OPERATING INSTRUCTIONS AND SYSTEM DESCRIPTION FOR THE

TEC-B-01

VOLTAGE CLAMP UNIT FOR BRIDGE AMPLIFIERS

VERSION 1.1
npi 2014

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1. Safety Regulations

<u>VERY IMPORTANT</u>: **Instruments and components supplied by npi electronic are NOT intended for clinical use or medical purposes (e.g. for diagnosis or treatment of humans), or for any other life-supporting system. npi electronic disclaims any warranties for such purpose. Equipment supplied by npi electronic must be operated only by selected, trained and adequately instructed personnel. For details please consult the GENERAL TERMS OF DELIVERY AND CONDITIONS OF BUSINESS of npi electronic, D-71732 Tamm, Germany.**

1) GENERAL: This system is designed for use in scientific laboratories and must be operated only by trained staff. General safety regulations for operating electrical devices should be followed.

2) AC MAINS CONNECTION: While working with npi systems, always adhere to the appropriate safety measures for handling electronic devices. Before using any device please read manuals and instructions carefully.
   The device is to be operated only at 115/230 Volt 60/50 Hz AC. Please check for appropriate line voltage before connecting any system to mains.
   Always use a three-wire line cord and a mains power-plug with a protection contact connected to ground (protective earth).
   Before opening the cabinet, unplug the instrument.
   Unplug the instrument when replacing the fuse or changing line voltage. Replace fuse only with an appropriate specified type.

3) STATIC ELECTRICITY: Electronic equipment is sensitive to static discharges. Some devices such as sensor inputs are equipped with very sensitive FET amplifiers, which can be damaged by electrostatic charge and must therefore be handled with care. Electrostatic discharge can be avoided by touching a grounded metal surface when changing or adjusting sensors. **Always turn power off when adding or removing modules, connecting or disconnecting sensors, headstages or other components from the instrument or 19” cabinet.**

4) TEMPERATURE DRIFT / WARM-UP TIME: All analog electronic systems are sensitive to temperature changes. Therefore, all electronic instruments containing analog circuits should be used only in a warmed-up condition (i.e. after internal temperature has reached steady-state values). In most cases a warm-up period of 20-30 minutes is sufficient.

5) HANDLING: Please protect the device from moisture, heat, radiation and corrosive chemicals.

2. TEC-B-01

2.1. Components

The following items are shipped with the TEC-B-01 system:

- ✓ TEC-B-01 amplifier
- ✓ Headstage
- ✓ Ground connector for headstage (2.6 mm)
- ✓ Power cord
- ✓ User manual

Optional accessories:

- ➪ Electrode holder
- ➪ Electrode holder adapter for mounting to a micromanipulator

- ➪ Passive cell model

2.2. System Description

The TEC-B-01 amplifier module is designed to be used together with a modified BA-1S, BA-01X or BA-03X bridge amplifier. In two-electrode CC or VC mode the current is applied through the current electrode connected to the TEC-B-01 and potential measurement is performed through the bridge amplifier. The active connection of the two amplifiers is indicated by the DUAL LED. The bridge amplifier has also an LED besides the display indicating two-electrode operation.
It is also possible to use the bridge amplifier as usual only with the electrode connected the BA-1S, BA-01X or BA-03X. For operation of the bridge amplifier using only one electrode, the MODE OF OPERATION switch of the TEC-B-01 has to be set into EXT or OFF position. In EXT position the TEC-B-01 can be used as current pump, e.g. for iontophoresis.

2.3. Description of the Front Panel

In the following description of the front panel elements each element has a number that is related to that in Figure 1. The number is followed by the name (in uppercase letters) written on the front panel and the type of the element (in lowercase letters). Then, a short description of the element is given.

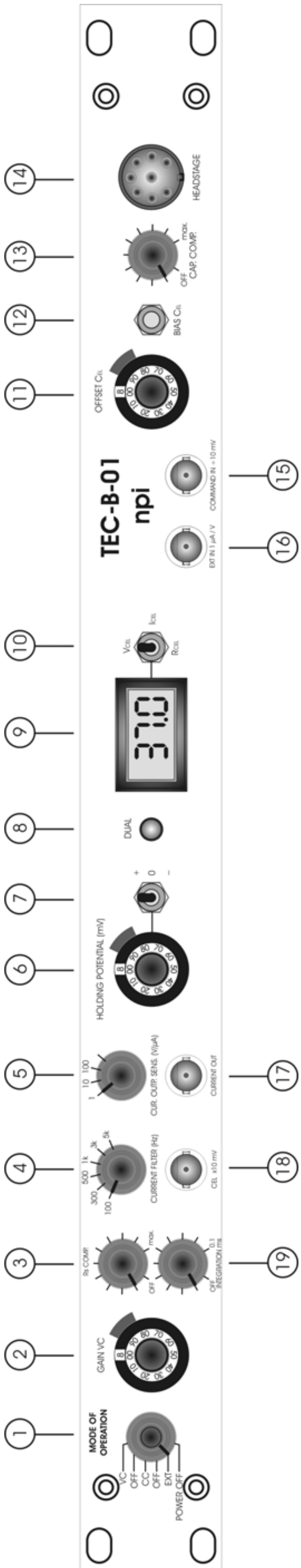


Figure 1: front panel view of the TEC-B-01

(1) MODE OF OPERATION switch

Switch to select the MODE OF OPERATION:

VC:	**V**oltage **C**lamp
OFF:	In this position the current injection of the amplifier is switched OFF, e.g. for operating the bridge amplifier as stand-alone device
CC:	**C**urrent **C**lamp with current injection through the current headstage

Note: In CC operation the current stimulus input of the bridge amplifier is used!

EXT.:	EXTernal control. For operating the TEC-B-01 amplifier as a constant current source, e.g. for iontophoresis, or for operating the bridge amplifier as stand-alone device
POWER OFF:	Power is turned OFF

(2) GAIN VC potentiometer

10-turn potentiometer to set amplification factor (GAIN) of the VC error signal. To keep the VC error as small as possible it is necessary to use high GAIN settings, but the system becomes unstable and begins to oscillate if the GAIN is set too high.

(3) Rs COMP. potentiometer

10-turn potentiometer to set the amount of series resistance compensation. Series resistance compensation improves the performance of the clamp system, especially if fast voltage-activated currents are recorded.

Important: Series resistance compensation is done by positive feedback in the control circuit, which can lead very quickly to stability problems. Therefore, tuning of the series resistance compensation has to be carried out with great care!

(4) CURRENT FILTER (Hz) switch

6-position switch to set the corner frequency of the CURRENT FILTER (100, 300, 500, 1k, 3k or 5k Hz) available at #**17**.

(5) CUR. OUTP. SENS. (V/µA) switch

Switch to set the amplification of the current signal at #**17** (0.1 V/µA, 1 V/µA, 10 V/µA).

(6) HOLDING POTENTIAL potentiometer

10-turn potentiometer for setting the HOLDING POTENTIAL in VC mode

(7) + /0 /- switch

Switch for setting the polarity of the HOLDING POTENTIAL in VC mode. In 0 position the HOLDING POTENTIAL is disabled, i.e. set to 0 mV.

(8) DUAL LED

LED indicating operation with a bridge amplifier in CC or VC mode.

(9) Display

LED display for the CURRENT passed through the CURRENT electrode in µA (XX.XX µA, switch #**10** in I_{CEL} position) or the potential at the current electrode in mV (XXXX mV, switch #**10** in V_{CEL} position) or the resistance of the current electrode (XX.X MΩ, switch #**10** in R_{CEL} position).

(10) V_{CEL} / I_{CEL} / R_{CEL} / switch

3-position switch for selection of the display mode:

V_{CEL}: potential of the current electrode is displayed
I_{CEL}: current value is displayed
R_{CEL}: resistance of the current electrode is displayed

(11) OFFSET potentiometer

10-turn potentiometer to cancel potential OFFSETs at the current electrode.

(12) BIAS C_{EL} trim pot.

Trim pot for adjusting the current headstage BIAS current.

Current Headstage Bias Current Adjustment for TEC-B-01

Caution: It is important that this tuning procedure is performed ONLY after a warm-up period of at least 30 minutes!

The tuning procedure should be performed regularly (at least once a month) with great care since the bias current changes over time and it determines the accuracy of the TEC-BA system.
The TEC-B-01 is equipped with a current source that is connected to the current injecting electrode and performs the current injection. This current source has a high-impedance floating output. Therefore, the zero point (the zero of the BIAS current) of the current source must be defined, i.e. without an input signal there should not be an output current.
The tuning procedure is done using the BIAS C_{EL} trim pot and one resistance of a few kΩ and one of a few MΩ. It is based on Ohm's Law (U = R * I).

If the headstage generates an output current, this current will cause a voltage deflection at a test resistor. If this test resistor has a low resistance of only a few kΩ, this voltage deflection originates only from a possible offset of the electrode, that can be cancelled using the OFFSET C_{EL} (#**11,** Figure 1) potentiometer.
Replacing the low resistance resistor by one of a much higher resistance may lead to another voltage reading at the digital display. This voltage deflection then originates only from the BIAS output current and is proportional to this output current according to Ohm’s law. Using the BIAS C_{EL} trim pot. the monitored voltage can be set to 0. This cancels the BIAS current.

Tuning procedure:

The tuning procedure is performed using high-value resistors. It cannot be performed with an electrode, since there are always unknown potentials involved (tip potential, junction potentials etc.).

Warning: **High voltage!** Always turn power off when working directly on the current headstage output.
❑

- ❑ Set the MODE OF OPERATION switch to OFF.

Important: The tuning procedure **must not** be done in VC mode!!

- ❑ Connect the electrode connector of the TEC-B-01 headstage to ground. If parasitic oscillations occur use a 10 kΩ resistor for grounding.
- ❑ Switch the digital display (#**9**) to V_{CEL} (potential output of the current electrode) using switch #**10**. Set the reading of the display to 0 using the OFFSET potentiometer (#**11**).
- ❑ After tuning the current electrode potential OFFSET simulate an electrode by replacing the 10 kΩ resistor with a much larger resistor (e.g. 1 MΩ).
- ❑ The digital display (and the CURRENT ELECTRODE potential connector (CEL x10mV) (#**18**)) now shows a voltage deflection that is related to the BIAS current of the headstage according to Ohm's Law. Cancel this voltage by tuning the BIAS C_{EL} trim pot (#**12**). The current is 0 if the voltage deflection is 0.

Note: Due to the characteristics of the high voltage OpAmps the V_{CEL} display may fluctuate around the baseline of 0 mV by some mV. With a 1 MΩ resistor (as used in the cell model) 1 mV corresponds to 1 nA. Keeping in mind that the display accuracy of the current is 10 nA in the last digit this is insignificant.

Important: The bridge amplifier it has a BIAS current as well and must be adjusted as described in the bridge amplifier user manual.

(13) CAP.COMP. potentiometer

Control for the capacity compensation of the CURRENT electrode or the signal connected to the EXT IN connector #**16** (potentiometer with OFF position, clockwise).

Note: For experiments with oocytes, it is recommended to switch the CAP.COMP potentiometer to OFF position.

Important: If the CAP. COMP is switched off, the BUZZ function for the C_{EL} is also disabled.

(14) **Current HEADSTAGE** connector

Connector for the current headstage.

(15) **COMMAND IN ÷10 mV** connector

BNC connector for an external COMMAND voltage in VC mode (sensitivity: ÷10 mV). The signal form remains unchanged.

(16) **EXT IN 1 µA/V** connector

BNC connector for connecting an external voltage signal. This can be used to operate the TEC-B-01 module as a constant current source, e.g. for iontophoresis. Scaling is 1 µA / V, i.e. 100 mV connected at EXT IN leads to a current of 100 nA at the current electrode.

(17) **CURRENT OUT** connector

BNC connector providing the current signal. The scaling is set by switch #**5**. The filter is set by switch #**4**.

(18) **C_{EL} x10 mV** connector

BNC connector providing the potential of the current electrode.

(19) **ms INTEGRATION** potentiometer

The integrator improves control performance for slower signals. Position OFF disables the integrator. Setting a time constant by turning the potentiometer clockwise converts the controller into a PI (proportional-integral) system. Time constant range is 10…0.1 ms.

2.4. Description of the Rear Panel

A cable is firmly connected for linking the TEC-B-01 amplifier to the bridge amplifier connector TO TEC-B-01 at the rear panel of the bridge amplifier.

CHASSIS
This connector is linked to mains ground (green / yellow wire, protective earth).

GROUND
This connector is linked to the internal system ground which has no connection to the 19" cabinet (CHASSIS) and the mains ground to avoid ground loops.

3. Operation

The TEC-B-01 can be operated as a stand-alone device or in conjunction with a modified bridge amplifier.

3.1. Stand Alone Operation

The TEC-B-01 can be used as a current pump, e.g. for iontophoresis. The MODE OF OPERATION switch (#**1**, Figure 1). A voltage signal is connected to the EXT IN BNC connector (#**16**, Figure 1). This voltage signal is converted into a current signal with a scaling of 1 µA / V. The (mostly rectangular) shape of the input signal can be influenced by the capacity compensation.

3.2. *Operation with Bridge Amplifier*

Figure 2: front panel of BA-03X

Together with a modified bridge amplifier the TEC-B-01 can be used for two electrode voltage clamp (TEVC) or two electrode current clamp (TECC) experiments. The scaling is adapted for current ranges normally used in experiments with oocytes.

Figure 3: Rear panel connector of the BA-03X for connection with TEC-B-01 (left). A DUAL LED on the front panel of the BA-03X indicates two electrode operation (right).

For two electrode operation the TEC-B-01 and bridge amplifier are connected with a cable at the rear panel. The operation mode switch (#**1**) is set to CC (for current clamp) or VC (for voltage clamp). The current electrode is connected to the TEC-B-01 headstage and the potential electrode to the headstage of the bridge amplifier.
In two electrode operation current injection is done with the current electrode (connected to the TEC-B-01) and potential measurement with the potential electrode (connected to the bridge amplifier. This applies to both CC and VC mode.
Two electrode operation is indicated by the DUAL LED at the TEC-01-B as well as by the DUAL LED at the bridge amplifier. Current display and current output at the bridge amplifier are disabled.

Voltage clamp:

In voltage clamp mode, the command signal is fed into the COMMAND IN BNC connector (**#15**) at the TEC-B-01 front panel.

Current clamp:

In current clamp mode, the command signal is fed into one of the STIMULUS INPU BNC connectors (**#23** or **#24**) at the BA-03X front panel.

3.3. *BUZZ or Penetration mode*

In two electrode configuration, the BUZZ module of the BA-03X works for both the potential electrode from BA-03X (P_{EL}) and the current electrode from TEC-B-01 (C_{EL}). The BUZZ select switch at the BA-03X front panel allows selection which electrode the BUZZ shall be applied to.

4. Simple cell model

4.1. Cell Model Description

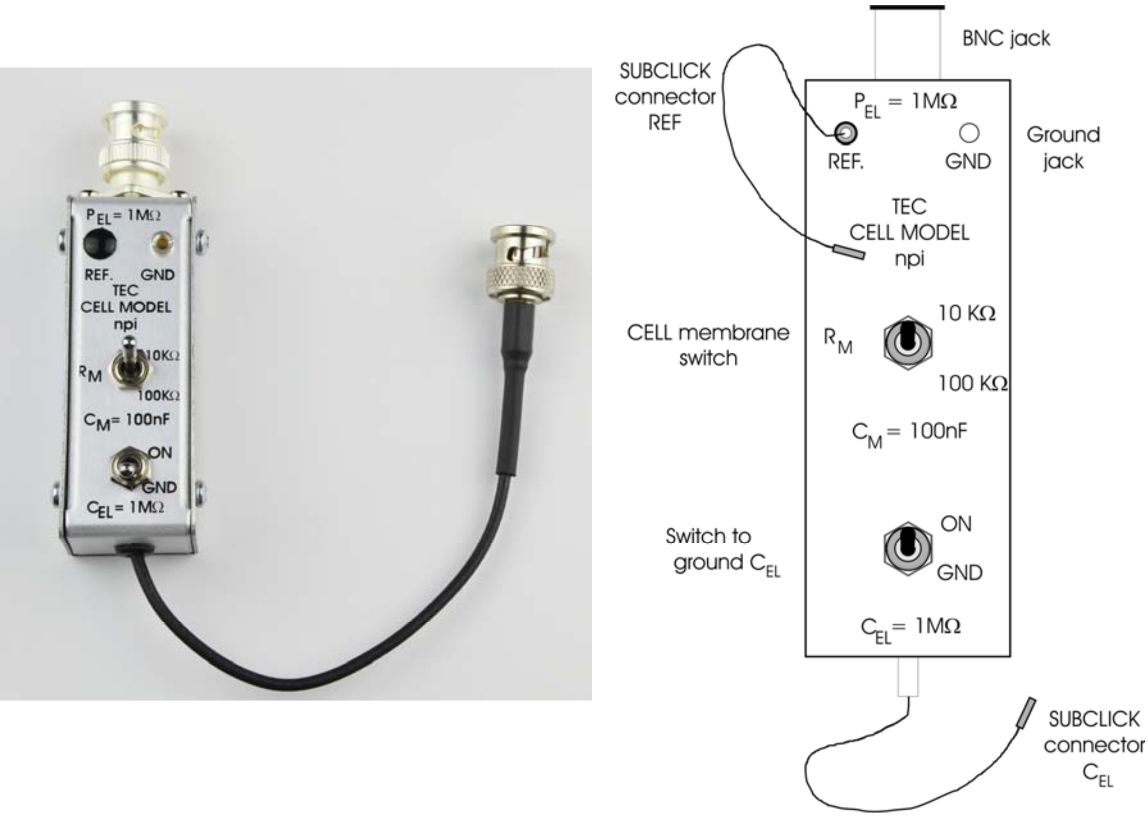


Figure 4: TEC passive cell model

P_{EL} BNC:	connector for the potential electrode, resistance: 1 MΩ
REF SUBCLICK:	subclick (SMB) connector for the reference electrode (optional)
GND:	ground connector
R_M:	switch for the cell membrane representing a membrane resistance of either 10 kΩ or 100 kΩ
C_M:	cell membrane capacity, always 100 nF
ON / GND:	switch to ground the current electrode, ON = C_{EL} inside the cell, GND = C_{EL} connected to ground (see also chapter 0)
C_{EL} SUBCLICK:	SMC connector for the current electrode, resistance: 1 MΩ (built as BNC connector for TEC-B-01).

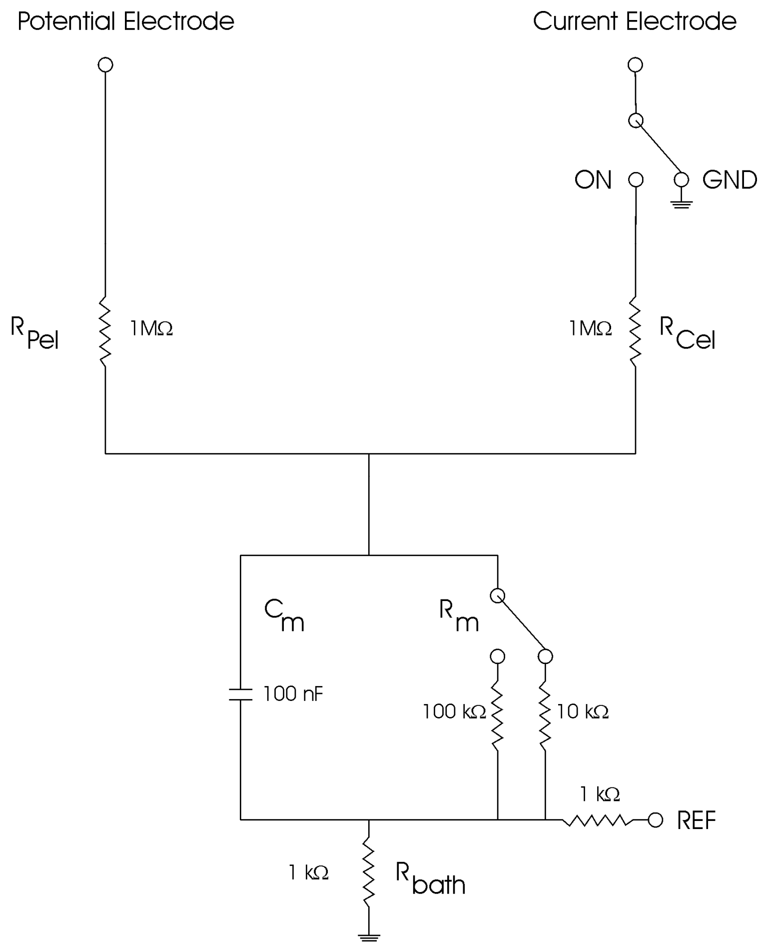


Figure 5: schematic diagram of the TEC passive cell model. For TEC-B-01, there is no REF connector and R_{bath} = 0 Ω.

4.2. Basic settings

Before using the TEC-B-01 always make the basic settings to avoid oscillations.

② **Basic settings**

- ❑ Turn all controls to low values (less than 1) and each symmetrical offset adjustment, i.e. C. HEADSTAGE BIAS CURRENT, CURRENT ELECTRODE OFFSET and POTENTIAL OFFSET potentiometers, in the range of 5 (zero position, see chapter 2.2). Set the CAPACITY COMPENSATION (#**13**) to OFF.
- ❑ Disable the HOLD unit by setting the + / 0 / - switch (#**7**) to 0.
- ❑ Set the MODE OF OPERATION (#**1**) to CC.
- ❑ Set the display to POTENTIAL ELECTRODE using switch #**10**.

Now the TEC-B-01 is ready for an initial check with the cell model.

4.3. Connections and Operation

Checking the Configuration with the Cell Model

- ❑ Make the basic settings (see chapter 4.2).
- ❑ Connect the P_{EL} BNC jack of the cell model to the BNC connector at the potential headstage.
- ❑ Connect the BNC jack C_{EL} to the connector at the current headstage.
- ❑ Switch the CELL membrane switch (see Figure 4) to the desired position.
- ❑ Set the GND switch (see Figure 4) to ON.
- ❑ Turn POWER switch of the amplifier on.

Now you can adjust the amplifier and apply test pulses to the cell model. Connection to both BNC connectors gives access to the cell via a potential and current electrode with 1 MΩ resistance. In the upper position, the R_M switch simulates a cell membrane with a resistance of 10 kΩ. In the lower position, a cell membrane with 100 kΩ is simulated. The membrane capacity is always 100 nF (see also chapter 5.5).

5. Test and Tuning Procedures

Important: The TEC-B-01 and BA amplifiers should be used only in warmed-up condition, i.e. 20 to 30 minutes after turning power on.

The following test and tuning procedures are necessary for optimal recordings. It is recommended first to connect a cell model to the amplifier to perform some basic adjustments and to get familiar with these procedures.

5.1. Current Headstage Bias Current Adjustment

Caution: It is important that this tuning procedure is performed ONLY after a warm-up period of at least 30 minutes!

The tuning procedure must be performed regularly (at least once a month) with great care since the bias current changes over time and it determines the accuracy of the TEC system.

The TEC-03X is equipped with a high-voltage current source that is connected to the current injecting electrode and performs the current injection. This current source has a high-impedance floating output. Therefore, the zero point (the zero of the bias current) of the current source must be defined, i.e. without an input signal there should not be an output current.

Since the high-voltage FET amplifiers that are used become warm from the internal heat dissipation and their characteristics are strongly temperature dependent, the calibration procedure has to be done periodically by the user.

The tuning procedure is done using the C. HEADSTAGE BIAS CURRENT control and one resistance of a few kΩ and one of a few MΩ or a cell model. It is based on Ohm's Law ($U = R * I$).

If the headstage generates an output current, this current will cause a voltage deflection at a test resistor. If this test resistor has a low resistance of only a few kΩ this voltage deflection is nearly zero, and a possible reading at the digital display originates only from a possible offset of the electrode, which can be cancelled using the (CURRENT ELECTRODE) OFFSET (#**11**) potentiometer. Replacing the low resistance resistor by one of a much higher resistance may lead to another voltage reading at the digital display. This voltage deflection then originates only from the BIAS output current and is proportional to this output current according to Ohm's law. Using the BIAS C_{EL} (#**12**) control the monitored voltage can be set to 0.

The tuning procedure is performed using high-value resistors or a cell model. It cannot be performed with an electrode, since there are always unknown potentials involved (tip potential, junction potentials etc.).

Warning: **High voltage!** Always turn power off when working directly on the current headstage output.

- ❑ Put the holding current switch to position 0 (+ / 0 / - switch, #**7**). If you use a cell model, only the C_{EL} and GND connectors must be connected.
- ❑ Set the MODE OF OPERATION switch to OFF.

Important: The tuning procedure **must not** be done in VC mode!!

- ❑ Connect the CURR.EL connector of the current headstage to ground. If parasitic oscillations occur use a 10 kΩ resistor for grounding. If you use a cell model set the ON / GND switch to GND.
- ❑ Switch the digital display (#**9**) to V_{CEL} (potential output of the current electrode) using the electrode selector (#**10**). Set the reading of the display to 0 using the potentiometer OFFSET (#**11**).
- ❑ After tuning the current electrode potential OFFSET connect the cell model (see chapter 4.2). If you do not use a cell model simulate an electrode by replacing the 10 kΩ resistor with a much larger resistor (min. 5-10 MΩ).
- ❑ The digital display (and the CURRENT ELECTRODE potential connector (C_{EL} x10mV (#**18**)) now shows a voltage deflection that is related to the BIAS current of the headstage according to Ohm's Law. Cancel this voltage by tuning the headstage BIAS C_{EL} potentiometer (#**12**). The current is 0 if the voltage deflection is 0.

Now the CURRENT OUTPUT (#**17**) and the CURRENT DISPLAY (#**9**) should also read 0.

5.2. Offset Compensation

If an electrode is immersed into the bath solution an offset voltage will appear, even if no current is passed. This offset potential is the sum of various effects at the tip of the electrode filled with electrolyte ("tip potential", junction potential etc.). This offset voltage must be compensated i.e. set to 0 carefully with the OFFSET controls (#**11** at TEC-01B and #**16** at BA-03X) before recording from a cell. The OFFSET compensation is done in CC mode of the amplifier. When adjusting the OFFSETs make sure that no current flows through the electrodes. Thus, it is recommended to disconnect COMMAND INPUT (#**15**) and to disable the HOLD unit (switch #**7** to 0).

Potential Electrode

- ❑ The potential of the potential electrode is read from the POTENTIAL DISPLAY (#**3**) of BA-03X. The display shows the potential of the potential electrode in XXX mV.
- ❑ Compensate the OFFSET with the OFFSET (#**16**) potentiometer from the BA-03X.

Current Electrode

- ❑ Switch the reading of the TEC-B-01 digital display (#**9**) to V_{CEL} using the electrode selector switch (#**10**). The display (#**9**) shows the potential of the current electrode in XXX mV.
- ❑ Compensate the OFFSET with the CURRENT ELECTRODE OFFSET (#**11**) potentiometer.

Note: If a cell model is connected the OFFSET controls should read values around 5, otherwise it is likely that the headstages or the amplifier are damaged. If microelectrodes are used unusual high OFFSETs are a sign of badly chlorinated silver wires or unwanted grounding of the bath.

5.3. Electrode Resistance Test

The electrode resistance is dependent on the tip diameter of the electrodes and may reveal whether electrodes are broken or clogged. Therefore, a resistance measurement test for the CURRENT ELECTRODE microelectrodes is included in the TEC-B-01. The respective test for the POTENTIAL ELECTRODE is included in the BA-03X. The test operates independently of any other adjustments, assuming that all microelectrodes are in contact with a grounded bath (zero potential). The measured resistance is independent of tip potentials and is automatically displayed on the respective digital display in MΩ. Furthermore, the electrode resistance can be tested even if the electrode is inside a cell!

The measurement is performed by applying square current pulses of ±10 nA to the respective microelectrode. The voltage deflection caused by this injection is recorded and processed to give a direct reading in MΩ on the digital display.

Important: The electrode resistance test is also a test of the correct function of the respective headstage.
The resistance test gives only a rough estimate of the electrode resistance. The value for the current electrode is dependent on the calibration of the current headstage (see chapter 5) and the reading is correct only in position x1.

Potential Electrode (BA-03X)

- ❑ Set the ELECTRODE RESISTANCE (#**13**) switch to CURRENT ELECTRODE. The upper digital display (#**10**) shows the resistance of the current electrode in XX.X MΩ.

Current Electrode (TEC-B-01)

- ❑ Push the ELECTRODE RESISTANCE PUSHBUTTON (#**33**). The left digital display (#**3**) shows the resistance of the potential electrode in XX.X MΩ.
- ❑

Important: Since the amplitude of the current pulses is relatively small (at least for oocytes) the electrode resistance can be checked even if the electrode is inside the cell!

5.4. Capacity Compensation

The frequency response of the potential electrode (low-pass characteristic due to stray capacities) is compensated for by a feedback circuit ("negative capacity" compensation, CAPACITY COMPENSATION) and a "driven-shield" arrangement (for an overview see Ogden 1994). Since **in oocyte experiments** microelectrodes are usually in the one MΩ range or below for most experiments it is **not required to use the CAPACITY COMPENSATION**.

The tuning of the capacity compensation control is performed using pulses applied to the COMMAND INPUT or pulses provided by the electrode resistance test circuit. The TEC-B-01 has to be in CC mode (see chapter 5.5).

With the cell model connected or the electrode in the bath the CAPACITY COMPENSATION control is turned clockwise until there is no artifact on the POTENTIAL OUTPUT P_{EL}.

Important: Capacity compensation is based on positive feedback. Therefore overcompensation causes oscillations which can damage the preparation or the recording electrodes. Therefore the control must be handled with care and before impaling a new cell it must be set to 0.

5.5. Testing Operation Modes

Current Clamp

The cell's response to current injections is measured in the current clamp (CC) mode. Current injection is performed by means of a current source connected to the current injecting microelectrode.

Important: In current clamp, the current stimulus is applied to the BA-03X. In DUAL mode, STIMULUS INPUT scalings are adapted for oocytes. STIMULUS INPUT (#**23**) is scaled to 0.1 µA/V and STIMULUS INPUT (#**24**) is scaled to 1 µA/V.

- Set the amplifier to CC mode using the MODE OF OPERATION switch (#**1**).
- If not already done tune the BIAS current to 0 (see chapter 5.1).
- Set the CURRENT OUTPUT SENSITIVITY (#**5**) to 1.
- Compensate the offsets of the current- and voltage electrode (see chapter 5.2).
- Set R_m at the cell model to 10k (see chapter 4.3).
- Set the holding current to –1 µA using the HOLD potentiometer (#**6**) (setting: 100, reading: -1.00 µA) and the HOLD current polarity switch (#**7**) (set to -).
- Make sure that the display selector switch (#**10**) is set to I_{CEL} and the ELECTRODE RESISTANCE test is not active.
- The POTENTIAL display (BA-03X) should read –10 mV (according to Ohm's law). The voltage at P_{EL} (#**36**) should be –100 mV.

Remember: The voltage at P_{EL} is the membrane potential multiplied by 10!

- Disable the holding current and apply a test pulse of 2 µA to the cell model by giving a voltage step of 2 V to STIMULUS INPUT (#**23**). The length of the test pulse should be at least 50 ms.
- You should see a potential step of 200 mV amplitude at POTENTIAL OUTPUT (#**36**). Due to the membrane capacity the step is smoothed.

Note: If you expect the POTENTIAL display to show the value of the potential step (in this case 20 mV amplitude) remember that the display is rather sluggish and may not display the right value (depending on the length of the step). The same is true for the CURRENT display.

Voltage Clamp

In voltage clamp mode, the membrane potential is forced by a controller to maintain a certain value or to follow an external command. That allows measurement of ion fluxes across the cell membrane. This is the most complex mode of operation with the TEC-B-01. Special precautions must be taken while tuning the control circuit in order avoid stability problems.

- Make sure that the amplifier works correctly with the cell model in CC mode (see above).
- Set the holding potential to –50 mV using the HOLD potentiometer (#**6**, setting: 050, reading: 050 mV) and the HOLD potential polarity switch (#**7**, set to -).

❑

- Set the CAPACITY COMPENSATION (#**13**) to 0 and the GAIN (#**2**) to 1.

- Enable the OSCILLATION SHUTOFF unit from BA-03X with a moderate THRESHOLD (DISABLED / RESET switch (#**32**) in middle position, OSCILLATION SHUTOFF LED green, THRESHOLD potentiometer set to a low value, but not to the most left position)
- Set the TEC-B-01 amplifier with the MODE OF OPERATION switch (#**1**) to VC mode.
- The BA-03X POTENTIAL display should show the holding potential of –50 mV and the TEC-B-01 display (I_{CEL}) the holding current of –5 µA (according to Ohm's law).
- It is very likely that the display shows a holding potential of slightly less than –50 mV because the controller is in GAIN ONLY mode and the GAIN is low. Increasing GAIN and activating the INTEGRATION will enhance the control loop and therefore increase accuracy.

Hint: If the system oscillates as soon as you switch to VC mode switch back to CC mode and check the settings. GAIN too high? CAPACITY COMPENSATION not 0? THRESHOLD potentiometer of the OSCILLATION SHUTOFF unit at the most left position? R_S-COMP. not switched OFF?

- Apply a test pulse of 20 mV to the cell model by giving a voltage step of 0.2 V to COMMAND INPUT (#**15**). The length of the test pulse should be at least 30 ms.
- You should see a potential step of 200 mV amplitude at BA-03X POTENTIAL OUTPUT (#**36**).

Note: If you expect the POTENTIAL display to show the value of the potential step (in this case +20 mV amplitude, i.e. –30 mV) remember that the display is rather sluggish and may not display the right value (depending on the length of the step). The same is true for the CURRENT display.

5.6. Tuning the VC mode

In VC mode there is the problem that the voltage step is often not strictly angular shaped. But, for instance, increasing the clamp speed by tuning the CAPACITY COMPENSATION of the potential electrode or increasing GAIN also increases noise. Therefore, the settings of the different parameters result always in a compromise between the stability, accuracy, noise and control speed. In this chapter we will give some practical hints, how to optimize the accuracy and speed of the clamp. The theoretical background of adjustment criteria is discussed in chapter 9 (see also Polder and Swandulla, 2001).

The main considerations are: Do I expect rapid or slow responses to voltage changes? How much noise can I accept? Is it possible to use electrodes with low resistance?

General: The speed and accuracy of the voltage clamp control circuit is mainly determined by the question how much current can be injected and how fast can this happen. Thus, the more current the system can inject within a short time the better the quality of the clamp (see chapter 9).

General Considerations

The key to accurate and fast recording is a properly built setup.

- Make sure that the internal system ground is connected to only one point on the measuring ground and originates from the potential headstage. Multiple grounding should be avoided; all ground points should originate from a central point. The electrode used for grounding the bath should have a low resistance so that it can pass large currents.
- Use electrodes with resistances as low as possible.
- Keep cables short.
- Check regularly whether cables and / or connections are broken.
- Make sure that chloriding of silver wires for the electrodes is proper and that there are no unwanted earth bridges, e.g. salt bridges originating from experimental solutions.

Only if no intracellular series resistance is considered TEC system can be tuned according to one of three optimization methods (see also chapter 9):

1. the “linear optimum” (LO) that provides only slow response to a command step and a maximal accuracy of 90-97%.
2. the "absolute value optimum" (AVO) that provides the fastest response to a command step with very little overshoot (maximum 4%) or
3. the "symmetrical optimum" (SO) has the best performance compensating intrinsic disturbance signals but shows a considerable overshoot (maximum 43%) to a step command.

Under consideration of an existing intracellular series resistance these methods cannot be applied. Instead, a series resistance compensation can be introduced to optimize clamp performance (see also chapter 9.2).

Three control modes are implemented to adapt the TEC-03X to the needs of the user:

1. NORMAL fits to many users. In this mode a good compromise between speed, accuracy, noise and stability is achieved. The normal mode can be optimized by the LO method (see above).
2. SLOW for relative slow recordings (e.g. ligand activated currents). In this mode accuracy and stability are increased while speed is decreased. Optimization is done according to the AVO- or SO method (see above).
3. FAST for very fast recordings (e.g. fast voltage activated currents). In this mode speed and accuracy are increased but the system is very sensitive with a higher noise level and tuning requires more experience. Optimization is done by adjusting the amount of current proportional gain of the SERIES RESISTANCE COMPENSATION and optimal positioning of the electrodes (see chapter 0).

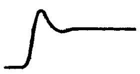
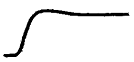
Important: First use a cell model for the tuning procedure. You will get familiar with the different settings and the consequences for the system without any damage to cells or electrodes.

Tuning Procedure

- ❑ Before you switch to VC mode tune all parameters related to the recording electrodes (offset, capacity compensation etc.) in CC mode, set GAIN to a low, save level and the control mode switch to NORMAL, GAIN ONLY (see chapter 5.5). Activate the OSCILLATION SHUTOFF unit!
- ❑ Switch to VC mode and apply identical test pulses to the cell model.
- ❑ The controller is now in P-mode (proportional only). Watch the potential output and increase the GAIN so that no overshoot appears.

If you a working on slow currents

- ❑ Switch the control mode switch to SLOW, INTEGRATION to activate the integrator. The controller is now in PI-mode (proportional-integral). Tune the GAIN again (see above).
- ❑ Watch the potential output and tune the time constant until the overshoot of the desired tuning method appears (see also Figure 6).

	Response to a command variable step	Response to a disturbance variable step
Linear optimum LO (aperiodic response) P-Controller	 slow response no overshoot	 slow response large deviation
Absolute value optimum AVO PI-Controller	 fastest response 4% overshoot	 slow response slight deviation
Symmetrical optimum SO Unsmoothed command variable PI-Controller	 fast response 43% overshoot	 very fast response slight deviation
Smoothed command variable	 slow response 8% overshoot	

LO
Only a P-Controller is used. The response to a command step is slow and has no overshoot (potential output). The response to a disturbance e.g. an activating channel is slow and has a large deviation.

AVO
A PI-Controller is used. The response to a command step is very fast with 4% overshoot (potential output). The response to a disturbance e.g. an activating channel is slow and has a slight deviation.

SO
A PI-Controller is used. The response to an unsmoothed command step is fast with 43% overshoot (potential output). The response to a disturbance e.g. an activating channel is very fast and has a slight deviation.

Figure 6: tuning VC according LO, AVO or SO. The potential output is shown.

If you a working on very fast currents

- ❑ Lower the GAIN by approximately 10%.
- ❑ Switch the control mode switch to FAST, SERIES RESISTANCE COMPENSATION to activate the series resistance compensation. Rise the amount of SERIES RESISTANCE COMPENSATION and watch the current output. The capacitive transient seen on the current trace should be mono-exponential. The critical compensation is achieved when the slow tail of the transient disappears. If you see ringing around the slow tail this a sign that the electrodes are not optimally positioned (see also chapter 0).

Hint: SERIES RESISTANCE COMPENSATION is done by positive feedback in the control circuit, which can lead very quickly to stability problems. Repositioning the electrodes is recommended whenever possible instead of extensive use of SERIES RESISTANCE COMPENSATION (see also chapter 0).

Note: With a standard cell model described in chapter 4 you cannot verify the advantages of the FAST mode (because no series resistance is simulated). Ask npi for a modified cell model with series resistance simulation.

Details of how to tune PI controllers and some theoretical aspects are described in chapter 9.2.

6. Positioning of Electrodes

The position of the electrodes plays an important role in tuning the clamp speed. The position of the current electrode is especially crucial for homogeneous charging of the membrane capacitance, one limiting factor of clamp speed. If the current electrode is placed just at the edge of the oocyte, i.e. with a small penetration depth, the part of the membrane close to the electrode will be charged more quickly than the membrane at the other side of the oocyte. Thus, the voltage controlled by the clamp is different. This leads to a capacitive transient with a slow tail (see Figure 7, right side). Placing the current electrode central in the oocytes i.e. with a large penetration depth, leads to a homogeneous charging of the membrane. In this case the capacitive transient can be kept short by critical compensating R_s (see Figure 7, left side).

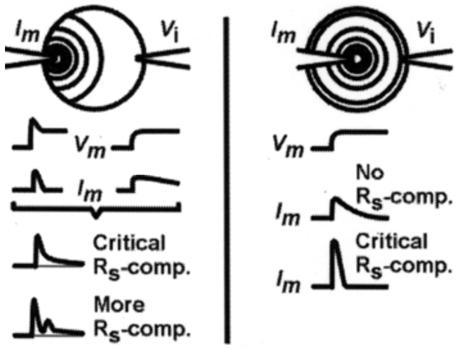


Figure 7: penetration depth of the current electrode and consequences for the clamp

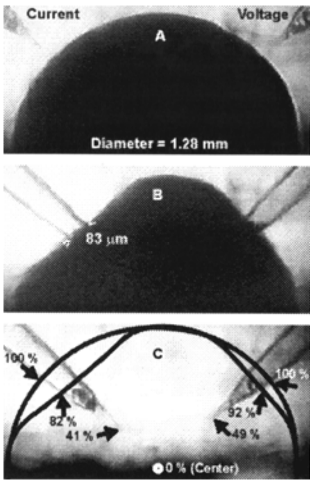


Figure 8: demonstration of electrode positioning

Figure 8 shows the side view of positioning the electrodes in a sequence of microphotographs. **A** shows the tips coming from 45° from above just before touching the oocyte and **B** the electrodes in their final position. The reconstruction in **C** gives the position relative to the center of the oocyte.

Figures are kindly provided by Nikolaus G. Greeff (Greeff, 2000).

7. Sample Experiment

In the following the basics of a simple experiment are described. It is assumed that all connections are built as described in chapter 4.3. Before starting remove the cell model.

Again: It is of major importance that the TEC-03X systems are used only in warmed-up condition i.e. 20 to 30 minutes after turning power on.

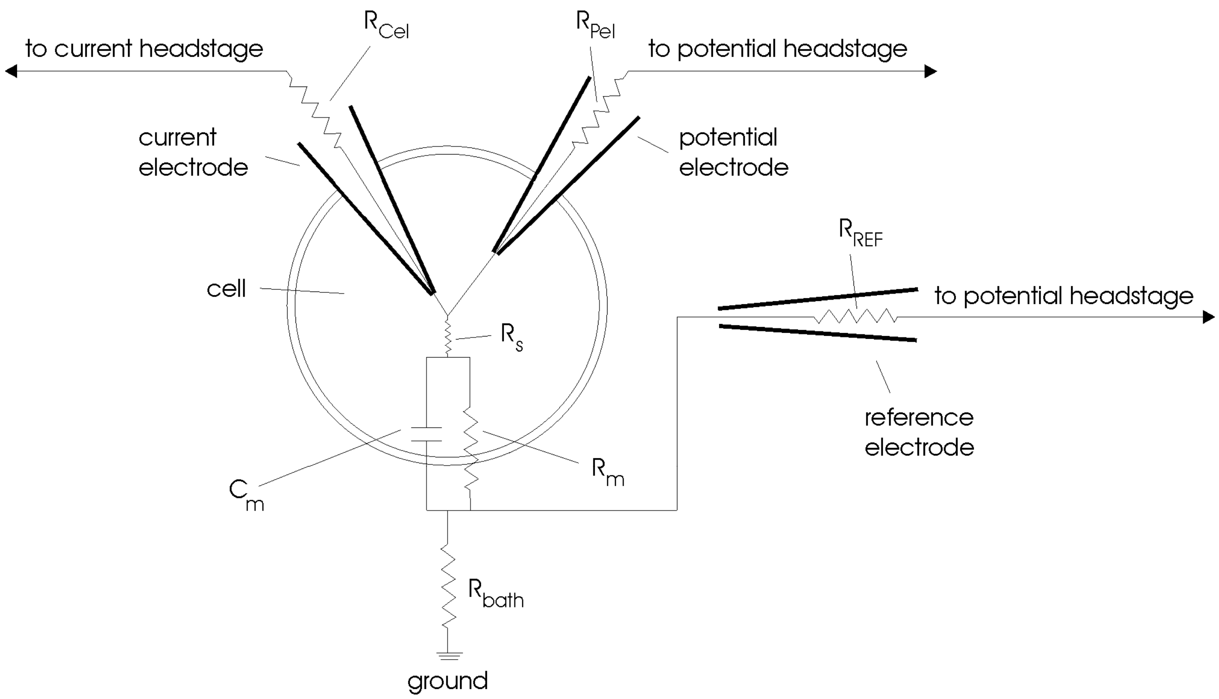


Figure 9: model circuit for voltage clamp recording from a cell, e.g. an oocyte using low-resistance electrodes
C_m: membrane capacity, R_m: membrane resistance, R_{CEL}: current electrode resistance, R_{PEL}: potential electrode resistance, R_{REF}: reference electrode resistance, R_s: series resistance

- ❑ Adjust BIAS CURRENT to 0 if necessary (see chapter 0).
- ❑ Turn off the amplifier!
- ❑ Reconnect the COMMAND INPUT.
- ❑ Connect the Ag-AgCl pellet or the agar-bridge for grounding the bath with GND at the potential headstage.
- ❑ Connect the reference electrode to REF. at the potential headstage (High Voltage TEC systems only).
- ❑ Insert potential- and current electrode into the electrode holders. Check if the silver wires of both electrodes are well chlorided and in contact with the electrode solution, and connect them to the respective headstages.
- ❑ Immerse both electrodes into the bath and make the basic settings (see chapter 4.2).
- ❑ Turn on the amplifier.

- ❑ Compensate the potential offset (for both electrodes, see chapter 5.2), measure the electrode resistance (for both electrodes, see chapter 5.3). The resistances should be 700 kΩ to 1-2 MΩ for the potential electrode and 500 kΩ to 1 MΩ for the current electrode. As mentioned above, it is usually not necessary to compensate for the electrode capacity (for the potential electrode, see chapter 5.4).
- ❑ Set the upper display to POTENTIAL ELECTRODE.
- ❑ Insert the potential electrode into the oocyte. The potential electrode is inside the cell if you read a membrane potential of about –50 mV to –60 mV. It's a good idea to activate the audio monitor. Then you can look through the microscope while hearing the membrane potential.

Remember: The membrane potential of the oocyte is strongly dependent on the condition of the oocyte (leaky or not), the experimental solutions and the membrane proteins (channels/transporters) that are expressed.

- ❑ If your reference electrode can be moved place it near the cell membrane and the potential electrode to optimize differential potential recording (High Voltage TEC systems only).
- ❑ Insert the current electrode into the oocyte. There are two indications that the current electrode is inside the cell:
  - If you apply a test current pulse to the cell, the potential read by the potential electrode changes according to Ohm's law.
  - If you switch the POTENTIAL display to CURRENT ELECTRODE (see chapter 5.2) you read the same membrane potential as read by the potential electrode.
- ❑ After penetration with both electrodes the voltage responses of the cell to the test pulses in CC mode should reflect the cell membrane resistance and time constant.
- ❑ Start the experiment.
- ❑ If you intend to work in VC mode tune the system in CC mode, then switch to VC mode and adjust the clamp as described in chapter 5.6.

8. Trouble Shooting

In the following section some common problems, possible reasons and their solutions are described.

Important: Please note that the suggestions for solving the problems are only hints and may not work. In a complex setup it is impossible to analyze problems without knowing details. In case of trouble always contact an experienced electrophysiologist in your laboratory if possible and connect a cell model to see whether the problem occurring with electrodes and "real" cells persists with the cell model.

Problem 1:
After immersing an electrode into the bath there is an unusual high potential offset.
Possible reasons:
1. The Ag-AgCl coating of the silver wire in the electrode holder is damaged
2. The Ag-AgCl pellet or Ag-AgCl coating of the silver wire in the agar-bridge are damaged
3. There is an unwanted GND-bridge e.g. caused by a leaky bath
4. The headstage or the amplifier has an error

Solutions:
1. Chloride the silver wire again
2. Exchange the pellet or chloride the silver wire in the agar-bridge
3. Try to find the GND-bridge and disconnect it e.g. by sealing the bath
4. Contact npi

Problem 2:
Even if no stimulus is given a current flows through the current electrode
Possible reason:
1. The BIAS current is not adjusted

Solution:
1. Adjust the BIAS current according the procedure described in chapter 0

Problem 3:
The system oscillates (see also *voltage clamp* in chapter 5.5)
Possible reason:
1. The capacitance of the electrode is overcompensated
2. There is too much series resistance compensation

Solution:
1. Turn the CAPACITY COMPENSATION potentiometer (#**13**) to the most left position and compensate the input capacitance again
2. Turn the SERIES RESISTANCE COMPENSATION potentiometer (#**3**) to a lower value

Problem 4:
With the cell model connected the R_{EL} display does not show the correct value (within a tolerance of 2%).
Possible reason:
1. The headstage has an error

Solution:
1. Contact npi

9. Appendix

9.1. Theory of Operation

The standard configuration for voltage clamping oocytes is the two electrode voltage clamp arrangement (Stühmer, 1992; Stühmer et al. 1992; Dietzel et al., 1992; Stühmer, 1998). In contrast to previously described clamp systems (for review see Smith et al., 1985) the amplifiers for oocyte clamping must meet special requirements since oocytes are very large cells with a high membrane capacity (up to 100-500 nF) and large membrane currents (up to 100 µA and more).

Voltage clamp instruments are closed loop control systems with two inputs external to the control loop. An electronic feedback network is used to force the membrane potential of a cell to follow a voltage command (setpoint input) as fast and as accurately as possible in the presence of incoming disturbances (disturbance input, correlated with the activities of the cell e.g. activation of ion channels). This is achieved by injecting an adequate amount of charge into the cell. The current injected by the clamp instrument is a direct measure of the ionic fluxes across the membrane (Ferreira et al., 1985; Jack et al., 1975; Ogden, 1994;Smith et al., 1985).

The performance evaluation and optimal tuning of these systems can be done by considering only the command input since the mathematical models (set point transfer function and the disturbance transfer function, see Froehr, 1985; Polder; 1984; Polder and Swandulla, 1990; Polder, 1993; Polder and Houamed, 1994; Polder and Swandulla, 2001) are closely related. Modern control theory provides adequate solutions for the design and the optimal tuning of feedback systems (Froehr, 1985).

Most voltage clamp systems are composed only of delay elements, i.e. elements which react with a retardation to a change. This type of closed loop systems can be optimized easily by adequate shaping of the "frequency characteristic magnitude" (***/F(jw)/***) of the associated transfer function ***F(s)*** (output to input ratio in the frequency domain = ***LAPLACE*** transform of the differential equation of the system, Polder and Swandulla, 2001).

Using controllers with a proportional-integral characteristic (PI-controllers) it is possible to force the magnitude of the frequency characteristic to be as close as possible to one over a wide frequency range ("modulus hugging", see Froehr, 1985; Polder; 1984; Polder and Swandulla, 1990; Ploder, 1993; Polder and Houamed, 1994; Polder and Swandulla, 2001). For voltage clamps this means that the controlled membrane potential rapidly reaches the desired command value.

The PI-controller yields an instantaneous fast response to changes (proportional gain) while the integral part increases the accuracy by raising the gain below the corner frequency of the integrator (i.e. for slow signals) to very high values (theoretically to infinite for DC signals, i.e. an error of 0%) without affecting the noise level and stability. Since the integrator induces a 0 in the transfer function, the clamp system will tend to overshoot if a step command is used. Therefore the tuning of the controller is performed following optimization rules which yield a well defined system performance (AVO and SO, see below).
The various components of the clamp feedback electronics can be described as first or second order delay elements with time constants in the range of microseconds. The cell capacity can be treated as an integrating element with a time constant *Tm* which is always in the range of hundreds of milliseconds.

Compared to this "physiological" time constant the "electronic" time constants of the feedback loop can be considered as "small" and added to an equivalent time constant ***Te***. The ratio of the "small" and the "large" time constant determines the maximum gain which can be achieved without oscillations and thus, the accuracy of the clamp. With the gain adjusted to this level the integrator time constant and "small" time constant determine the speed of response of the system.

The clamp performance can be increased considerably if the influence of the current injecting electrode is excluded as far as possible from the clamp loop since the electrode resistance is nonlinear. This is achieved if the output of the clamp system is a current source rather than a voltage source. In this case the clamp transfer function has the magnitude of a conductance (A/V). Other advantages of this arrangement are that the clamp current can be determined by a differential amplifier (with no need of virtual ground, see Greeff and Polder, 1997; Polder and Houamed, 1994) and that the bandwidth of the feedback system can be altered easily (e.g. for noise suppression during simultaneous patch clamp recordings, see Stühmer, 1992; Stühmer et al. 1992; Stühmer and Parekh, 1995).

This output circuit is equipped with large bandwidth high voltage operational amplifiers. To avoid deterioration of clamp performance caused by electrode overload the output current has to be limited by an electronic circuit to a safe level. With electrodes in the range of one MΩ and a voltage of ±150 V, the maximum current will be 150 µA. With this current a cell with a capacity of 100 nF can be depolarized by 100 mV in approximately 100 µs, which comes close to the theoretically possible speed of response, without any detectable deviations from the command level. With an output compliance of 225 V and a x2 or x5 range current injecting headstage, currents up to 500 µA can be injected (see Greeff and Polder, 1997; Polder and Houamed, 1994).

The accuracy of a two electrode clamp system and the speed of response is determined by the cell capacity, the resistance of the current injecting microelectrode (that limits the maximum amount of injected current) and the equivalent time constant and accuracy of the potential recording and feedback electronic systems. Therefore, the design of the potential recording site is very important. A differential potential registration with a reference electrode that registers the bath potential minimizes errors due to resistances outside the cell in series with the cell membrane. Driven shield and capacity compensation circuits are used to improve the speed of response.

In some cases, a series resistance compensation circuit (for series resistance inside the cell) which adds a current proportional gain can improve the clamp performance considerably (Greeff and Polder, 1997; Greeff, 2000; Greeff and Kühn, 2000). The use of such a circuit enhances the speed of response and improves the accuracy of the clamp system. But the noise level is also increased because both circuits are positive feedback loops.

In addition to the elements of the clamp loop itself, this oocyte clamp amplifier has some additional units that facilitate experiments such as electrode resistance test units, oscillation shut-off unit, adequate output signal amplification, filtering and display units, facility for compensating capacitive currents, etc.

9.2. Tuning Procedures for VC Controllers

The initial settings (see *voltage clamp* in chapter 5.5) guarantee only a stable clamp that is not very accurate and insufficiently rapid for certain types of experiments, e.g. investigation of fast voltage-activated ion channels or gating currents. Thus, for successful and reliable experiments, it is necessary to tune the clamp loop.

Only if no intracellular series resistance is considered tuning of the clamp is performed according to optimization methods. It depends on the type of experiment to which method one should follow (see below).

- “**L**inear **O**ptimum” (LO)

  with this method only the proportional part of the PI controller is used. The response to a command step is slow, but produces no overshoot. The response to a disturbance is also slow with a large deviation of the membrane potential. Clamp accuracy is a maximum of 90-97% (Finkel and Redman, 1985). Therefore, this method should only be used only if it is very important to avoid overshoots of the membrane potential.

- "**A**bsolute **V**alue **O**ptimum" (AVO)

  uses the PI controller and provides the fastest response to a command step with very little overshoot (maximum 4%). The response to a disturbance is of moderate speed and the amplitude of the deviation is only half the amplitude obtained with LO. It is applied if maximum speed of response to a command step is desirable e.g. if large voltage activated currents are investigated.

- "**S**ymmetrical **O**ptimum" (SO)

  uses also the PI controller and has the best performance compensating intrinsic disturbance signals. The response to a command step shows a very steep rise phase followed by a considerable overshoot (maximum 43%). The response to a disturbance is fast and the amplitude of the deviation is in the same range as with the AVO method. The overshoot can be reduced by adequate shaping of the command pulse by a delay unit (Froehr, 1985; Polder and Swandulla, 1990; Polder and Swandulla, 2001). This method is preferred for slowly activating currents, such as those evoked by agonist application.

The upper speed limit for all optimization methods is determined by the maximum amount of current which the clamp system can force through a given electrode (see chapter 9.3).

Practical Implications

In the following some practical implications of the theory discussed earlier in this chapter are outlined. It is assumed that you have read the last chapters, that all connections are set up as described in chapter 4.3 and that the system is in VC mode with the initial settings described in chapter 5.5.
Although most of the parameters of the control chain are not known during an experiment it is possible to tune the clamp controller by optimizing the response to a test pulse applied to the COMMAND INPUT. The main criterion of tuning is the overshoot seen at the potential output. Since the SO method provides the tightest control it will be most sensitive to parameter settings and requires much experience.

Note: The transitions between the optimization methods are blurred and the tuning procedure is adapted to the experimental requirements. Often, the adequate tuning of a clamp system can be tested by specific test signals (e.g. stimulus evoked signals, etc.).

Very important: All parameters that influence clamp performance (microelectrode offsets, capacity compensation, etc.) must be optimally tuned before starting the PI controller tuning procedure (see chapter 5). Always activate the OSCILLATION SHUTOFF unit.

The tuning procedure involves the following steps:

Again: The main criterion of tuning is the amount of overshoot seen at the potential output.

Tuning of the proportional gain

- ❑ Use the command input without smoothing and apply adequate, identical pulses to the cell (e.g. small hyperpolarizing pulses).
- ❑ The controller is in P-mode (proportional only). Watch the potential output and rise the GAIN so that no overshoot appears (LO method). The response to a command step is slow and has no overshoot (potential output). The response to a disturbance, e.g. synaptic input or an activating channel, is slow and has a large deviation.

Since the integral part of the controller is disconnected a steady state error in the range of a few percents will be present.

Tuning the integrator (SLOW mode)

- ❑ Reconnect the integrator to form the complete PI controller by setting the voltage clamp control mode switch to SLOW.
- ❑ Apply adequate test pulses without filtering.
- ❑ Adjust the integrator time constant (#**19**) to achieve the overshoot of the selected optimization method (4% with the AVO method and 43 % with the SO method). With the AVO method the response to a command step is very fast with 4% overshoot (potential output). The response to a disturbance, e.g. an activating channel is slow and has a slight deviation. With the SO method the response to an unsmoothed command step is fast with 43% overshoot (potential output). The response to a disturbance, e.g. an activating channel, is very fast and has a slight deviation.

Now the steady-state error must disappear.

Note: If the SO is used, an external command input filter can be used to smooth the command signal and consequently reduce the overshoot according to the requirements of the experiment (see also Figure 6).

Tuning the series resistance compensation (FAST mode)

The optimization methods mentioned above cannot be applied if series resistance is present and has to be considered.
Generally, the upper speed limit for all optimization methods is determined by the maximum amount of current which the clamp system can force through a given electrode. In experimental situations where very high clamp speed is desirable (e.g. recording of gating currents), the clamp speed can be improved additionally by optimizing the position of the electrodes and using SERIES RESISTANCE COMPENSATION (see also Greeff and Polder, 1998; Greeff and Kühn, 2000).

Since SERIES RESISTANCE COMPENSATION is done by positive feedback in the control circuit, its use can lead very quickly to stability problems. Therefore, the clamp speed should be improved first through conventional methods.
The optimal positioning of the electrodes, especially of the current electrode is important for best SERIES RESISTANCE COMPENSATION (see also chapter 0). By placing the electrode in the center of the oocyte, the membrane capacity is charged homogeneously. The capacitive current transient is mono-exponential and the amplifier can be tuned without ringing around the slow tail of the transient (Greeff, 2000).

- ❏ Before the experiment make sure that the electrodes are in optimal position (see Figure 8). If you have a micromanipulator that can remember positions you can first position the electrodes without the oocyte. Then the position is saved and the electrodes are drawn back, the oocyte is placed and the electrodes are brought back into the saved position.

- ❏ Set the voltage clamp control mode switch to FAST.
- ❏ Apply adequate test pulses without filtering.
- ❏ Tune the amount of SERIES RESISTANCE COMPENSATION (#**13**), while watching the current output. The capacitive transient of the current should show a mono-exponential decay. Overcompensation is indicated by a ringing after the first peak of the capacitive transient (see also Figure 7 in chapter 0, left side). This is also a sign that electrode position is not optimal.

9.3. Speed of Response and Linearity of the Capacitive Transients

For the investigation of voltage activated channels with voltage clamp instruments, some special techniques for eliminating the capacitive and leak currents have been introduced, such as the P/4 or more general P/N protocol (see Rudy and Iverson, 1992 for overview). For these protocols the speed and linearity of response of the clamp system is of great importance.

As outlined in chapter 9.1 the TEC systems are designed following a control theory procedure called "modulus hugging" (see Froehr, 1985; Polder, 1984;Polder and Swandulla, 1990, Polder and Swandulla, 2001). The procedure requires a PI (proportional-integral) controller. This procedure is applicable to control systems composed of an element with one "large" time constant T_m and many "small" time constants T_i. These "small" time constants can be added to an "equivalent" time constant T_e.

In case of the TEC control chain the "large" time constant is formed by the time constant of the cell membrane (several hundred of milliseconds) and the sum of "small" time constants resulting from the microelectrodes and the electronics (a few ten microseconds).

Note: Here only the proportional part of the PI controller is considered. Possible improvement of clamp performance due to series resistance compensation (see Ogden, 1994; Smith et al., 1990, Greeff, 2000; Greeff and Kühn, 2000 for details) is not considered.

General Considerations

For the TEC systems the "small" time constants are at least two orders of magnitude below the "large" time constant: The "large" time constant is the time constant of the membrane and the equivalent time constant is composed of the time constants of the electrodes, amplifiers etc.

$T_m = R_m * C_m$, $T_e = \Sigma T_i$ with

T_m = "large time constant
R_m = membrane resistance
C_m = membrane capacity
T_e = "equivalent" time constant
T_i = "small" time constant

The performance of a clamp system can be improved if a voltage controlled current source is used for the current injecting electrode. In this case, the very large time constant (hundreds of milliseconds) formed by the electrode resistance and the cell capacity can be ignored, because the output of the clamp circuit is a current that flows regardless of the resistance of the injecting microelectrode (Smith et al., 1990). Thus, the performance of the clamp is no longer dependent on the electrode resistance (as long as the current source is not saturated). In this case the clamp gain has the magnitude of a conductance [A/V].

The proportional gain of the clamp system can be calculated as follows (Froehr, 1985; Polder, 1984):

$K = C_m / 4 * T_e$ Linear optimum (LO), aperiodic response, no overshoot
$K = C_m / 2 * T_e$ Modulus optimum (MO or AVO, respectively), 4% overshoot, fastest rise time
The optimal gain for a VC experiment is in between these two values. The overshoot can be reduced by low-pass filtering of the command pulse.

The speed of response of the clamp in case of the modulus optimum can be calculated as:
$T_r = 4.7 * T_e$, $T_s = 8.4 * T_e$ with

T_r - time until the membrane potential reaches for the first time 100% of the command pulse
T_s - time to reach steady state within a tolerance of 2%.

T_s is roughly the duration of the capacitive transient. For a system with dampened overshoot T_r approaches T_s.

From these formulas, it is clear that the performance of the clamp is determined by T_e. T_e is determined by the time constant of the current injecting electrode i.e. by the electrode resistance, stray capacities, cable capacities etc. Shielded cables have capacities of 60 - 110 pF / m, connectors and pipette holders add a few picofarads. The potential electrode is equipped with a driven shield and a capacity compensation circuit. Therefore, this time constant is always much smaller than the time constant associated with the current electrode. The time constants of the operational amplifiers are small and can be neglected.

Example

A cable of 10 cm has a capacity of approximately 10 pF, with the stray capacities in the headstage and an electrode resistance of 1 MΩ (cell model). This gives a time constant of 10 – 30 µs (corner frequencies of 5 – 15 kHz). With C_m = 100 nF and T_e = 20 µs (8 kHz bandwidth), the gain can be calculated as:
LO: K = 1.25 mA / V
MO: K = 2.5 mA / V

The standard TEC current source has a calibration of 10 µA / V. This means that the gain stages related to the GAIN control on the front panel must provide a gain between 125-250. In the TEC system the gain amplifier is composed of two stages: x10 (fix) and 1 - 100 (variable). The maximum gain of the variable gain stage can be set with an internal trim potentiometer.

If a command step of 150 mV is applied, the output of the first stage is 1.5V, while the second stage goes into saturation if the gain values calculated above are used. Therefore, the capacitive transients will have large nonlinear components.
A response with no saturation effects is obtainable only with command signals below 100 mV. With larger membrane capacities the saturation effects start even earlier, because a higher gain is required. In this situation, systems with higher output compliance and / or headstage with x2, x5 or x10 ranges must be used to improve clamp response. In this case the saturation effect of the gain amplifier is avoided (Polder and Houamed, 1994; Greeff and Polder, 1997; Polder et al., 1997).

The speed of response (with x1 headstage and 150 V output) from the point of view of control theory is:

$T_r = 94\ \mu s$
$T_s = 168\ \mu s$

Maximum speed of response

The speed of an ideal VC system is limited only by the maximum current delivered by the current source:

$[dUm/dt]_{max} = U_{max} / (C_m * R_{EL})$
$[dUm/dt]_{max} = 150\ V / (0.1\ \mu F * 1\ M\Omega) = 1500\ V / s = 1.5\ mV / \mu s$

It would last 100 µs to reach 150 mV, provided that the clamp has an ideal characteristic.

Now the minimum bandwidth of a real clamp system necessary for "ideal" behavior can be calculated:

$T_s = 8.4 * T_e = 100\ \mu s$ gives $T_e = 12\ \mu s$; $BW = 1 / (2 * \pi * Te) = 13\ kHz$
with BW = bandwidth

If we assume that T_e is determined by 70 - 80% by the time constant of the current electrode (i.e. T_{el} = 10 µs if T_e = 12 µs) it is clear that with electrode resistances in the range of 500 kΩ the total capacity related to the current injecting electrode can be maximum 20 pF. In this case the maximum cable length is 15 - 20 cm.

A cable of 0.5 - 1.5 m has a capacity in the range of 50 - 200 pF. With such a capacity and an electrode resistance of 1 MΩ, T_e is in the range of 50 - 200 µs and the speed of response would be in a range of 0.5 - 2 ms!

Conclusions:

1. For adequate VC experiments a clamp gain of 1 - 5 mA / V (i.e. 100 - 500 internal gain with a current source calibration of 10 µA / V) is necessary. Therefore, with pulse amplitudes of 100 - 200 mV the operational amplifiers in the gain stages will be saturated causing nonlinear components in the capacitive transients.
2. The maximum speed of response is determined by the cell capacity, the maximum available current and the command amplitude.
3. The real speed of response is determined by the time constant associated with the current injecting electrode. It is strongly dependent on the length of the cable that connects the headstage with the electrode holder.

Important: The speed of response and the linearity of the capacitive transients can be improved considerably if a current headstage with a steeper gain (x2 = 20 µA / V, x5 = 50 µA / V) is used especially in combination with a higher output voltage of ±225 V (TEC 225 System) and an improved series resistance compensation (Dietzel et al., 1992; Polder and Houamed, 1994; Greeff and Polder, 1997; Greeff and Kühn, 2000).

10. Technical Data

<u>Modes of Operation</u>

CC:	Current Clamp mode
VC:	Voltage Clamp mode
OFF:	Current- and Voltage Clamp disabled, BA amplifier operational
EXT:	External mode
MODE selection:	by rotary switch

Headstages

Potential headstage

Bridge amplifier headstage is used for potential measurement

Current headstage

Operating voltage: ±45 V
Input resistance: >10^{12} Ω
Electrode connector: BNC, shield is grounded
Ground: 2.4 mm connector or headstage enclosure
Size: 23 x 70 x 26 mm, enclosure grounded
Holding bar: diameter 8 mm, length 10 cm

Current range:

45 µA / 1 MΩ

Current electrode parameter controls:

Offset compensation: ten-turn control, ±500 mV

Electrode Resistance Test:

obtained by application of square current pulses ±10 nA, display XX.X MΩ, selected by switch

Current Outputs:

Filtered output: sensitivity: set by current range switch, with low-pass Bessel filter, output impedance 249 Ω
Current range switch: 0.1 µA/V, 1 µA/V or 10 µA/V
DISPLAY: XX.XX µA

Current Output Filters:

One-pole low-pass Bessel filter
6 corner frequencies: 100, 300, 500, 1k, 3k or 5k Hz.

Current Clamp (current stimulus input from bridge amplifier):

Inputs: 0.1 µA/V, 1 µA/V
Input impedance: >100 kΩ
HOLD: X.XX µA, ten-turn digital control with -/0/+ switch, maximum 10 µA.

Voltage Clamp:

Input sensitivity: ÷10 mV
Input impedance >100 kΩ
HOLD: XXX mV, ten-turn digital control with +/0/- switch, maximum 1000 mV