Electronic Instruments for the Life Sciences

APPENDIX

TUNING CAPACITY COMPENSATION IN SEC AMPLIFIER SYSTEMS

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For accurate measurements in switched mode, it is essential that the capacity of the electrode is fully compensated.

Important: Wrong compensation of electrode capacity leads to errors in measurements done in switched mode of the amplifier (see Figure 2).

Microelectrode selection: Electrodes must be tested before use. This is done by applying positive and negative current pulses. Electrodes that show significant changes in resistance (rectification) cannot be used for intracellular recordings. By increasing the current amplitude the capability of the electrode to carry current can be estimated. The test current must cover the full range of currents used in the experiment. For details see (3).

Switching frequency is a key parameter of discontinuous single electrode clamp (dSEVC) systems. The switching frequency determines the accuracy, speed of response, and signal-to noise ratio of the dSEVC system (3)(6). Since its launch in 1984, one of the outstanding features of the SEC series of single electrode voltage / current clamp systems has been the ability to record routinely with high switching frequencies in the range of tens of kilohertz, regardless of the microelectrode resistance (1). Principles of the dSEVC technique are found in (1)(2).

Looking back: In the early eighties, when the design of the SEC 1L system was started, single electrode clamping began to gain importance beside the two classical intracellular methods: bridge recording or whole cell patch clamp recording. The great advantage compared to the whole cell recording method using a patch amplifier was the elimination of series resistance due to the time sharing protocol. No current flow during voltage recording means no interference from the series resistance regardless of its value. Voltage clamp recordings became possible with sharp microelectrodes in deep cell layers. The historical weak point of this method was the low switching frequency due to the fact that stray capacities around the microelectrode could not be compensated sufficiently.

The SEC systems provided a solution for this problem. With their improvements on capacity compensation electronics, they could be used with switching frequencies of tens of kHz even with high resistance microelectrodes. What are the technical principles that make possible such high switching frequencies?
In SEC systems a special protocol is used to rapidly compensate the microelectrode. Figure 1 shows the compensation scheme of a sharp microelectrode immersed 3 mm in cerebrospinal fluid. Here the increase in speed can be seen clearly. Recordings under such conditions and possible applications have been presented in several papers (e.g. (3)).

Criteria for the selection of the switching frequency

Which are the most important criteria for the selection of the switching frequency? This question was analyzed in detail by M. Weckstrom and colleagues (4)(5). They presented a formula that describes the conditions for obtaining reliable results during a switching single electrode clamp:

$f_e > 3f_{sw}$, $f_{sw} > 2f_s$, $f_s > 2f_f > f_m$

f_e: upper cutoff frequency of the microelectrode
f_{sw}: switching frequency of the dSEVC
f_s: sampling frequency of the data acquisition system
f_f: upper cutoff frequency of the lowpass filter for current recording,
f_m: upper cutoff frequency of the membrane.

Example (6): With the time constant of 1-3 µs recorded for the electrodes used in this study, f_e is 80-160 kHz, the selected switching frequency of the dSEVC was 30 - 50kHz (calculated range is 25-53 kHz), data were sampled at 10 kHz and the current signals have been filtered at 5 kHz. These settings are currently used for recordings in many labs.

The principle of operation in switched mode is shown below.

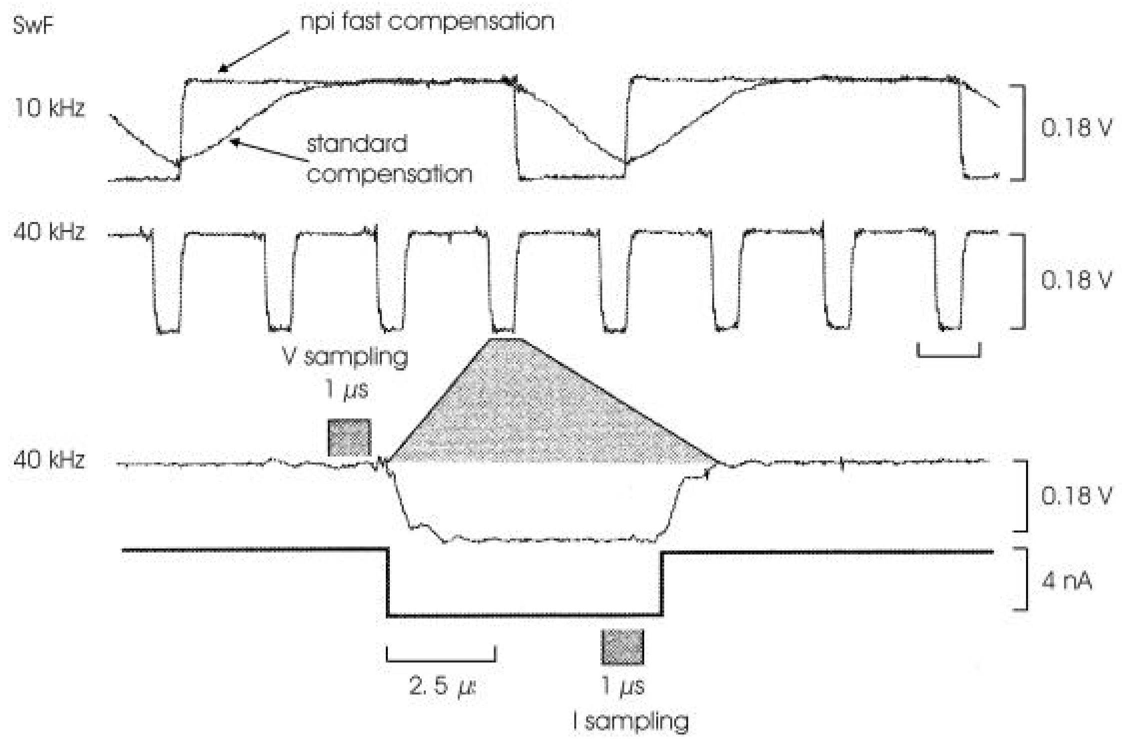

Figure 1: Microelectrode artifact settling.

Compensation of stray capacities with a SEC 05 amplifier. The upper trace shows the comparison between the standard capacity compensation and the fast compensation of the SEC systems. After full compensation the settling time of the microelectrode is reduced to a few microseconds allowing very high switching frequencies (here: 40 kHz, middle and lower trace). The microelectrode was immersed 3 mm deep in cerebrospinal fluid. Microelectrode resistance: 45 MΩ, current: 1 nA, duty cycle 25%. SwF: switching frequency.
Original data kindly provided by Prof. Diethelm W. Richter, Goettingen. For details see (3).

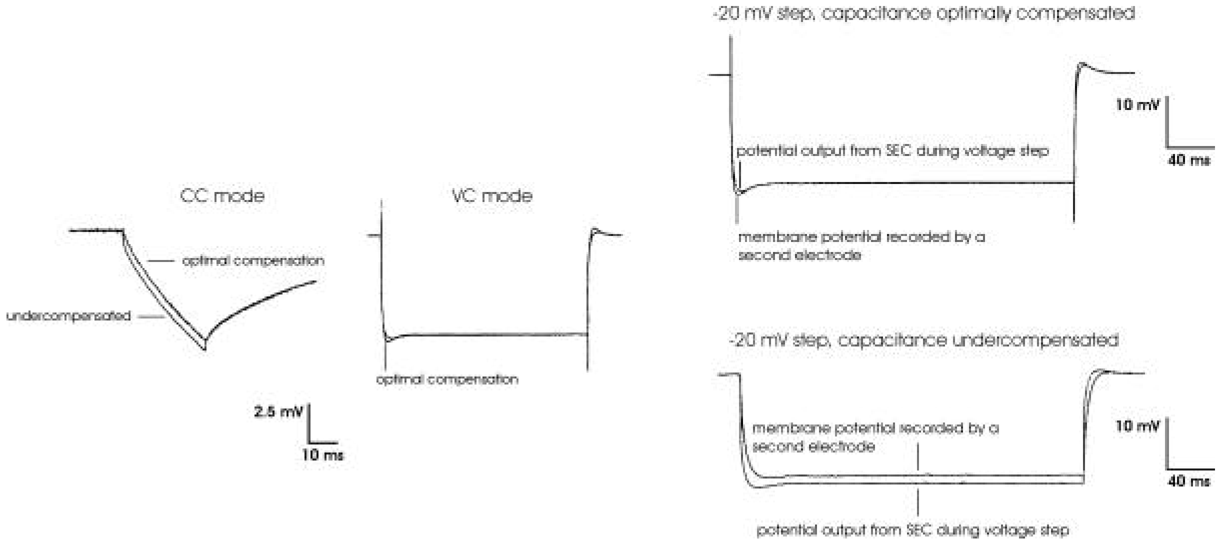

Figure 2: Errors resulting from wrong compensation of the electrode capacity.
Original data kindly provided by Ajay Kapur. For details see (7).

Tuning Procedure (see also chapter “Getting Started”, pages 14, 15):

First part: basic setting

In SEC systems the capacity compensation of the electrode is split into two controls, the coarse control in the headstage and a the fine control at the front panel of the amplifier. The aim of the first part of the tuning procedure is to set the coarse capacity compensation at the headstage, so that an optimal, wide range of CAP.COMP. at the amplifier is achieved.

- ❑ Insert the electrode into the electrode holder and connect it to the amplifier.
- ❑ Immerse the electrode, as deep as it will be during the experiment, into the bath solution.
- ❑ Set the CAP.COMP. control at the amplifier (potentiometer #**24** at the front panel) to a value around 2 and turn COARSE CAPACITY COMPENSATION at the headstage to the leftmost position. Select a DUTY CYCLE as desired (#**24** at the front panel).
- ❑ Connect the BNC connector ELECTRODE POTENTIAL OUTPUT at the rear panel to an oscilloscope and trigger with the signal at BNC connector SWITCHING FREQUENCY (also at the rear panel). The oscilloscope should be in external trigger mode. The time base of the oscilloscope should be in the range of 250 µs.
- ❑ Set the amplifier in CC mode and select the lowest switching frequency (1 to 2 kHz)
- ❑ Apply positive or negative current to the electrode using the HOLDING CURRENT control (potentiometer #**21** at the front panel).
- ❑ You should see a signal at the oscilloscope similar to those in Figure 3. Turn the COARSE CAPACITY COMPENSATION carefully clockwise until the signal becomes as square as possible (lower diagram in Figure 3).

Important: If you use a model cell (e.g. to train yourself in adjusting the capacity compensation) the capacity of the model cell is always present. Thus, you will get an approximately square shaped signal with a slight slope as shown in Figure 4 (lower panel).

- ❑ Increase the switching frequency to at least 15 kHz. The amplitude and shape of the signal should not change considerably.

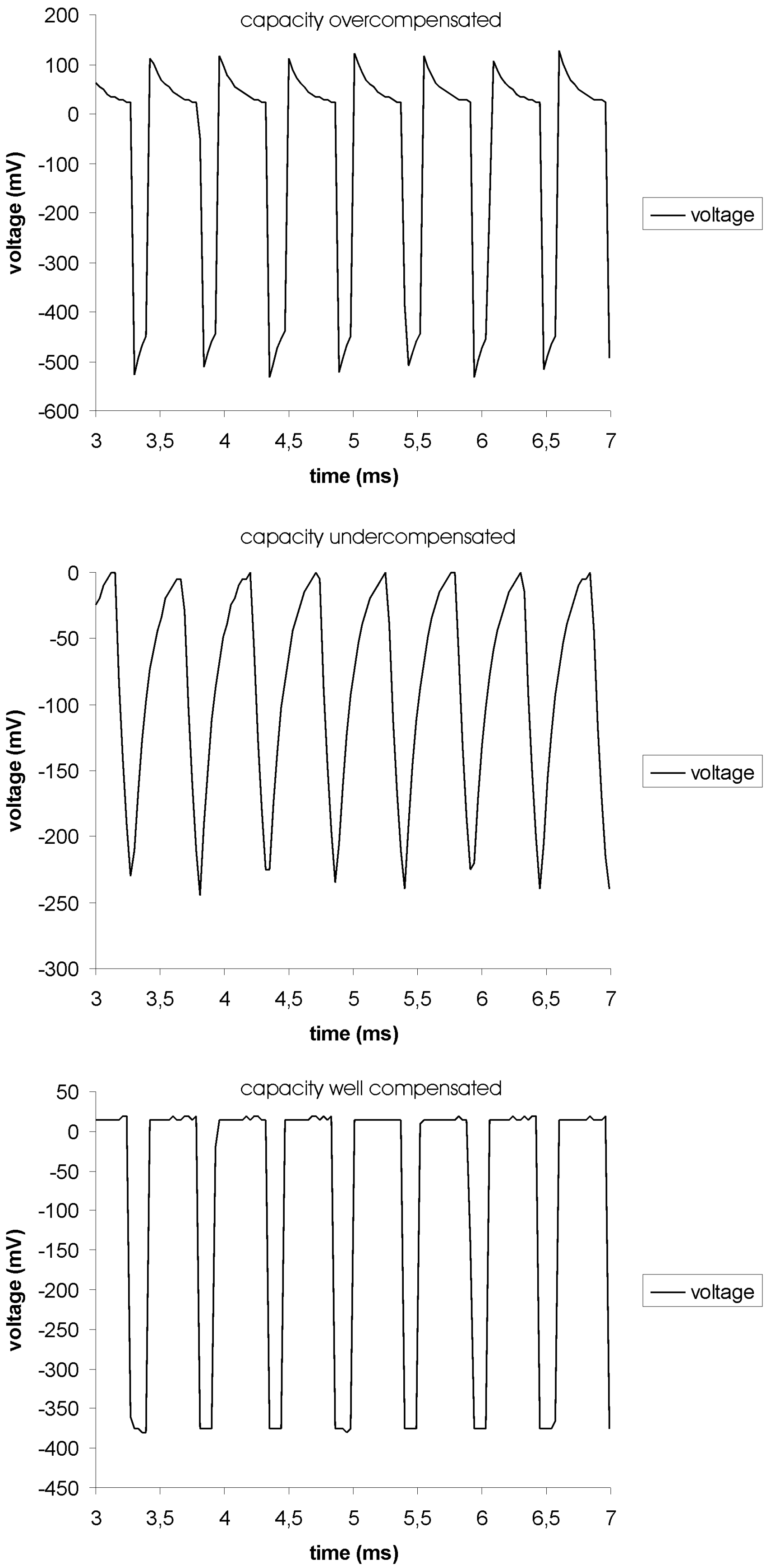

Figure 3: Tuning of the coarse capacity compensation with an electrode (resistance100 MΩ) in the bath. Time course of the signal at ELECTRODE POTENTIAL OUTPUT is shown (holding current: -1 nA, duty cycle: ¼, switching frequency: 2 kHz).

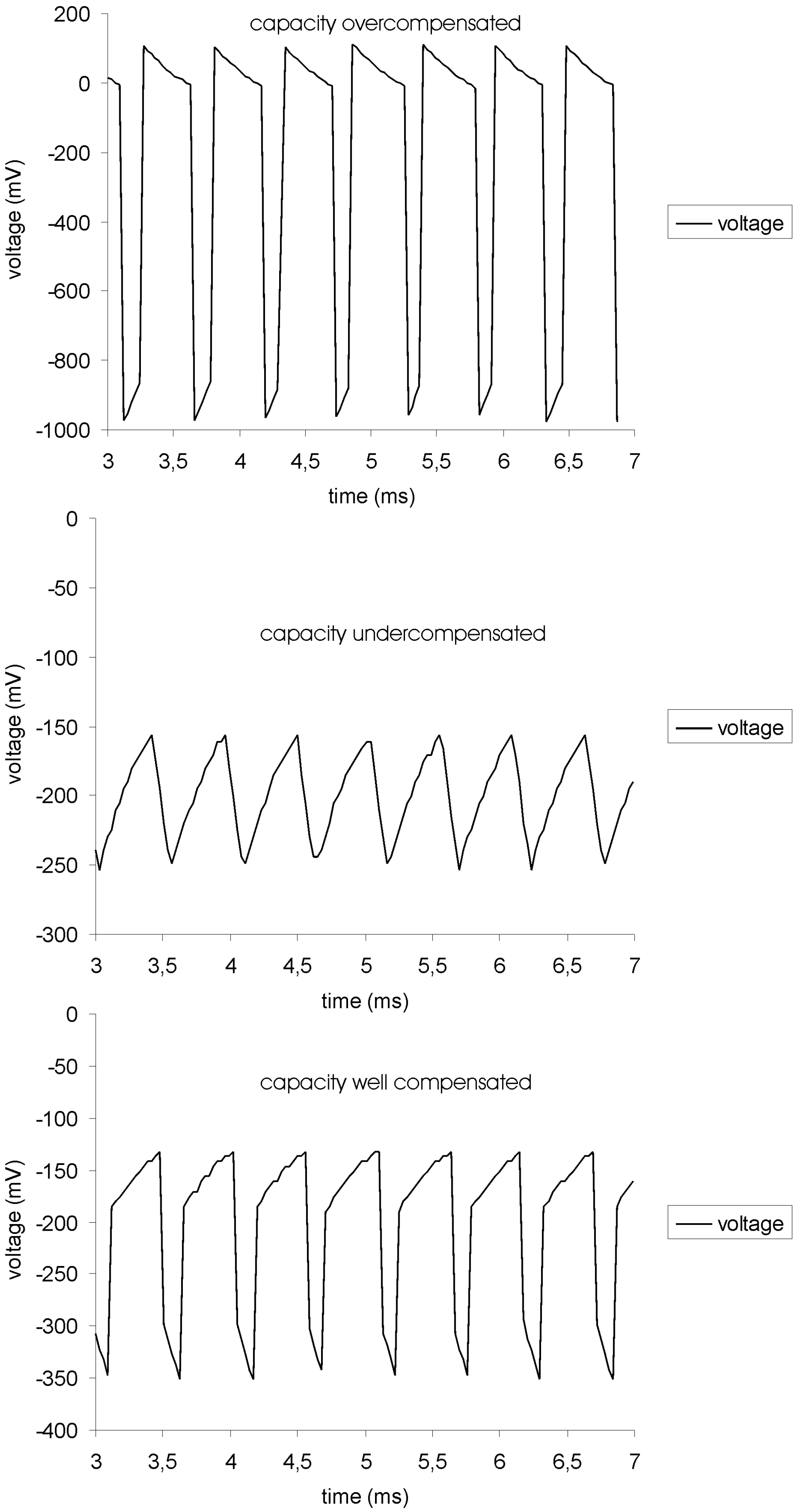

Figure 4: Tuning of the coarse capacity compensation. Time course of the signal at ELECTRODE POTENTIAL OUTPUT is shown (holding current: -1 nA, duty cycle: ¼, switching frequency: 2 kHz). A model cell was connected (electrode resistance 100 MΩ).

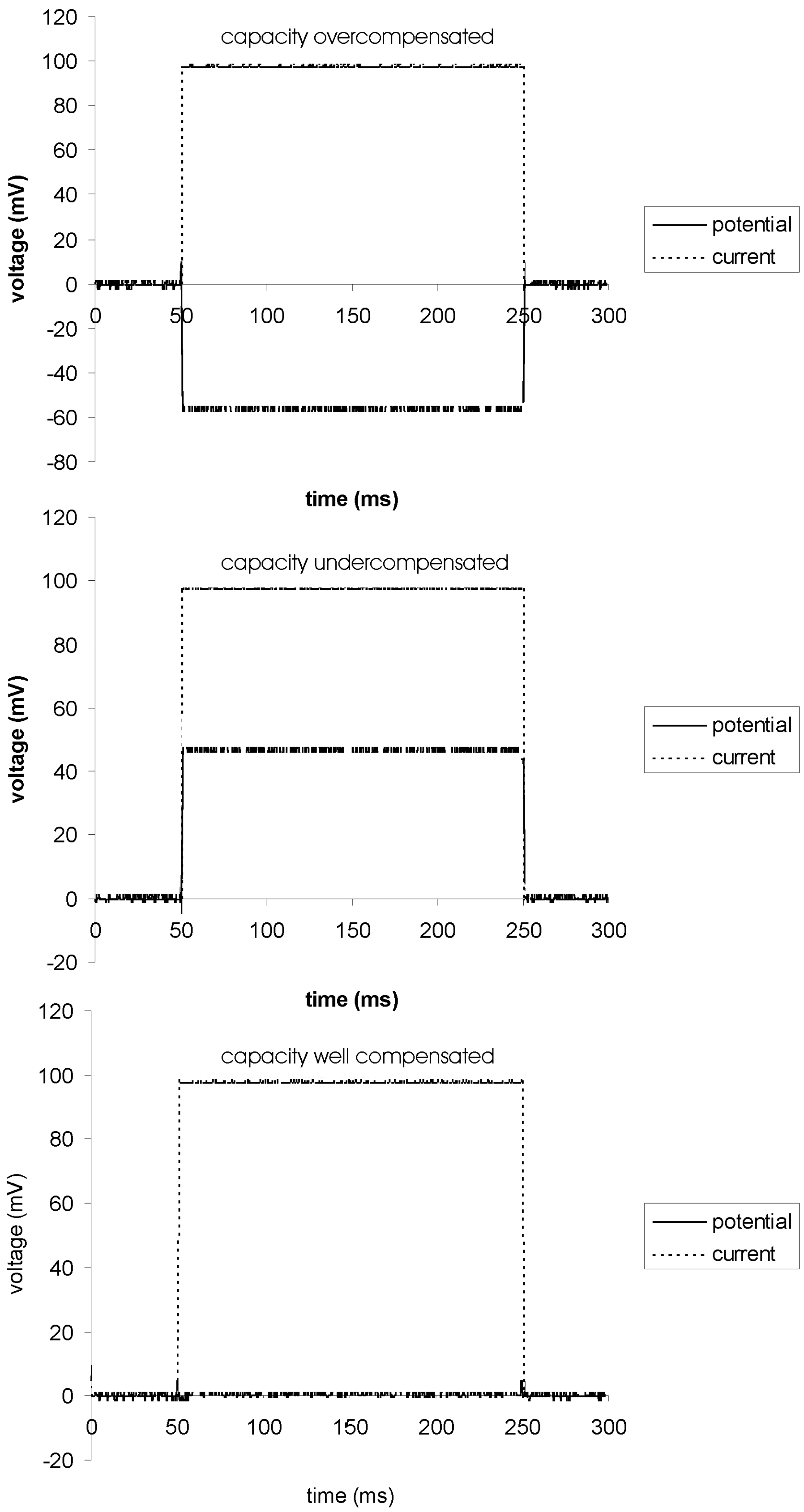

Figure 5: Capacity compensation of the electrode in the bath (electrode resistance: 100 MΩ, Current stimulus: 1 nA, duty cycle: ¼, switching frequency: 2 kHz). Current stimulus and electrode potential are shown.

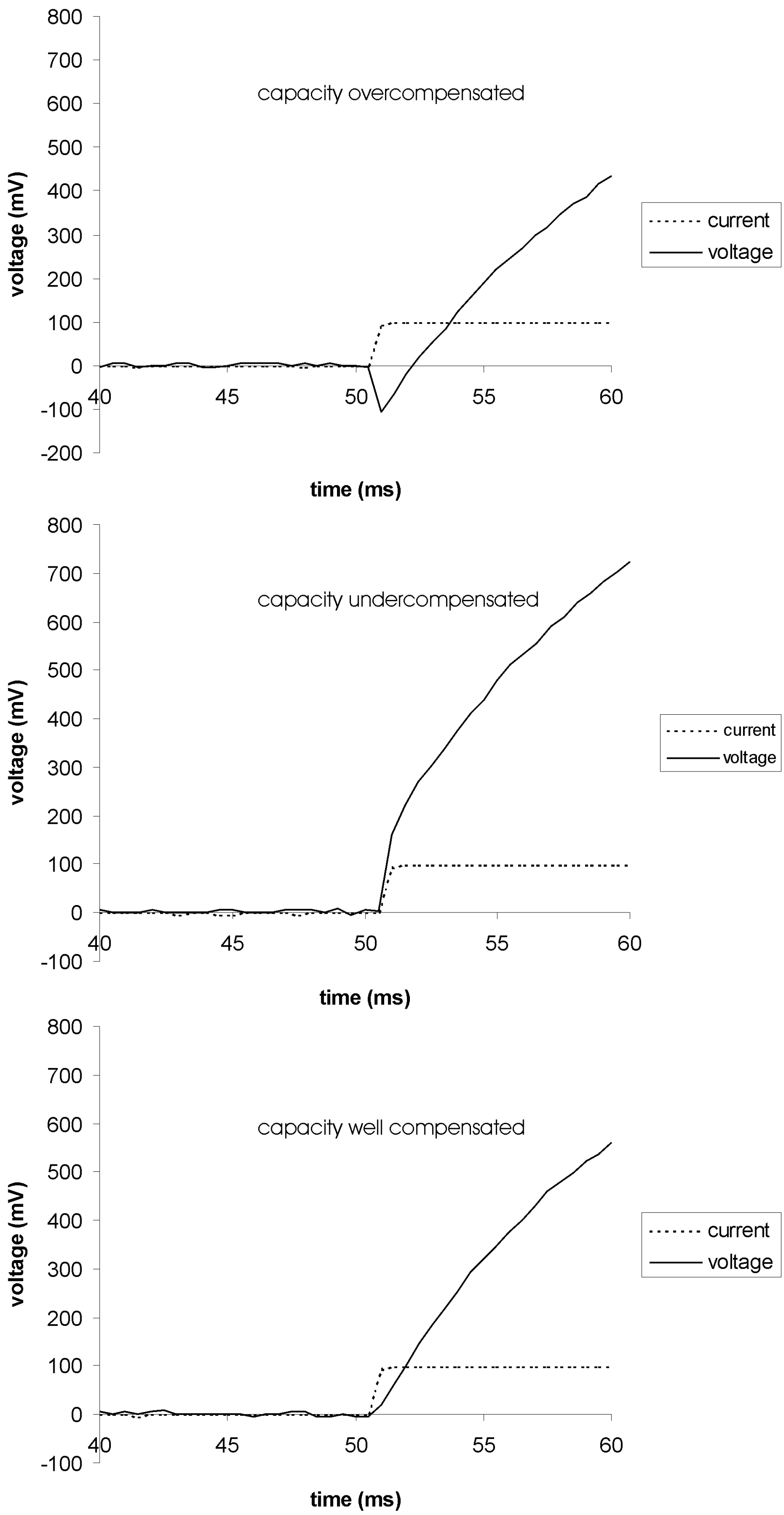

Figure 6: Capacity compensation of the electrode using a model cell (electrode resistance: 100 MΩ, current: 1 nA, cell membrane: 100 MΩ, 100 pF, duty cycle: ¼, switching frequency: 2 kHz). Current stimulus and membrane potential are shown.

Second part: fine tuning

Now the basic setting of the CAPACITY COMPENSATION is achieved. Since the electrode parameters change during the experiment (especially after impaling a cell), it is necessary to fine tune the CAPACITY COMPENSATION during the experiment using the CAP.COMP. control on the amplifier. To get familiar with this, connect a cell model and go through the following steps (the procedure is the identical with a "real" cell).

- ❑ Connect POTENTIAL OUTPUT and CURRENT OUTPUT (front panel) to another oscilloscope.
- ❑ Set SWITCHING FREQUENCY to the desired value (>15 kHz) and DUTY CYCLE to the desired value.
- ❑ Set the HOLDING CURRENT to zero. With the amplifier in CC mode, apply square pulses of a few nA (or a few tens of pA for patch recordings) to the cell. Negative current pulses are recommended. If you apply positive current pulses, be sure only to elicit ohmic responses of the cell membrane, i.e. pulses should not elicit openings of voltage gated channels.
- ❑ The POTENTIAL OUTPUT should show the ohmic response of the cell membrane, without an artifact, as illustrated in Figure 6 and Figure 7.

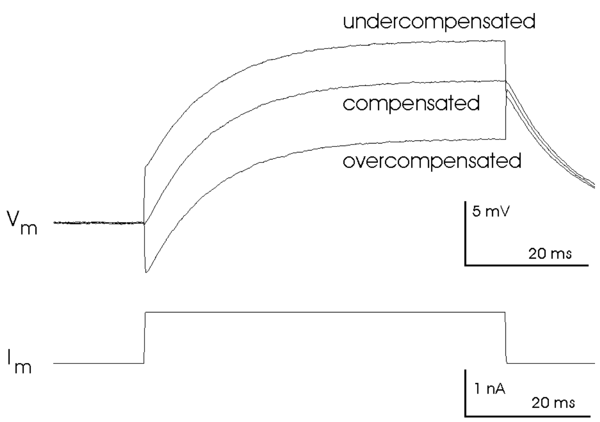

Figure 7: Capacity compensation of the electrode inside a cell. Current stimulus and membrane potential are shown.

Hint: The results of this procedure look very similar to tuning of the bridge balance. If the BRIDGE is balanced accurately no differences in the potential outputs should occur when switching between CC- and BRIDGE mode.

Important: Always monitor the OUTPUT from ELECTRODE POTENTIAL OUTPUT at the rear panel, using a second oscilloscope. The signals must be always square. If not, CAPACITY COMPENSATION has to be readjusted or the switching frequency must be lowered.

References:

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