s rate theory assuming instantaneous transitions:

$$\text{state i} \underset{k_{ji}}{\overset{k_{ij}}{\rightleftharpoons}} \text{state j} \qquad k_{ij} = k_{ij}^{0}\, e^{\frac{\delta_{ij} z_{ij} e_0 (V+V_{off})}{k_B T}} \qquad k_{ji} = k_{ji}^{0}\, e^{-\frac{(1-\delta_{ij}) z_{ij} e_0 (V+V_{off})}{k_B T}}$$

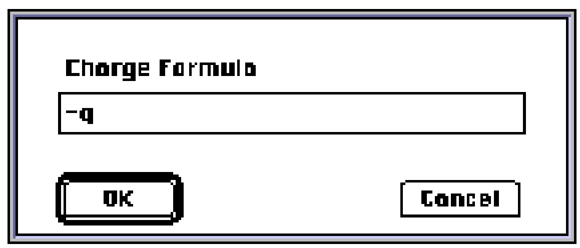
where zero rates k_{ij}^{0} and k_{ji}^{0} are transition rates at zero voltage, δ_{ij} is the relative position of the activated state in the electric field relative to states i and j, z_{ij} is the effective valence translocated during the transitions $i \rightarrow j$ and $j \rightarrow i$, e_0 is the unitary electronic charge, k_B is Boltzmann's constant, and T the absolute temperature. All these values are specified with the following dialog.

For clarity reasons, only the transitions to one particular state are displayed at one time. This particular state is selected by the radio button in the left column.

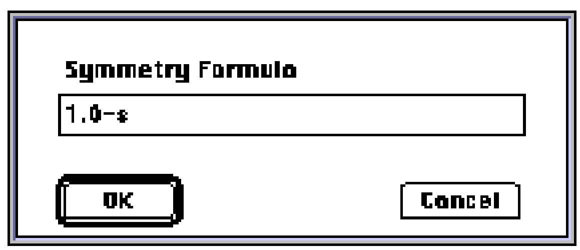


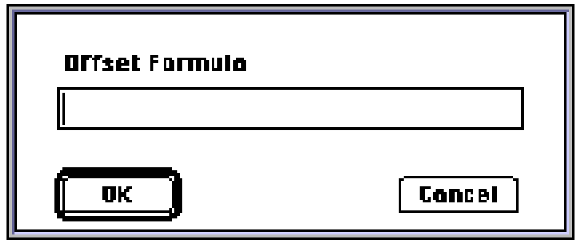
Edit: Selector for the display of a subset of transition rates.

Charge [e0]: If this button is clicked, the transition charge z_{ij} can be specified by a text formula with the dialog shown on the right. If the formula string is empty, the charge is zero. The result of the interpreted formula is shown in the near left item. Unspecified charges are indicated by a dash.



Symmetry: If this button is clicked, the symmetry factor δ_{ij} can be specified by a text formula with the dialog shown on the right. If the formula string is empty, the symmetry factor is zero. The result of the interpreted formula is shown in the near left item. Unspecified symmetry factors are indicated by a dash.

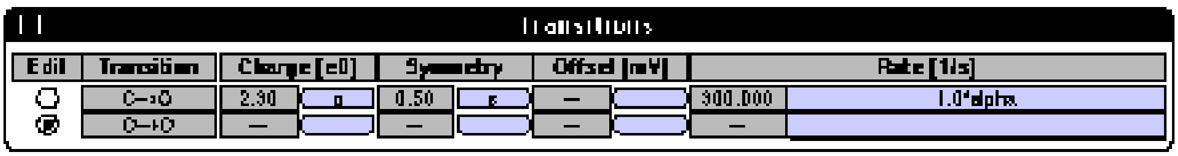




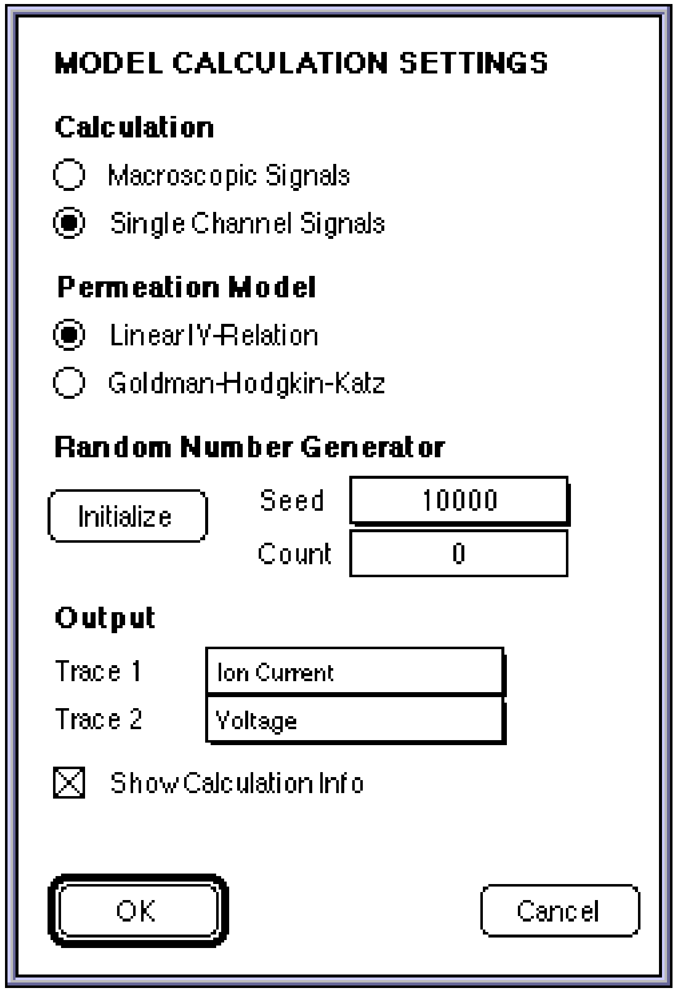
Offset [mV]: If this button is clicked, the voltage offset V_{off} can be specified by a text formula with the dialog shown on the right in units of mV. If the formula string is empty, the offset is zero. The result of the interpreted formula is shown in the near left item. Unspecified offsets are indicated by a dash.

Rate [1/s]: Text formula string for the zero transition rate k_{ij}^{0} in units of 1/s. If the formula string is empty, the rate is zero. The result of the interpreted formula is shown in the near left item. Unspecified rates are indicated by a dash.

In the dialog above, all the transitions to the first state C (closed state) are shown since the first radio button is selected. In the following dialog the second radio button is selected so that all the transition to the second state O (open state) are visible.



Model Calculation Settings



Additional parameters for calculations, which are not stored in the model file, however, are specified in this dialog. It is called from the *Model* drop-down menu. If the dialog is terminated by clicking the button "Cancel", all the settings remain unchanged.

Calculation: Select the kind of signal to be calculated. Two basic kinds of signal are possible: macroscopic and single-channel signals.

Permeation Model: Specify the single-channel I-V relationships for current signals. Linear or GHK (Goldman-Hodgkin-Katz) I-V function is available (see also *PulseFit Manual, Chapter 5 - Series Fit*).

Random Number Generator: The calculation of single-channel signals is based on a direct description of the stochastic process, which makes use of random numbers. The generation of random numbers is initialized with an arbitrary number called "Seed". For subsequent independent signals, however, it is recommended not to re-initialize the generator between calculations. The random number generator of *PulseSim* is based on the implementation of L'Ecuyer (see Press et al., 1994; Steffan, 1998). It allows the generation of approximately 10^{18} pseudo-independent numbers. The present amount of generated numbers during the calculation after initialization is shown by the item "Count". After re-initialization this number is set to zero.

Output: Two signals are stored in the first ("Trace 1") and second trace ("Trace 2") of the *Pulse* data structure. The signal type is selected from a submenu with the following entries:

- **Voltage:** Time course of the voltage determined from the pgf sequence.
- **Ion Current:** Ionic Current based on the right equation, where i_i is single-channel current level of state i and N_C is the number of identical channels. The Numerical methods for the calculation of the occupancy probability function $p_i(n\Delta t)$ of all the N_S states are briefly summarized in Steffan et al., 1998.

$$I(n\Delta t) = N_C \sum_{i=1}^{N_S} \hat{i}_i \, p_i(n\Delta t)$$

- **Gating Current:** Gating Current based on the right equation, where k_{ij} is the transition rate; q_{ij} the transition charge movement between state i and j and N_C is the number of identical channels. Numerical methods for the calculation of the occupancy probability function $p_i(n\Delta t)$ of all the N_S states are briefly summarized in Steffan et al., 1998.

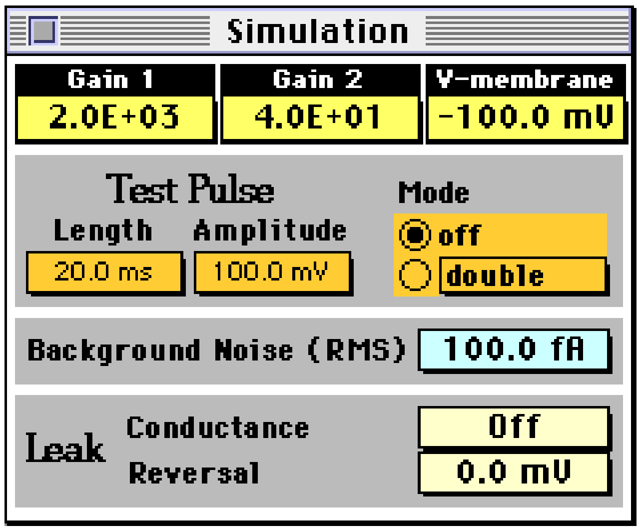
$$I_g(n\Delta t) = N_C \sum_{i=1}^{N_S} \sum_{j=1}^{N_S} p_i(n\Delta t) k_{ij} \, z_{ij} \, e_0$$

- **Total Current:** Sum of ionic and gating current.
- **State Probability:** Occupancy probability function of a particular state, which is selected in the *States* window of the model editor dialogs (see above). If more than one state is selected, the sum of the probabilities is calculated.

Various output during the calculations is written to the terminal window *Notebook* if the item "Show Calculation Info" is checked and if the *Notebook* window is open.

Simulation

The purpose of the *Simulation* dialog is to control the calculation of step responses based on segment-oriented pool sequences defined in the *Pulse Generator* dialog (see *Pulse Manual, Chapter 11 - Pulse Generator*). Since this function is similar to that of an amplifier dialog in *Pulse*, the design of this dialog is similar. Calculated signals are stored in the data tree of the *Replay* window if the “Store” button in the oscilloscope window is checked.



Gain 1, Gain 2: Scaling factors for the storage of the first and second trace. The factors equal the “Gain 1” and “Gain 2” entries of a sweep record and can therefore also be edited after storage in the *Replay* dialog (see *Pulse Manual, Chapter 12 - Replay*).

V-membrane: Sets the desired holding voltage (see also *Pulse Manual*).

Test Pulse: The function of these items are almost identical to that of an real amplifier and can be used for testing or for demonstration purposes. Note that calculation can be very time consuming dependent on the active model. Therefore, the test pulse function is only recommended for relatively fast computers.

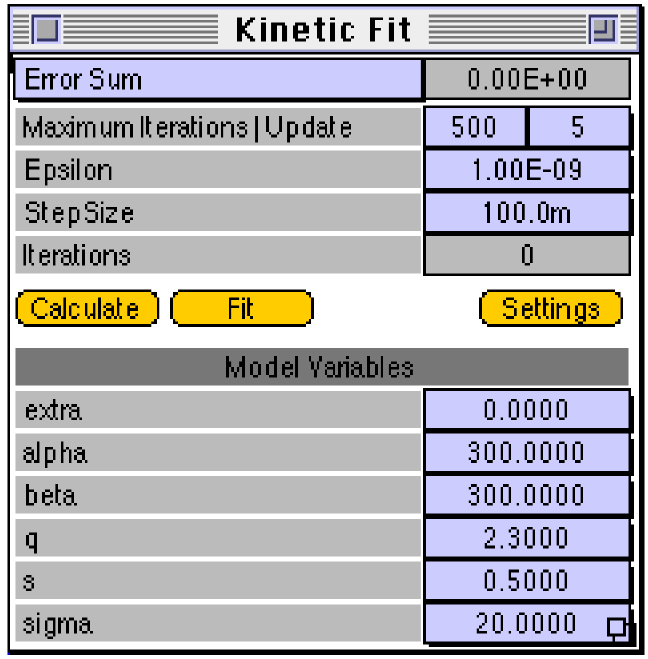
Background Noise (RMS): Sets the standard deviation value of Gaussian distributed noise, which will be superimposed to the calculated signal.

Leak: If “Conductance” is not zero, i.e. “Off”, a leak current I_L will be superimposed to calculated current

$$I_L(n\Delta t) = g_L\left[V(n\Delta t) - E_{rev}^L\right]$$

signals. A linear I-V relationship of the leak is assumed, where g_L is the specified “Conductance” value and E_{rev}^L is the specified reversal voltage (“Reversal”).

Kinetic Fit



The *Kinetic Fit* dialog consists of two sections: data fit control and “Model Variables”.

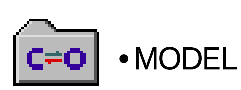
In the section “Model Variables” a list of the used-defined variables is shown. It is a copy of the variable list in the model editor dialog “Variables”. This variable list is updated on-line during data fits. As a helpful feature, the variables can also be edited here without calling the model editor dialog. This can be used for manual data fits, for example.

The data fit control functions are used to set the parameters for optimization procedures. In the initial version of *PulseSim*, only the Simplex algorithm, which is also used by *PulseFit*, is available. Therefore, most of the fit control functions are almost identical to that of *PulseFit* and are only briefly described here (see *PulseFit Manual, Chapter 4 - Sweep Fit*).

Fit Control Functions

Error Sum: Squared deviation between fit and data. This value is used as residual for the fit algorithm.

Maximum Iterations: Maximum number of fit iterations. When this number is reached, the fit will be terminated.



Update: Number of fit iterations between window updates. With every update, a backup of the present model file is stored in the “•MODEL” (MacOS) or “SimModel” (Windows) directory within the *PulseSim* directory. If the model directory does not exist, a new directory will be automatically created. The present date is appended to the file name.

Epsilon: Criterion for fit termination.

Step Size: Initial relative step size for changing fit parameters.

Iterations: Iterations counter.

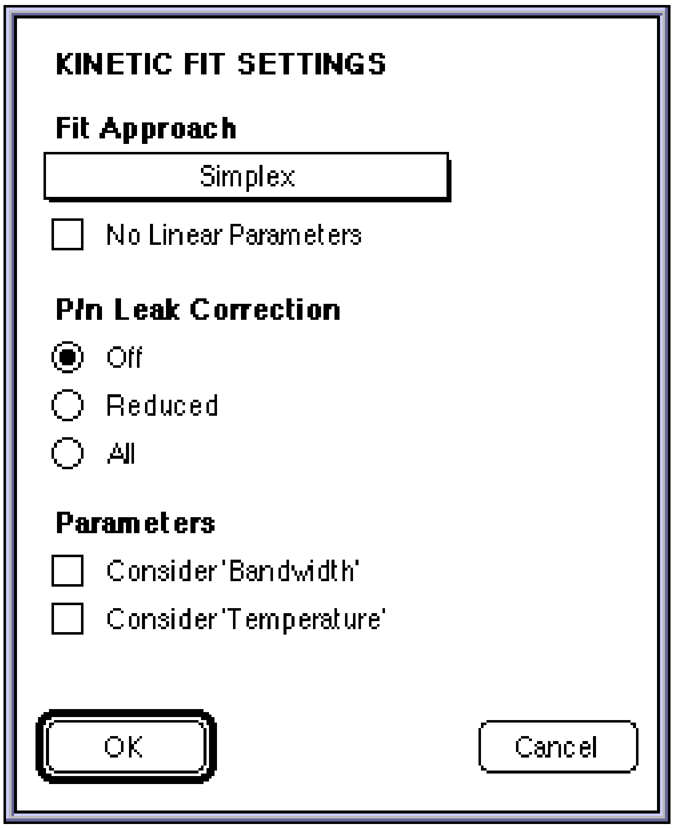
Calculate: Run kinetic calculation according to the pgf-template as part of the data file. This operation is performed either on individual sweeps/series/groups, depending on the selection or marks in the input tree and the cursor settings. The model predictions are superimposed to the data displayed in the oscilloscope window, i.e. the voltage trace, the leak-corrected current trace and the leak trace. The deviation between the first data trace and the first calculated trace is calculated and the "Error Sum" item (see above) is updated.

Fit: Run fit by iterative kinetic calculations.

Settings: Calls a dialog for specifying various additional parameters.

Kinetic Fit Settings

Additional parameters for both, calculation and fit control are specified in this dialog. If the dialog is terminated by clicking the button "Cancel", all the settings remain unchanged.



Fit Approach: Optimization algorithm used for data fits. In the present version of *PulseSim*, only the Simplex method is selectable.

No Linear Parameters: Before each iteration step, the number of channels is determined by linear regression (if "Channels" is selected for fit in the model editor dialog "Parameters"). If this item is checked, the number of channels is also varied by the non-linear fit approach (see also Steffan, 1998).

P/n Leak Correction: In order to fit the model to the "real" linearly leak-corrected data, both the test data and the *p/n* trace have to be calculated based on the model before comparison (for details see Steffan et al., 1998). Three options are available:

- **Off:** Leak correction is not considered for calculations and data fits.
- **Reduced:** Only one or two (in the case of alternating leak pulses) leak responses are calculated, no matter how many leak pulses are specified.
- **All:** Leak responses are calculated and averaged according to the specified number of leaks in the pgf-file.

Parameters: Interface to *Pulse* parameters.

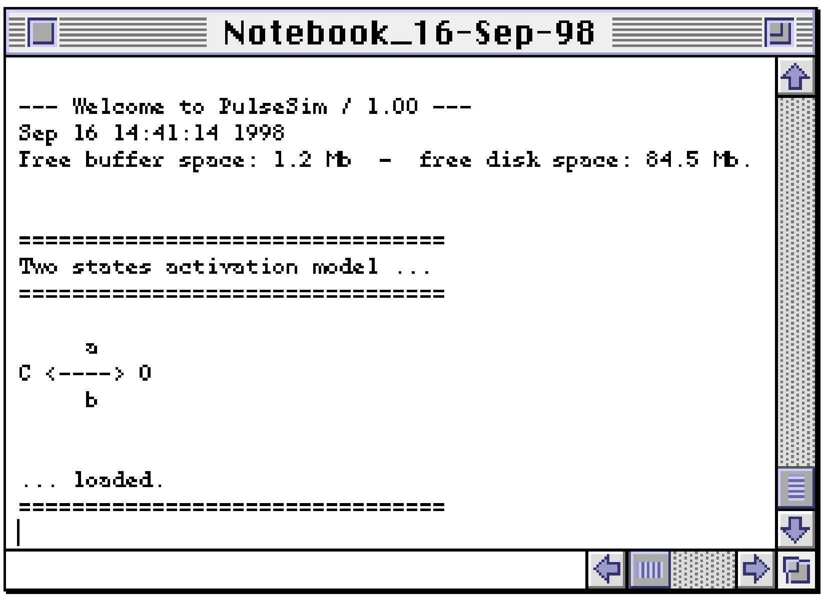
- **Consider 'Bandwidth':** If this item is selected, the cut-off frequency parameter of the model is automatically updated according to the bandwidth parameter of the series which is currently processed. The series parameters can be edited in the *Replay* dialog (see *Pulse Manual, Chapter 12 - Replay*).
- **Consider 'Temperature':** The temperature parameter of the model is automatically updated. Similar function as "Consider Bandwidth".

4. The First Simulation

In this chapter it is briefly described how a very simple first simulation with *PulseSim* could look like. The required computing time used for kinetic calculations of *PulseSim* mainly depends on the size and the parameter values of the model. The importance of the computer speed can therefore not be overestimated. Here, a simple two states model, as it was also used in the previous chapter to describe the model editor dialogs, is used. With this simple model one should be able to reiterate these examples even with a very slow computer. The basic steps for using most of the features of *PulseSim* are outlined.

Setting Up the Model

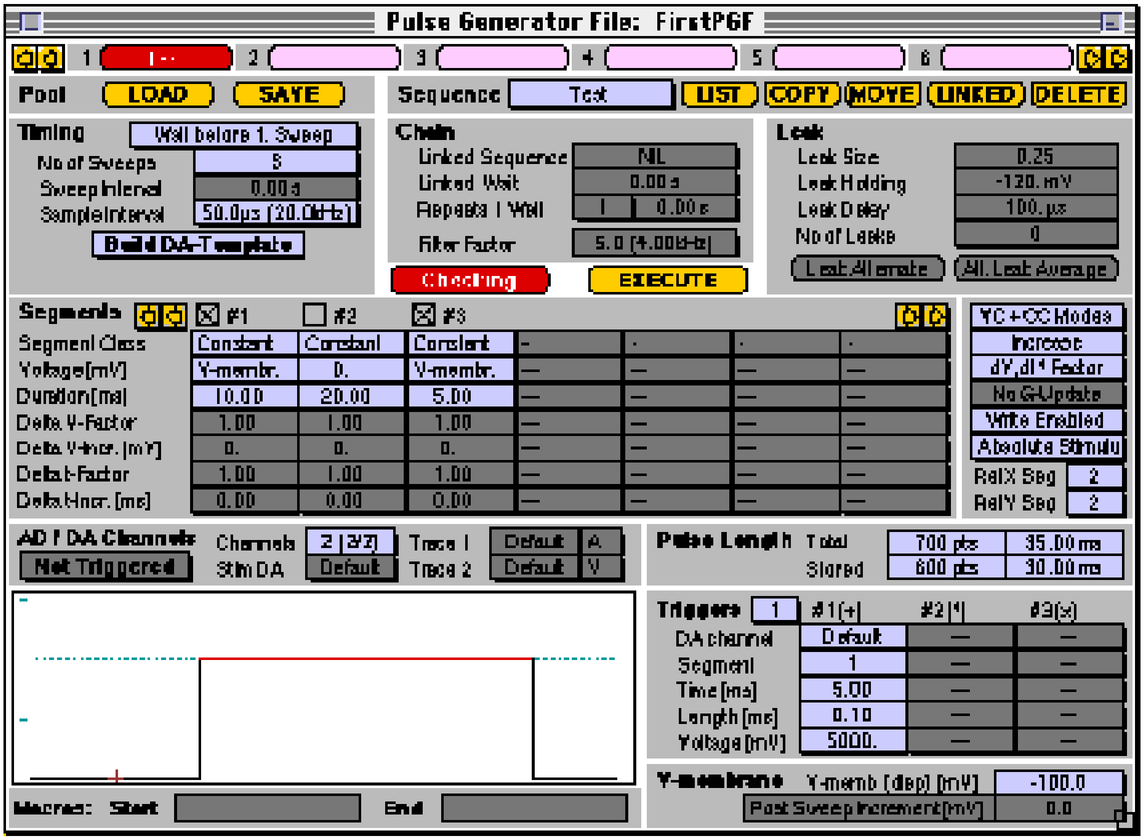
Start *PulseSim* and select the entry “Load Default CO-Model” in the *Model* drop-down menu (see *PulseSim Manual, Chapter 2 - Files and Menus*). If the notebook window is open (see *Pulse Manual*), the information shown on the right will be written to the notebook.



Use the model editor dialogs to adjust the model parameters such that the model is identical to the model described in the chapter *Dialogs* (α =300, β =300, etc.). Alternatively, the two states model can also be loaded from the folder “•MODEL” (MacOS) or “SimModel” (Windows) within the *PulseSim* folder. The file name of the model is “CO_Model”.

Generate a Pulse Protocol

Similar to a “real” experiment, a pulse protocol has to be generated in the pulse generator dialog (see *Pulse Manual*). Adjust the parameters of a simple test pulse with three segments according to the depicted dialog below. Note that the number of leak pulses is zero, that two AD / DA channels are specified (i.e. two calculated traces will be stored), and that the sample interval is 50µs so that 600 data points will be calculated. The number of sweeps is three.



A pgf-file that contains this pulse template can also be found in the "•DATA" (MacOS) or "SimData" (Windows) folder within the *PulseSim* directory. The file name is "SimPGF.pgf". It can be loaded by clicking the button "LOAD" in the pulse generator dialog.

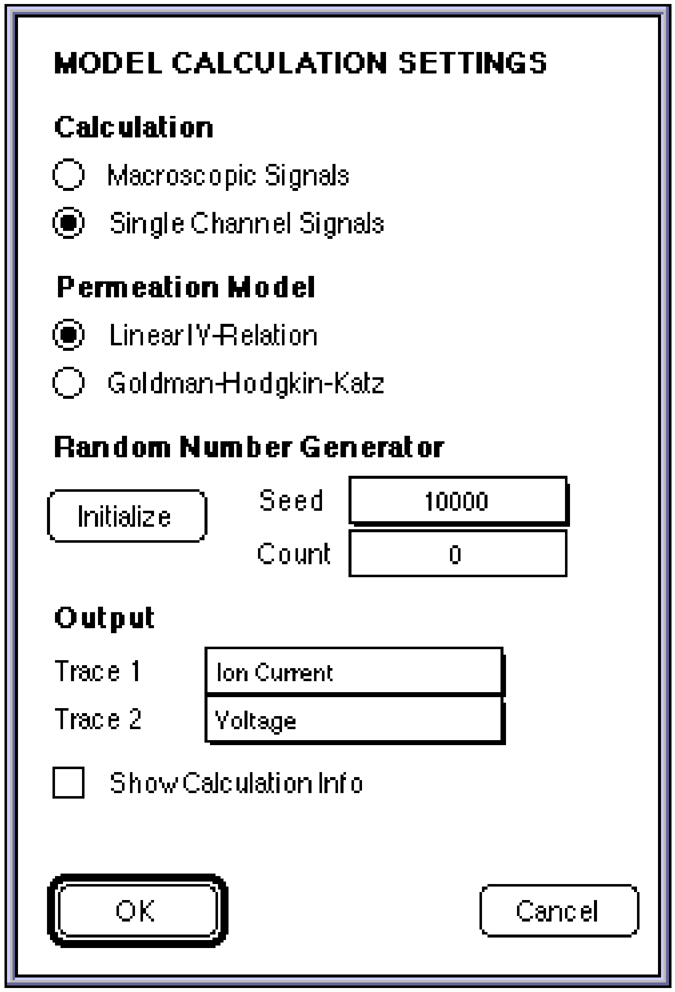
Simulation Control

Set "Gain 1" and "Gain 2" in the *Simulation* dialog to 2000 and 40, respectively. The item *V-membrane* shows the holding voltage, which is used for the first and third segment of the specified *Test* sequence. Set this value to -100 mV; the arrows keys can be used similarly to a real amplifier control. Background noise should be 100 fA.

Create a new file with the name “FirstSim” in a user directory and activate the button *Store* in the oscilloscope window.

Single Channel Simulation

Now we can start to do a first simulation. In order to “acquire” single-channel current responses, additional model parameters have to be adjusted as follows.



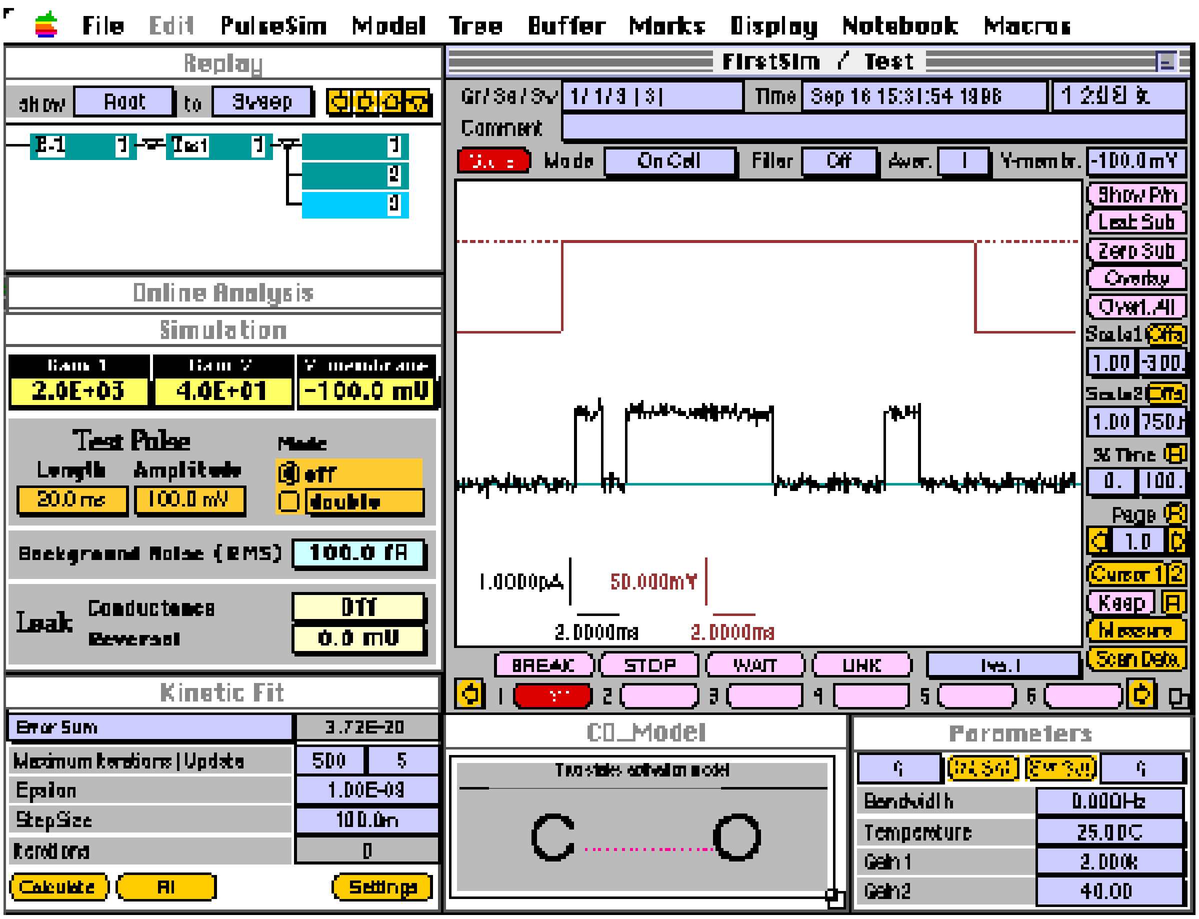
Open the *Model Calculation Settings* window (selected from the drop-down menu *Model*). Select the radio button *Single Channel Signals*, initialize the random number generator with a seed of 10,000 and set *Trace 1* to *Ion Current* and *Trace 2* to *Voltage*. Close this window with the return key or with the button “OK”. Otherwise all modifications will be ignored.

Check *Show Zero Line* and *Second Trace* as the *Backround Trace* in the drop-down menu *Display*. All other display features should be deactivated (not-checked).

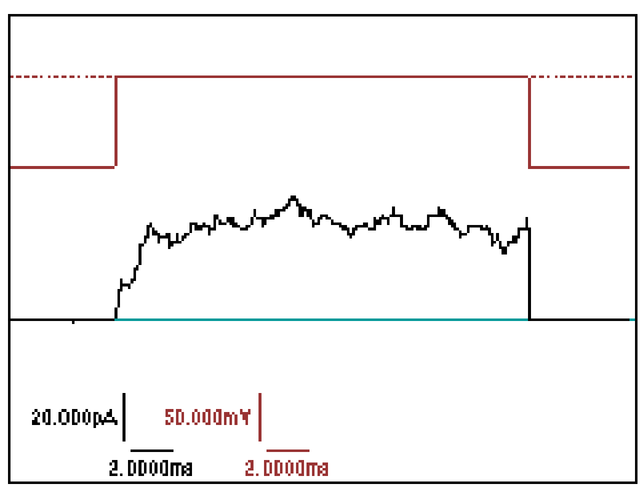
Switch to the oscilloscope window and set *Scale 1* to 1.0; -0.3 and *Scale 2* to 1.0; 0.75. The result after execution of the sequence “Test” is shown in the screen picture below.

Three single-channel current responses were calculated and stored in the data tree of the *Replay* window. The last current response is still shown in the oscilloscope window. Additionally, the voltage trace as used for calculation is stored and displayed as second trace. The third current response indicates that at -100 mV the channel is closed most of the time, so that only the background noise is obtained (i.e. the open probability is very low, as expected). At the test voltage of 0 mV the channel randomly opens three times with random open sojourn times.

For comparison, these data can also be found as part of the “SimDemo” file.



Simulation of Fluctuating Currents



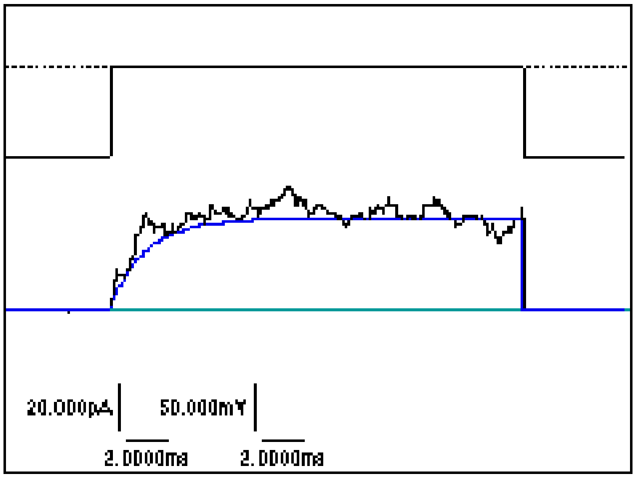
If more than one channel is in a patch, fluctuating current responses are obtained. They can be simulated by the superposition of independent single-channel responses.

Increase the number of channels to 50 in the *Parameters* dialog window as part of the model editor, which is called from the drop-down menu *Model*. Switch to the *Simulation* window an decrease *Gain 1* to 100. Set the number of sweeps to 1 in *Pulse*

Generator window and execute the sequence "Test" again. The calculated traces will be stored in a new series of the *Replay* window. The resulting oscilloscope window is depicted on the right.

The fluctuations of the current response result from the random opening and closing of the "channels". Note, that the background noise was not changed for this simulation. It is negligible small at this gain level, however.

Calculation of Macroscopic Currents



In the following, the stored fluctuating current response is used to demonstrate the calculation of macroscopic currents based on "acquired" data.

Switch to the *Replay* window and select the sweep which correspond to the previous simulation. Switch to the oscilloscope window and activate the button *Overlay*. Switch to the *Online Analysis* window and set Left B and Right B to 0% and 125%, respectively. Call the *Model Calculation Settings* window from the drop-down menu *Model* and select the radio button *Macroscopic Signals*. Switch to the *Kinetic Fit* window and select the button *Calculate*.

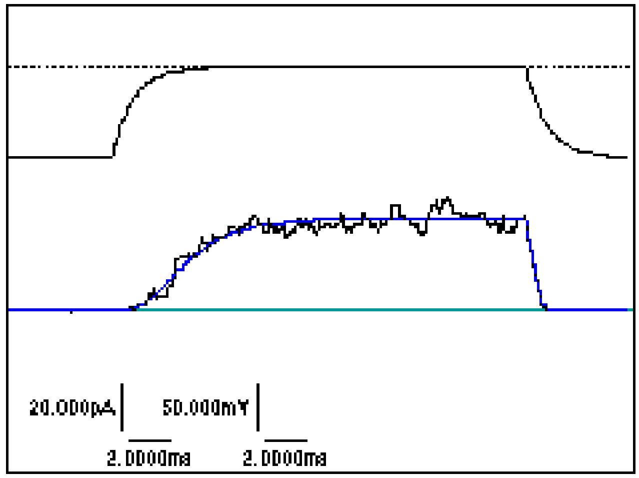
The current response is now calculated within the specified cursor range according to the pgf-information of the stored data. The resulting macroscopic current corresponds to the average of an infinite number of experiments and therefore shows no fluctuations. This theoretical result is superimposed to the "acquired" data as depicted on the right where the trace color corresponds to the color of fit traces, which can be specified in the *Configuration* window (see *Pulse Manual*). Note that the voltage trace is also superimposed to the second trace of the stored data. In this example they must be identical, of course.

Considering Non-ideal Voltage Steps

The same model calculations as shown before shall be performed considering a non-ideal voltage step. In *PulseSim*, the rise time of the voltage is described by an exponential function.

Open the *Parameters* window as part of the model editor from the drop-down menu *Model* and set *Tau 1* to 1ms. The algorithm for the simulation of single-channel currents will be identical as in the case of an ideal voltage step. However, the algorithm for the calculation of the theoretical macroscopic current will be different (for details see Steffan et al.; Steffan, 1998). The results are described in the following. The data can also be found in the file "SimDemo".

When all the calculations are performed exactly as described before, the oscilloscope picture as shown on the right will be obtained.



The time course of the voltage increase and decrease following an exponential function as required. The time course of the fluctuating current, which results from simulated single-channel currents, clearly match the time course of the theoretical macroscopic current.

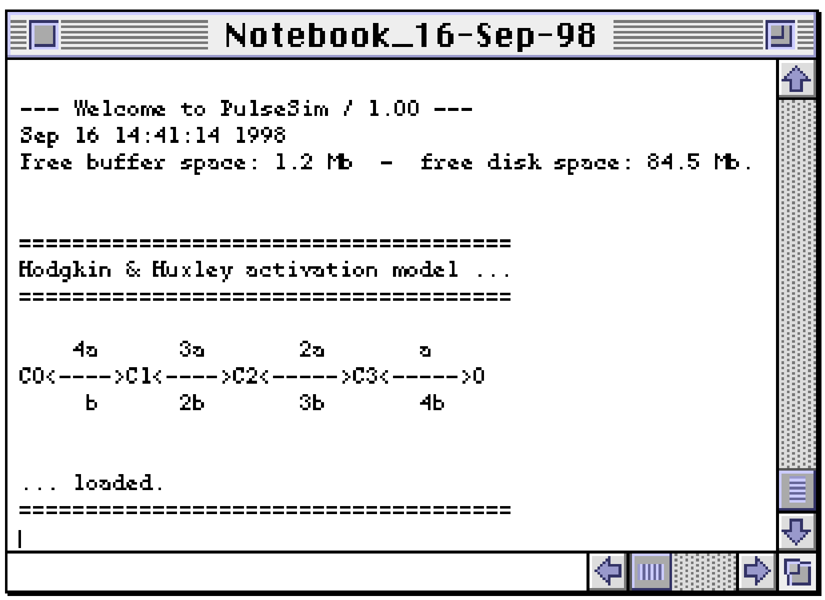
5. The First Modeling

In this chapter it is briefly described how a very simple first modeling analysis with *PulseSim* could look like. It is shown step by step how a build-in model of *PulseSim* is extended and how the parameters of such a user-designed model can be fit to experimental data. As an initial example, the “Default HH-Model” is extended to describe the voltage-dependent activation and inactivation of Kv1.4 potassium channels where the experimental data have been acquired with two-electrode voltage clamp methods.

$$C \underset{\beta}{\overset{4\alpha}{\rightleftharpoons}} C \underset{2\beta}{\overset{3\alpha}{\rightleftharpoons}} C \underset{3\beta}{\overset{2\alpha}{\rightleftharpoons}} C \underset{4\beta}{\overset{\alpha}{\rightleftharpoons}} O \underset{\delta}{\overset{\gamma}{\rightleftharpoons}} I$$

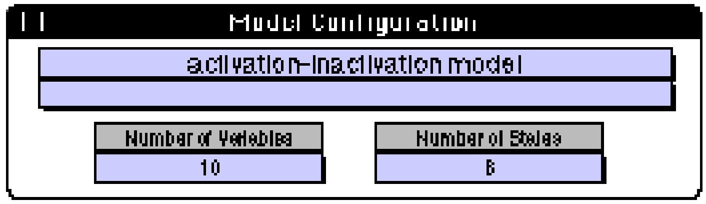
A simplified model for voltage-gating of potassium channels with inactivation is shown on the right where the activation pathway corresponds to the n^4-formalism of Hodgkin and Huxley and where the open state (“O”) is followed by just one inactivated state (“I”) (see Zagotta and Aldrich, 1990). The forward and backward transitions to and from the inactivated state shall be only slightly voltage-dependent and have the zero rates γ and δ, respectively.

Setting up the Model

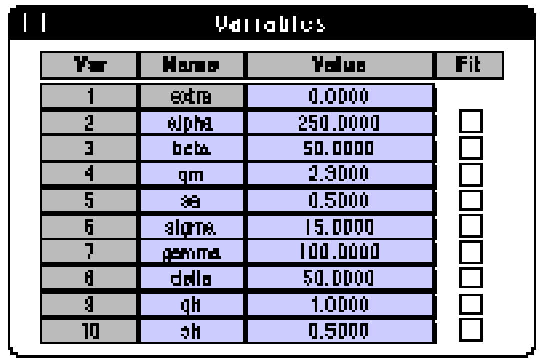


Start *PulseSim* and select the entry “Load Default HH-Model” in the *Model* drop-down menu (see *PulseSim Manual, Chapter 2 - Files and Menus*). If the notebook window is open (see *Pulse Manual*), the information shown on the right will be written to the notebook. In order to extend this model by one state with voltage-dependent transition rates, four additional model parameters are required: the zero rates γ and δ, the corresponding transition charge q_h and the symmetry factor s_h of the assumed energy barrier of the transition.

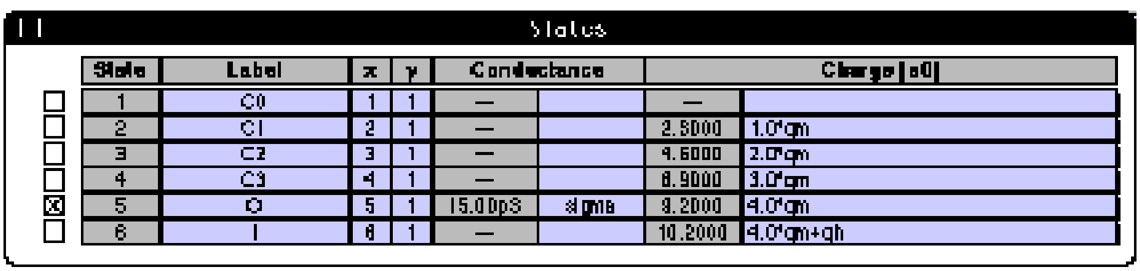
Open the *Model Configuration* dialog window from the *Model* drop-down menu and increase the number of states by one and the number of variables by four. The comment line could be changed to “activation-inactivation model”, for example



Open the *Variables* dialog window from the *Model* drop-down menu where four additional variable items can be found. Entitle these variables “gamma”, “delta”, “qh” and “sh” and set their default values to 100, 50, 1 and 0.5, respectively. In the rightmost column, the variables, which shall be varied during a data fit, have to be checked. This will be done later, however.

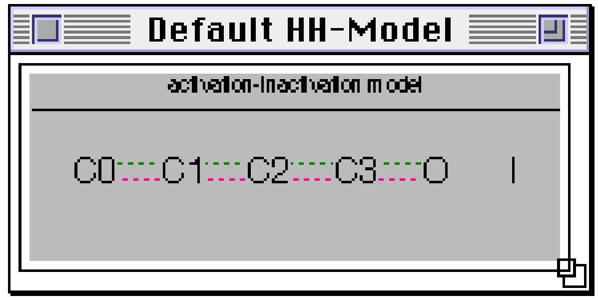


Now the inactivation state can be specified in the *States* dialog as shown below. The state label is “I” and shall be depicted right from the state “O” so that the x-y-coordinates of “I” are 6 and 1, respectively.

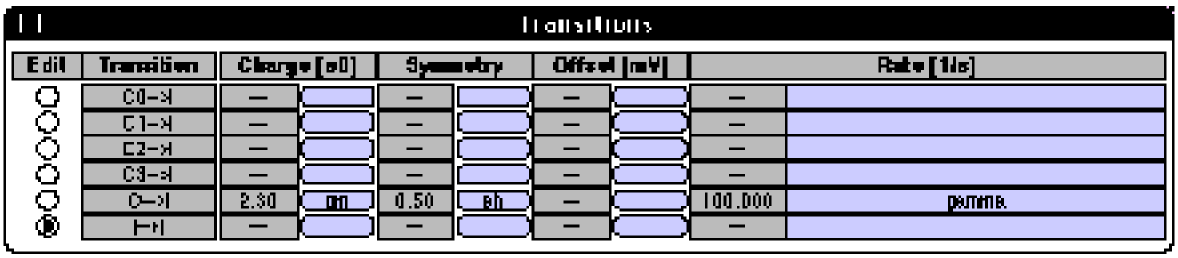


The total charge movement from state C0 to state I is $(4q_m + q_h)$. Type this formula string in the text item “Charge[e0]”. According to the current variables values, this formula equals 10.2 as shown in the near left item.

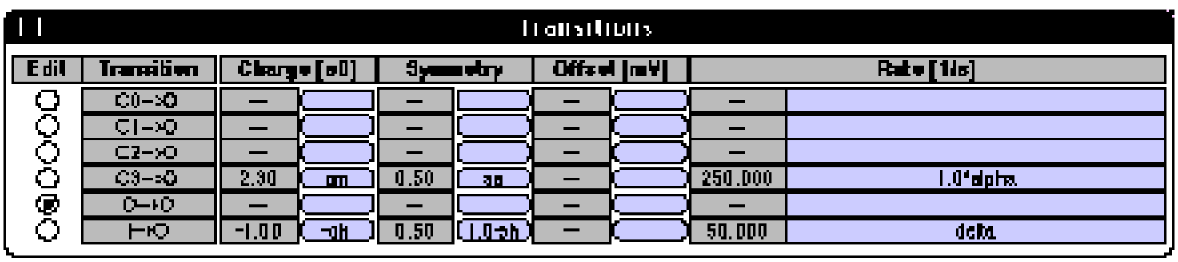
The results of these modifications can be seen on-line in the *Model* window. So far, the transition rates between states I and O were not specified so that the corresponding dashed lines are still missing. These rates are specified in the dialog window *Transitions* as described in the following.



All transitions to the inactivated state can be specified by clicking on the radio button nearby the label”I”. In this simple model, only one transition to state I exists. Type the formula strings in the row labeled by “O-->I“ as depicted below.

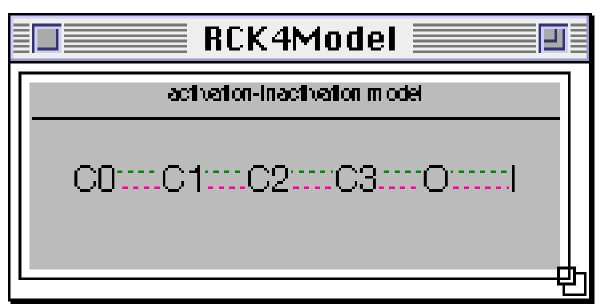


Click on the radio button nearby the label ”O” so that all the transitions to the open state can be modified. Type the formula strings of the transition “I-->O”.



Note: The charge movement for the backward transition is negative. The corresponding symmetry factor must be specified such that the sum of the forward and backward symmetry factor equals one.

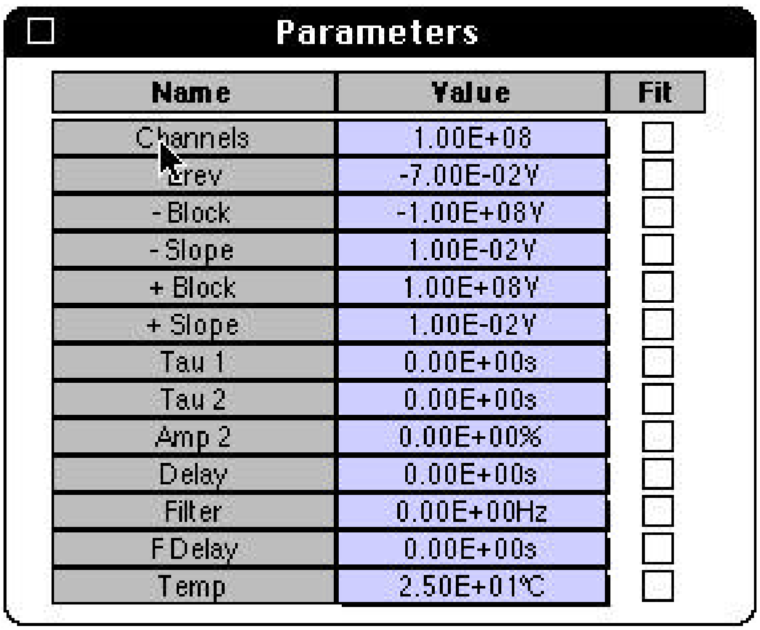
If the transitions were specified properly, the corresponding dashed lines will appear in the model scheme depicted in the *Model* window. Save the model using the file name “RCK4Model” by selecting the menu entry “Save as...” in the *Model*



drop-down menu. The *Model* window title will be updated automatically as shown on the right.

Specification of Experimental Parameters

Once the model is setup, a data file has to be opened. The model parameters have to be adjusted according to the experimental data. These parameters can be kept fixed during a data fit or they are left free to vary: check Fit box.



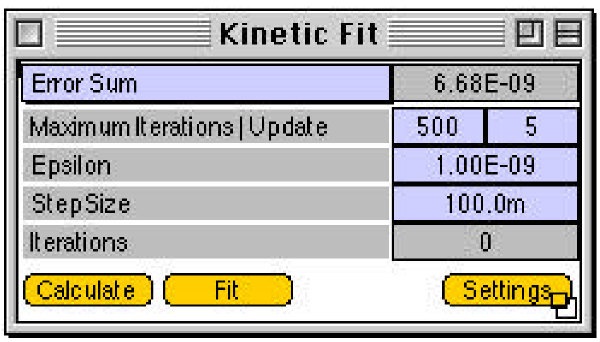
The parameters have the following meaning:

- **Channels:** Number of channels.
- **Erev:** Reversal potential.
- **-Block:** Half-maximal voltage of block at negative potentials (single Boltzmann function). This is used to account for non-linear single-channel iV curves; saturation at negative voltages.
- **-Slope:** Slope factor associated to “-Block”.
- **+Block:** Half-maximal voltage of block at positive potentials (single Boltzmann function). This is used to account for non-linear single-channel iV curves; saturation at positive voltages.
- **+Slope:** Slope factor associated to “+Block”.

- **Tau1, Tau2, Amp2:** Time constants and relative amplitude of the voltage rise time function.
- **Delay:** Delay between theoretical and real voltage trace.
- **Filter:** Low-pass filter frequency.
- **FDelay:** Extra delay introduced by low-pass filter.

Calculation Settings and Data Fit Control

The type of single-channel iV has to be selected using the “Calculation Settings …” menu (see Chapter 4). In order to get an idea whether or not the selected parameters are reasonable, a data trace should be selected by highlighting it. By means of the “Calculate” button in the “Kinetic Fit” window a data trace is calculated according to the model parameters and the template of the data file. The result is superimposed in blue. Before doing a serious data fit, one should try to adjust the most crucial parameters by hand.

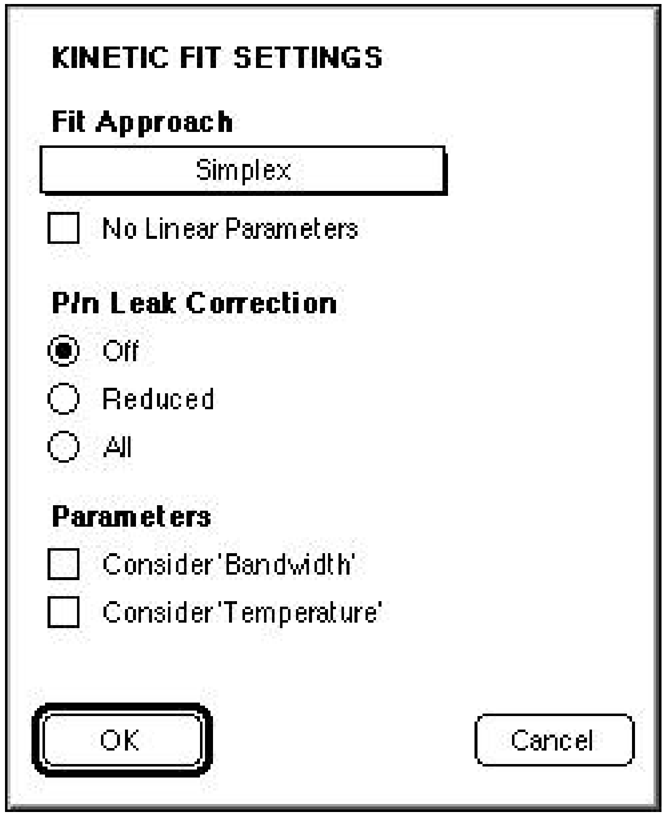


The Kinetic Fit dialog is used to setup fit parameters.

- **ErrorSum:** This is the method used to calculate a residuum.
- **Maximum Iterations / Update:** Here the maximal number of fit iterations has to be specified. After “Update” iterations the fit results are displayed.
- **Epsilon:** Convergence criterion for the Simplex fit.
- **StepSize:** Initial deviation of the fit parameters. 100m means that before the first iteration the Simplex algorithm alters the parameters by maximal 10%.
- **Iterations:** Counter for the number of iterations.
- **Calculate:** Calculate traces based on the highlighted traces.

- **Fit:** Start Simplex data fit. Fit the selected data traces in the cursor bounds.
- **Settings:** Additional Settings for the fit (see below).

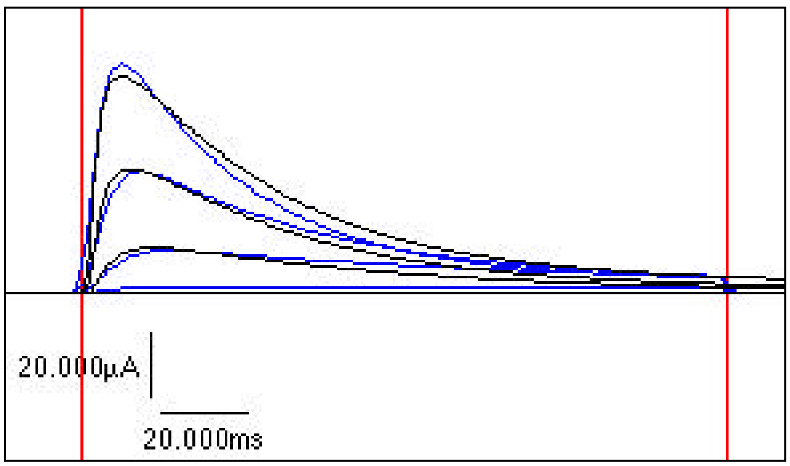
Kinetic fit settings:



- **Fit Approach:** Currently the only option is Simplex fit.
- **No Linear Parameters:** By default linear parameters (i.e. number of channels) are calculated directly. This option, which makes the fit faster, can be disabled.
- **P/n Leak Correction:** Off: no leak currents are calculated. Reduced: only one set of leak currents are calculated if “NumberLeaks” is greater than 1 (or greater than 2 for alternating leaks). All: The complete leak template is calculated.
- **Parameters:** Read *Bandwidth* and *Temperature* information from input file. This is important if various data traces with different *Bandwidth* and/or *Temperature* are to be fitted simultaneously.

Example of Fit

In the example shown below we used two-electrode voltage clamp recordings from oocytes injected with mRNA coding for Kv1.4 channels. We selected four data traces from an IV family (at -50, -30, -10, and +10 mV). These traces are here shown in black. A simple activation model (see above) was fitted within the indicated cursor bounds. The blue traces are the fit results after 16 iterations. Note that these data are heavily affected by filtering and slow voltage clamp. Therefore, the selections made for the parameters “Filter”, “Delay”, and “FilterDelay” are very important.



The fit procedure can be interrupted with the ESC key.

6. References

Colquhoun D., and A. G. Hawks. 1995. The principles of the stochastic interpretation of ion--channel mechanisms. in Single Channel Recording. Sakmann B., and E. Neher. Plenum Press.

Colquhoun D., and A. G. Hawks. 1995. A Q--Matrix cookbook. How to write only one program to calculate the single--channel and macroscopic predictions for any kinetic mechanism. in Single Channel Recording. Sakmann B., and E. Neher. Plenum Press.

Hodgkin A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.)* **117**: 500-544.

Sigworth F. J. 1993. Voltage gating of ion channels. *Quart. Rev. Biophys.* **27**: 1-40

Steffan R., C. Hennesthal, and S. H. Heinemann. 1998. Voltage--dependent ion channels: analysis of nonideal macroscopic current data. *Meth. Enzym.* **293**: 391-419.

Steffan R. 1998. Modellierung und Simulation nichtstationärer Stromantworten spannungsabhängiger Ionenkanäle. Dissertation University of Stuttgart. Shaker Verlag Aachen. In press.

Zagotta W. N., and R. W. Aldrich. 1990. Voltage-dependent gating of *Shaker* A-type potassium channels in *Drosophila* muscle. *J. Gen. Physiol.* **95**: 29-60.

Appendix

Build-In Models

In the following the build-in models are briefly described. They can be loaded from the drop-down menu *Model* and serve as starting points for the development of more sophisticated models. The number of channels of all the default models is one and the symmetry factors of energy barriers is 0.5 (see chapter *Model Editor, Transitions* of this manual). The model “Parameters” are not active, i.e. no single-channel IV-block, no filtering, and ideal voltage rise time, for example.

Default CO-Model

$$C \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} O$$

A simple two-states model with one closed (“C”) and one open (“O”) state. The transition rate from C to O is α, the transition rate from O to C is β, where $\alpha = 500/s$ and $\beta = 50/s$.

Default HH-Model

$$C \underset{\beta}{\overset{4\alpha}{\rightleftharpoons}} C \underset{2\beta}{\overset{3\alpha}{\rightleftharpoons}} C \underset{3\beta}{\overset{2\alpha}{\rightleftharpoons}} C \underset{4\beta}{\overset{\alpha}{\rightleftharpoons}} O$$

The HH-model corresponds to the n^4-formalism of Hodgkin and Huxley for voltage-dependent potassium channels. It consists of three closed states “C” and one open state “O”. All the forward transitions have rate α, all the backward transitions have rate β, where $\alpha = 250/s$ and $\beta = 50/s$. The statistical rate factors result from the assumption of independent transitions, i.e. no cooperativity between the four subunits. These factors are part of the transition formula strings where α and β are “Variables”.