Current Clamp Recordings with PATCHMASTER™ Software

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TALK OVERVIEW

• EPC 10 revision T hardware overview
• Intracellular recordings with high resistance electrodes
• Current clamp recording
• Voltage clamp recording using AP as stimulus
• Online analysis – AP functions
What can we measure:

- Membrane resting potential
- Spontaneous action potentials
- Elicited action potentials
Extended features

The EPC 10 USB Revision “T”:

- increases the voltage stimulus range to 2V in voltage clamp mode
- increases the voltage measuring range to 5V in current clamp mode
- increases the current injection capability in current clamp by a factor of 5
- lower noise red star headstage

Low Noise!
- 31 fA @ 1 kHz
- 120 fA @ 5 kHz
EPC 10 Hardware Controls

- Current Gain
- CC Mode
- C-fast
- C-slow
- Bridge
- Filter2
- I-membrane
- CC Gain
- Gentle Switch
- V-mon Gain
- Filter1
- Ext Command
Gentle CC-Switch

- OFF - I-membrane will be set to 0pA
- ON - I-membrane will be set to the proper value to keep the membrane voltage at the commanded voltage in voltage clamp
CC-Gain & Current Gain

Four gain ranges for setting maximum current injection

CC-gain ranges of 0.1 or 1.0 pA/mV will operate in the medium gain range

CC-gain ranges of 10.0 or 100.0 pA/mV will operate in the low gain range
-> V-mon Gain

Measured voltage can be amplified by either a factor of 10 or 100 before digitization
The External CC-stim input will be added to the internal stimulus (I-membrane and PGF).

The External CC-stim input is scaled by CC- Gain value.
Filter 1

Automatically set

- 10kHz when CC-gain = 0.1 or 1.0pA/mV
- 30kHz when CC-gain = 10.0 or 100.0pA/mV
Filter2 can be applied to:

- Current signal
- Bypassed
- Voltage signal
Bridge Balance

A balanced bridge is the ability to inject current through the resistance of the pipette while still measuring the true membrane voltage.

When switching modes:
- Rs Comp is transformed to Bridge
- Bridge is initially OFF
→ Bridge Balance settings

Time constant - vary based on CC-Gain range settings

Percentage control - % of R-Series used for compensation

Maximum access resistances that can be compensated:
- 0.1pA/mV and 1.0pA/mV CC-gain: 500MΩ
- 10pA/mV and 100pA/mV CC-gain: 5MΩ
C-fast compensation

In VC mode perform Auto C-fast to neutralize capacitance of the electrode

CC-mode:

Automatic C-fast compensation is disabled

C-fast is reduced by 0.5pF to reduce possibility of oscillation and potentially the destruction of the cell membrane
→ C-slow compensation

Perform Auto C-slow in VC mode to:

- Cancel any remaining capacitive transients
- Get initial estimates of membrane capacitance and series resistance

CC-mode:

C-slow compensation is automatically turned OFF

WHY? C-slow affects cellular parameters which will distort the voltage signal
Current clamp recording mode

- The input of the headstage acts as a high impedance voltage follower
- The feedback resistor is used for stimulation
- I-membrane is set
- Internal stimulation stopped
- Filter1 is set to Bessel 10kHz or 30kHz
- C-slow = Off
- C-fast reduced by 0.5pF
- Rs-compensation = “bridge balance”
Example - high resistance electrodes

Top: high resistance $\sim 50 \, \text{M}\Omega$

Middle: a clogged electrode or an “ideal” On Cell configuration

Lower: impaled cell with high access resistance
Electrode in bath
Test Pulse is executed
Current and voltage acquired
Setup to Reset
R-memb ~ 50MΩ
Example - high resistance electrodes

Have Gigaseal
Auto C-Fast
Example - high resistance electrodes

Impale cell

Auto C-slow
Example - high resistance electrodes

Current clamp mode

Enable Test Pulse
Example - high resistance electrodes

Set Bridge values to $10\mu s$ and 100%
Current clamp recording - AP

Action Potential

- Depolarization phase
- Repolarization phase
- Threshold
- Resting potential
- Refractory period
- Stimulus
Current clamp recording

PGF sequence to record an action potential.
Current clamp recording

PGF sequence to record an action potential train
Current clamp recording

PGF sequence to elicit action potentials
→ Using an AP as stimulus

To export the voltage trace:
Select the trace from data tree
Replay menu select Export Trace - As Stimulus Template
Enter file name
Using an AP as stimulus

To use the file template we need to create a pgf sequence:

Select Copy

Enter AP_template as new sequence
Using an AP as stimulus

A few minor adjustments

For DA channel 1 “load from file template” needs to be activated
Using an AP as stimulus

Change PGF type to Voltage Clamp
Using an AP as stimulus

Results!

Current

Stimulus
AP Online analysis

The AP Analysis is designed for analysis of evoked action potentials.
AP Online analysis

Y-axis parameters:

- Baseline
- AP Amplitude
- Repolarization Amplitude
- Up Slope
- Down Slope
AP Online analysis

X-axis parameters:

- Time to Repolarization Amplitude
- Time to AP Amplitude
- Rise Time
- Rise Time Delay
- Decay Time
- Decay Time Delay
- Decay Tau
AP Online analysis

Sweep of at least three segments:
- baseline segment
- stimulus segment
- waiting segment

Stimulus segment is marked as Relevant X-and Y-segment
AP Online analysis

Cursor ranges

start of the second segment

end of the third segment
AP Online analysis example

X Function

Y Function

Measurements
- Extremum
- Maximum
- Minimum
- Mean
- Integral
- Variance
- Slope
- Peak Amplitude

Analysis Functions

AP Analysis
- Baseline
- AP Amplitude
- Time to AP Ampl
- Repol Ampl
- Time to Repol Ampl
- Rise Time
- Up Slope
- Rise Time Delay
- Decay Time
- Down Slope
- Decay Time Delay
- Decay Tau

Analysis Functions

- Timer
- Time to Min
- AT_ampl
- DecayTau

Cursor Bounds (%)

X-Y Seg. Offset

Trace #

Notebook

Fit

Trace 1

Trace 2

Highlights

- Maximum
- NEW
- DELETE
- MOVE

List

Value not stored
AP Online analysis example

Create Graph

- Select x and y parameters
- Specify axis scaling
- Select graph position
Sub-threshold depolarization
AP Online analysis – APD$_{80}$

Action Potential

$\text{APD}_{80} = a - b$

80% Rise Time Delay
80% Decay Time Delay

Resting potential
Online analysis – APD$_{80}$
Online analysis – $\text{APD}_{80}$

$\text{APD}_{80}$
Online analysis – APD$_{80}$

Create Graph

- Select x and y parameters
- Specify axis scaling
- Select graph position
Online analysis – $\text{APD}_{80}$
### Summary

- Proper C-fast and C-slow compensation before switching to CC mode defines initial parameters.
- When adjusting Bridge Balance start with an initial value of 100%.
- To initiate an Action Potential the current injection (stimulus) must be above the threshold.
- Variety of online analysis functions are available, including Math functions and user defined Equations.
Thank you!