USING THE SWAM IIc PATCH-CLAMP AMPLIFIER:

A TUTORIAL

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C_m recordings using SWAM IIc

The SWAM IIc patch-clamp/lock-in amplifier enables two types of membrane capacitance (C_m) recordings, compensated and uncompensated (Lindau & Neher; 1988, Zorec et. al.; 1991). In the **compensated recording** the bulk of cell capacitive current is compensated (i.e. cancelled) and small changes in cell capacitance are measured (in the range of fF or lower, known also as microscopic membrane capacitance measurements) . With the correct phase angle setting of the lock-in amplifier the two lock-in amplifier outputs of the SWAM IIc become proportional to small changes in combined conductance (access and membrane conductance, influenced by membrane capacitance) – G and cell membrane capacitance - C, respectively. In the **uncompensated recording** (measuring range tens of pF, known also as macroscopic membrane capacitance measurements) the four outputs from the patch-clamp/lock-in amplifier (DC current (I_{DC}), voltage (V_m), imaginary (Y_{im}) and real (Y_{re}) admittance) together with the estimated reversal potential provide parameters used in the on-line calculation of the membrane capacitance (C_m), membrane conductance (G_m) and access conductance (G_a).

Here we present a step-by-step tutorial on how to make both types of recordings. The examples described below mimic a typical neuroendocrine cell, e.g. the anterior lobe pituitary cell or the chromaffin cell. Note that the settings (gain, amplitude, frequency of the sine wave and other parameters) for other cell types can differ significantly and depend on cell size and the type of active membrane conductances present in the cell membrane.

Setting-up the recording system

Equipment

In addition to the SWAM IIc patch-clamp/lock-in amplifier, you will also need a 2-4 channel digital oscilloscope that has a roll mode option, a stimulator or a function generator, a low pass filter (4 channels, 1 channel minimum – for DC current in uncompensated recordings), a PC, an A/D converter (National Instruments, CED...) and appropriate software (CELL, Cap3, WinEDR...) for the desired type of capacitance measurements to be carried out.

BNC outputs

The BNC outputs of the SWAM IIc are $\mathbf{I_{mon}}$ (current, filtered by the **kHz** filter control), **10 Vp** (pipette voltage, scaled up by a factor of 10), **G 10 S/V** (conductance or real admittance (Y_{re}), filtered by the **Hz** filter control) and **C** $\mathbf{10^{-3}}$ **F/V | 10 S/V** (capacitance or imaginary part of admittance (Y_{im}), the setting selected by the **C** switch, filtered by the **Hz** filter control). On the rear panel there is a single output, which also provides unfiltered $\mathbf{I_{mon}}$. See also legend to Figure 1.

Connections

As shown in Figure 1, connect the output of the stimulator or a function generator to the SWAM IIc's **STIM** input. For the compensated mode of membrane capacitance recording connect the SWAM IIc outputs $\mathbf{I_{mon}}$ and **Vp** to the oscilloscope inputs. For the uncompensated mode of membrane capacitance recording connect these outputs also to the LP filter (filter set typically at 10 Hz). Connect the filter outputs with the A/D converter inputs. **C** and **G** outputs have to be low-pass filtered as well (again typically set at 10 Hz) before being connected to the oscilloscope and the A/D converter inputs, respectively. Do not forget to connect the microscope and the ground connector (**GND**) on SWAM IIc with a low resistance cable. The Faraday cage and the metal base plate are best connected to the common

ground of the entire system through a separate low resistance cable. The peak-to-peak (p-p) noise of the open (empty) headstage should be approx. 3 pA when the 1 GΩ headstage feedback resistor is used (the patch-clamp gain is in the region from 2 nA/V – 50 pA/V, and the LP filter is set to 3kHz or 10 kHz).

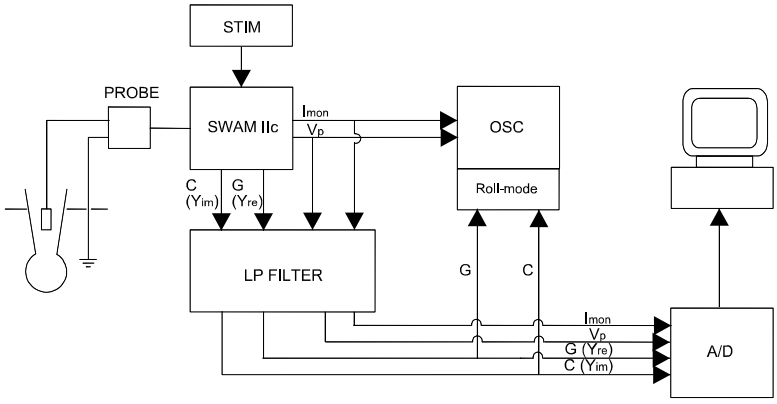

Figure 1: Schematics of the set-up for capacitance measurements.

The set-up for both compensated and uncompensated recordings is basically identical. However, in the compensated recording the G and C outputs (filtered) of the SWAM IIc provide readings of membrane capacitance and membrane conductance and access conductance, while in the uncompensated recording the four BNC outputs (I_{mon}, Vp, C and G) have to be filtered and acquired into the PC for real-time calculation of the passive cell parameters Cm, Ga and Gm. The G and C outputs in the uncompensated recording represent real (Y_{re}) and imaginary (Y_{im}) admittance signals.

Reading the gain settings

The gain of the $\mathbf{I_{mon}}$ output is given by the knob in **A/V**. From these values the alternative notation in V/A can easily be calculated (e.g. 50 pA/V means 20 mV/pA and vice versa; see Table 1).

Table 1: Conversion of gain settings between A/V and V/A (used on List and Axon patch-clamp amplifiers). Note that the units in A/V are in line with the patch-clamp headstage function, which is a current-to-voltage converter.

pA/V	mV/pA
0.5	2000
1	1000
2	500
5	200
10	100
20	50
50	20
100	10
200	5
500	2
1000	1
2000	0.5

The **Vp** output is scaled-up by a factor of 10 (10 mV output equals 1 mV at the pipette).

The gain of the **G** and **C** outputs can be determined by multiplying the patch clamp amplifier gain (knob **GAIN A/V**) by the gain of the lock-in amplifier (knob **x GAIN A/V**) and the factor, displayed near the BNC connector (for G **10** and for C either **10^{-3}** –used in compensated C_m recordings, or **10** –used in uncompensated C_m recordings). When the patch-clamp gain (**GAIN A/V**) is set to 50 pA/V and lock-in gain (**x GAIN A/V**) to 100, a 1 nS change will give a voltage change of 20 mV at the C or G outputs. (Note: the option 10 S/V for C output is selected only for uncompensated recordings.) When the option 10^{-3} F/V is selected (for compensated recordings) the C output must also be multiplied by the omega (ω) factor (0.5 or 1). Therefore, with the settings as above and with the lock-in frequency of 800 Hz (ω factor equals 0.5), the calculation goes as follows: 50 p(A/V) · 100 · 0.5 · 10^{-3} and yields the output in F/V; 1 V output equals 2.5 pF; or 1 pF equals 400 mV.

Functional check of the system: Using model cells

Model cells – theory
To test the SWAM IIc amplifier, three different model cells are provided (model cells: A, B, C). Each of the model cell is an electrical approximation (equivalent circuit made of resistors and capacitors) of the recording configurations, encountered in sequence in a typical patch-clamp experiment. Model cell A represents a pipette in bath (Figure 2(i)). Model cell B is the electrical equivalent of the cell-attached configuration (Figure 2(ii)). In this configuration the pipette (a resistor with some stray capacitance) is attached to the patch of the cell membrane. A good seal is represented by high value resistance (a ~GΩ resistor). Model cell C is the electrical approximation of the whole-cell configuration (Figure 2(iii)). The membrane can be described (or modelled) by a parallel combination of a resistor (channel conductances etc.) and a capacitor (lipid bilayer). This is connected in series to the headstage input via a resistor representing the patch pipette. Note that physical size of any electrical model cell results in unavoidable »contamination« by stray capacitance (shown by dotted lines in Fig. 2 (ii and iii)), ranging ~ 1 – 3 pF and must be taken into account. Therefore, the values given in the Figures provide an indication of the range of the values that should be expected in measurements**. For this reason it is important to note that these model cells are not suitable for calibration of the instrument since the measured values of these components may differ significantly from the precise, declared values of the individual electrical components.** The most reliable is the value of the resistor (model cell A), which should agree with the declared value (when checked) within ± 5%. The values of parameters for the other two model cells which also include capacitors are less accurate and therefore more likely to deviate from given values to a greater degree (± 20 %).

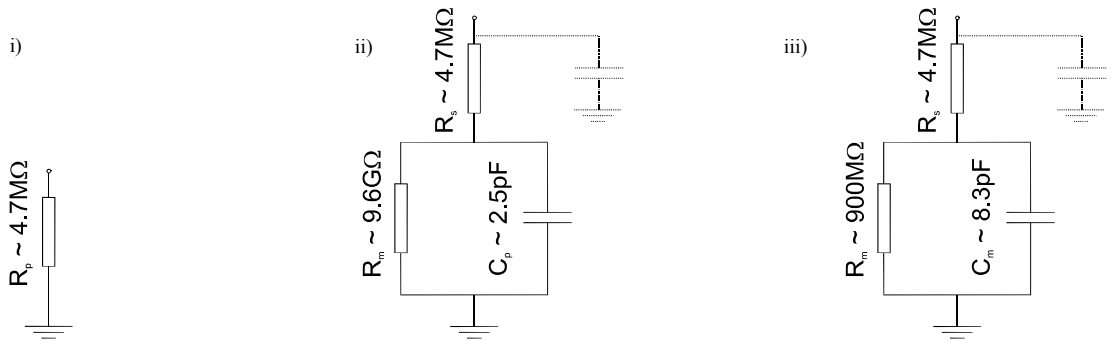

Figure 2: The model cell circuits i) pipette immersed in the bath, ii) cell-attached configuration, iii) whole-cell configuration.

i) Model cell A - pipette in bath. **ii)** Model cell B - cell-attached configuration. **iii)** Model cell C - whole-cell configuration. The values of the elements can differ slightly. Note that the combined stray capacitance of the model cell case, crocodile clip and connectors (dotted lines in (ii) and (iii)) can be as high as 1 – 2 pF.

Model cell A ("pipette in bath")

Plug in the Model cell A into the headstage input connector (2 mm diameter jack). Connect the reference plug into the headstage reference connector (0.5 mm diameter jack) and with the model cell by using the crocodile clip on the cable provided. Select **VC** mode and set the **GAIN A/V** to 50 pA/V. Apply a square voltage pulse with the amplitude of 5 mV (note that the amplitude is the product of the stimulator voltage pulse amplitude and the value on the **STIM** knob of the SWAM IIc). Observe the shape of the current which should resemble the trace in Figure 3(i). Calculate the resistance of the Model cell A from the applied voltage and the measured current using the Ohm's law. Compare calculated resistance with the resistance given in the model cell specification sheet. The values should match within ± 5%. Try the same procedure with different patch-clamp amplifier gains and different square-wave stimulus amplitudes. Observe if the shape of the signal is stable. Only the amplitude of the current should change (proportionally to the gain and amplitude of the voltage pulse) and not the shape. If you select mode **TR** and press the **RESET** switch, you should observe the signal resembling the trace on Figure 3(ii). Note that in this mode of operation, the $\mathbf{I_{mon}}$ is held at 0 pA. In this configuration the reading of the **mV** LCD display should read 0.00 mV. In a measurement using saline filled glass pipettes this reading may be different from zero because of junction potentials. These can be corrected by pressing the **RESET** switch and turning the knob **OFFSET mV**.)

Figure 3: Current trace ($\mathbf{I_{mon}}$) observed on oscilloscope with the Model cell A.

For the recording the **GAIN A/V** was set to 50 pA/V, **STIM** knob to 0.001, stimulator voltage pulse amplitude to 500 mV and duration of the pulse to 7 ms. The filtering frequency for the $\mathbf{I_{mon}}$ was set to 3 kHz. **i)** Current observed in the **VC** mode. **ii)** Current observed in the **TR** mode and with the **RESET** switch pressed. Dotted line denotes zero current level.

Model cell B (cell-attached configuration)
Next, connect the Model cell B (cell-attached configuration) into the headstage. Set the **GAIN A/V** to 50 pA/V. Observe the shape of the current when applying a voltage stimulus. With the square-wave stimulus you should observe a transient current at the beginning and at the end of the stimulus (Figure 4(i and iii)). Try to cancel the transients with $\mathbf{C_{fast}}$ potentiometer (Figure 4(ii and iv)) and the knob **µs**. Repeat the same procedure with a sine-wave stimulus (set the **x GAIN A/V** to 100 and **x 10^3/s OMEGA** control to 1; Figure 4(v and vi)). Compare the readings on the $\mathbf{C_{fast}}$ cancellation potentiometer with those specified for the Model cell B shipped. The values will match within ± 20%. Change the patch-clamp amplifier gain and the voltage amplitude (square or sine wave) and observe the signal.

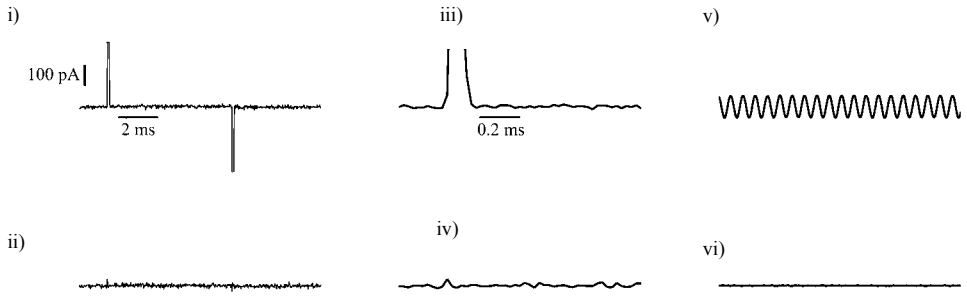

Figure 4: Voltage induced currents in the cell-attached configuration using Model cell B.

Uncancelled (**i**, **iii** and **v**) and cancelled capacitive current (**ii**, **iv** and **vi**). The **GAIN A/V** was set to 0.2 nA/V, the filtering frequency for the $\mathbf{I_{mon}}$ was set to full. The settings used for the square-wave stimulus were 500 mV and 7 ms for the amplitude and duration, respectively, and the value of 0.05 on **STIM** input of the SWAM IIc was used. For the sine-wave stimulus (**v** and **vi**) the **x GAIN A/V** was set to 100, **x 10^3/s OMEGA** control to 1. The **GAIN A/V** in this case was set to 50 pA/V and the filtering frequency for the $\mathbf{I_{mon}}$ to 3 kHz. The time and amplitude scales are the same for all recordings, except for the panels **iii** and **iv**, which show the expanded epochs windowed on panels **i** and **ii** at a higher time resolution.

Model cell C (whole-cell configuration)
Last, connect the Model cell C (whole-cell configuration) into the headstage. Set the **GAIN A/V** to 0.1 nA/V. Apply the square voltage pulse (5 mV) and observe the shape of the current (Figure 5(i and iii)). Cancel the capacitive transients by turning $\mathbf{C_{slow}}$ and $\mathbf{G_s}$ potentiometers (Figure 5(ii and iv)). Note that there may be some stray capacitance left, which should be cancelled by $\mathbf{C_{fast}}$ and **µs** knob. Repeat the same procedure with a sine-wave stimulus (**x GAIN A/V** set to 100; Figure 5(iii and iv)). Turn the potentiometers $\mathbf{C_{slow}}$ and $\mathbf{G_s}$ back to zero and use the software CELL or Cap3 to measure the capacitance and conductance of the Model cell (take care of the zero phase angle settings; see the section Uncompensated recording, below). Compare the values of the Model cell C measured by the software with the ones obtained previously by cancellation controls. All values should match within ± 20%. Try the procedure with different patch-clamp and lock-in amplifier gains and different square-wave stimulus amplitudes (for example: 5 – 50 mV; select the voltage amplitudes so that the maximum current does not exceed 5 times the **GAIN A/V** value). Observe if the shape of the signal is stable.

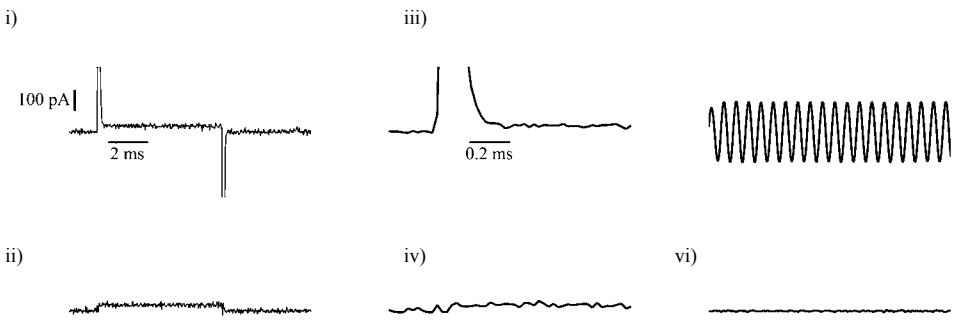

Figure 5: Voltage-induced currents in the Model cell C in the whole-cell configuration.

Uncancelled (**i**, **iii** and **v**) and cancelled voltage-induced currents (**ii**, **iv** and **vi**). The **GAIN A/V** was set to 0.2 nA/V, the filtering frequency for the $\mathbf{I_{mon}}$ was set to full. The settings used for the square-wave stimulus were 500 mV and 7 ms for the amplitude and duration, respectively, whereas the value of 0.05 on the **STIM** input of the SWAM IIc was used. For the sine-wave stimulus (**v** and **vi**) the **x GAIN A/V** was set to 100, **x** $\mathbf{10^3}$**/s OMEGA** control to 1. The **GAIN A/V** in this case was set to 0.1 nA/V and the filtering frequency for the $\mathbf{I_{mon}}$ to 3 kHz. The time and amplitude scale is the same for all panels except for panels **iii** and **iv**, which show the framed signal epochs on panels **i** and **ii** at a higher time resolution. Note the difference in time constant of the transient in Figures 4(iii) and 5(iii).

Compensated C_m recording (measurements in the fF range)

Junction potential compensation

Fill the glass pipette and the holder with appropriate saline. Insert the pipette into the holder and plug the pipette-holder assembly into the headstage input jack (2mm diameter connector). Submerge the Ag/AgCl reference electrode in the bath and connect it with the headstage reference jack (0.5mm diameter connector). Set the **GAIN A/V** to 0.5 nA/V, the **kHz** knob to 3 kHz and select **VC** mode. Submerge the pipette into the bath. Select either **GATE** or **OFF** mode and apply a continuous square-wave stimulus with the amplitude of a few mV (e.g. a 500 mV amplitude square waveform and **STIM** scale factor of 0.01 would result in a square pulse amplitude of 5 mV at the pipette). Observe the current and turn the potentiometer **OFFSET mV** until the steady-state $\mathbf{I_{mon}}$ output equals 0 pA (select the polarity by the adjacent ± switch). With this procedure junction potentials are compensated for. There is a second method for the junction potential compensation, which is especially helpful when high patch-clamp amplifier gains are used. This method is as follows: Select **TR** mode, which acts as a slow current-clamp and is used to nullify the current output. With a slow time constant the current will gradually drop to 0 pA. By pressing the **RESET** switch you can speed up the performance of the **TR** mode. The shape of the current will change from a square wave pulse to the one in Figure 3(ii). The steady state value of the signal on $\mathbf{I_{mon}}$ will equal 0 pA. The value shown on the left **mV** LCD display reports the junction potentials. While pressing the **RESET** switch turn the **OFFSET mV** potentiometer until the junction potentials equal 0 mV (LCD displays 0). Then change to the **VC** mode. After the junction potential compensation calculate the pipette resistance. This is easiest to do by changing the amplitude of the voltage pulse until the current output amplitude equals some round, easily measurable amplitude like 1 nA. This equals 2 boxes on the oscilloscope with the **GAIN A/V** set to 0.5 nA/V and 1 V/div on the oscilloscope. In this way the value of the pipette voltage step in mV numerically equals the pipette resistance in MΩ. (Note: The output of the stimulator in this case is 100 times higher than the pipette voltage.)

Giga-seal formation
Gently approach the cell with the pipette until contact is established. Proceed to the cell-attached configuration by forming a giga-seal using gentle suction. When the giga-seal is formed the shape of the current should resemble the trace of the Model cell B (Figure 4(i and iii); cell-attached configuration). Turn the **V hold** control to adjust the desired constant voltage, displayed on the LCD **PIPETTE VOLTAGE mV** (e.g. –70 mV). Select the polarity with the adjacent ± switch. Check if the seal is of good quality (the resistance should be at least 1 GΩ or higher). Calculate the resistance from the DC current (e.g. 50 pA of DC current at –50 mV holding voltage equals 1 GΩ).

Cell-attached mode
Compensate the pipette stray capacitance by turning the $\mathbf{C_{fast}}$ potentiometer and the **μs** knob (the time constant; see Fig. 4(ii and iv)). Switch off the square wave stimulus and apply the sine wave stimulation by turning the **MODE** knob to **ME**. Select the **x GAIN A/V** of 10 and the command **x 10^3/s OMEGA** of 1. Turn the **Hz** filter knob to 10 Hz. You should observe the sine wave resembling the trace in Figure 4(vi). Re-check the $\mathbf{C_{fast}}$ compensation and proceed to the whole cell configuration (Figure 5(v)).

Whole-cell mode
Turn the **pF** knob to 10 pF, to select the 0 – 10 pF range of the $\mathbf{C_{slow}}$ potentiometer. By breaking the patch with abrupt suction, the whole-cell recording configuration is obtained. With the multiturn controls $\mathbf{C_{slow}}$ and $\mathbf{G_s}$ compensate the cell capacitance and access conductance as described previously for the Model cell C (Figure 5(vi)). Decrease the **GAIN A/V** to 2 nA/V and increase the **x GAIN A/V** to 1. Again compensate the cell capacitance and series conductance in case some sinusoidal current still remains. (If the shape of the signal with the sine wave stimulation significantly deviates from a pure sinusoid (it has dents on the upward or downward slope), you probably either saturated the amplifier, in which case you also hear the saturation buzzer alarm, or the distortion of the signal is due to the activation of some sort of voltage dependent conductances. For the latter case three possible solutions exist: a) decrease the amplitude of the sine wave (i.e. **x GAIN A/V**; also the solution for the amplifier saturation), b) add a channel blocker for the voltage-activated conductance to the internal or external solution, or c) exclude the permeating ion from the solutions). Start the recording. Press the **ΔC** switch to activate a calibration pulse of 100 fF (turning the switch adds 1% of the $\mathbf{C_{slow}}$ range to the $\mathbf{C_{slow}}$ compensation) and adjust the phase angle with the **PHASE** control. Phase angle in degrees is displayed on the adjacent **PHASE DEGREES** LCD. Observe the **G** and **C** outputs on the oscilloscope. The phase angle is set when the calibration pulse appears as a negative-going deflection only on trace C with no projection onto trace G. The amplitude of the deflection, as it appears on the oscilloscope, depends on the $\mathbf{C_{slow}}$ and $\mathbf{G_s}$ positions. It is however always equal to 100 fF with 10 pF $\mathbf{C_{slow}}$ range (1% of the entire $\mathbf{C_{slow}}$ range). The continuous calibration pulses can be applied automatically by turning the knob **MODE** from **ME** to **ADJ**. After the phase adjustment is complete, return the knob to **ME**. Record the **C** and **G** outputs. If the cell capacitance or series conductance values change substantially (i.e. if the sine wave reappears and exceeds some chosen level, for example 1nA p-p), re-compensate and check for any phase changes by applying calibration pulses. With larger G_s and C_{slow} excursions the attenuation factor of the recording configuration is bound to change. The new amplitude of the calibration pulse, as it appears on **C** output, has to be taken into account for the subsequent part of the record until the next capacitive current compensation.

Uncompensated recording

Recording software and software set-up
For the uncompensated recording appropriate software is needed for the calculation of the parameters. For this purpose various programs exist like CELL, Cap3, WinEDR etc. The programs are similar in operation and can be interchanged and selected according to one's needs and wishes. First, they all use the same algorithm published by Lindau & Neher (1988) and Gillis (1995). Second, they all need 4 inputs (DC current (I_{DC}), voltage (V_m), imaginary (Y_{im}) and real (Y_{re}) admittance) that have to be connected with the A/D converter input BNC connectors. The positions on the A/D converter BNC connector board are either fixed (Cap3) or can be freely selected (WinEDR etc.). For the appropriate wiring scheme consult the manual or the Help utility of the selected software. Next, all programs require gain factors of the inputs (in V/nS or mV/nS, mV/pA, etc.). In addition to the gain factors of the input parameters, you have to provide the program with the values of the lock-in frequency and cell's reversal potential. The value of the reversal potential (V_{rev}) has to be estimated. It is preferably determined in separate set of experiments with the same cell type and the same pipette and bath solutions as used in actual C_m-recording experiments. Note that V_{rev} can change during the recording and the value in the program is only an approximation of the actual value. This inaccuracy does not influence the calculation of C_m as long as G_a stays much larger than G_m (see Gillis, 1995). However, care must be taken that the values of the estimated reversal potential, given to the program, and the pipette holding voltage are not equal since that would produce zero in denominator of one of the equations used for C_m calculation (see Lindau and Neher, 1988). The software calculates the parameters (C_m, G_a and G_m) in real time and saves them in a file. The additional requirement for these measurements is also that the phase of the patch-clamp amplifier is set so as to compensate for any phase shifts produced by the amplifier (mainly due to the low-pass filter; see next section). Contrary to uncompensated recordings, the compensated recording can be done with any software that enables continuous recording of at least two channels (**C** and **G**).

Initial settings
Make appropriate connections between reference and measuring electrodes and the headstage (see the section Compensated C_m recording). Set the **GAIN A/V** to 50 pA/V, **kHz** knob to 3 kHz and select **VC** mode. Check the initial phase of the patch clamp amplifier. Apply a sine wave stimulus by turning the **MODE** knob to **ME**. Turn the potentiometer $\mathbf{C_{fast}}$ a few times and observe the real (Y_{re}; **G**) and imaginary (Y_{im}; **C**) output of the lock-in amplifier. Downward deflections should appear only on the imaginary trace. If this is not the case press the **PHASE** switch repeatedly (or constantly) to adjust the phase angle of the amplifier so that when turning the $\mathbf{C_{fast}}$ potentiometer clockwise only the negative deflections on imaginary output are observed with no projections onto the real output. (Caution: selection of a different patch-clamp low-pass filter (**kHz**) will change the phase angle setting!) When this is done, submerge the pipette into the bath solution. Select **GATE** mode and apply a continuous square-wave stimulus with the amplitude of 0.1-1 mV (e.g. 500 mV amplitude square waveform and **STIM** scale factor of 0.001 would give 0.5 mV square pulses). Compensate for junction potentials and measure the pipette resistance as described in the section Compensated recording – Junction potential compensation.

The recording
With the pipette in gentle contact with the cell go into the cell-attached configuration by forming a giga-seal. Turn the **V hold** control until the desired voltage is read on the **PIPETTE VOLTAGE mV** LCD (for example –80 mV). Calculate the resistance from the

given voltage amplitude of the square wave stimulus and observed current. Compensate the pipette stray capacitance by turning the $\mathbf{C_{fast}}$ potentiometer and **µs** knob. Switch off the square wave stimulus and turn the **MODE** button from **GATE** to **ME**. Select the **x GAIN A/V** control 100, **x 10^3/s OMEGA** control 1 and **Hz** control 10 Hz. Re-check the $\mathbf{C_{fast}}$ compensation and start the software (CELL, Cap3, WinEDR). Proceed to the whole cell configuration. The software is now recording the parameters required to calculate the cell's passive parameters C_m, G_m and G_a.

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