Electronic Instruments for the Life Sciences

made to measure

OPERATING INSTRUCTIONS AND SYSTEM DESCRIPTION FOR THE

ELC-03XS

UNIVERSAL AMPLIFIER

for EXTRA & INTRACELLULAR RECORDING, SINGLE CELL STIMULATION and ELECTROPORATION

VERSION 3.1
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1. Safety Regulations

VERY IMPORTANT: 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.

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.

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.

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.

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.

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

2. Introduction

"Loose patch" recordings (or "loose seal" recordings [Roberts & Almers, 1992]) are used to record from single excitable cells without damage, i.e. without a direct access to the cell interior. The first recordings were made around 1960 from muscles cells by Alfred Strickholm long time before "tight seal" recording was invented by Erwin Neher and Bert Sakmann twenty years later: "*A method has been developed permitting measurement of membrane impedance and current, as a function of transmembrane potential, at small, electrically isolated regions of the muscle cell surface without microelectrode impalement.*" [Strickholm 1961].
The loose seal has a resistance of a few ten to a few hundred MΩ, and it creates an electrically isolated access to a single neuron. This isolated area can be used for precise recording, stimulation or drug and dye application on the single cell level without damaging the cell [Babour & Isope, 2000]. In contrast to tight seal recordings the same electrode can be reused for recording from several cells, which is a great advantage.
Since its beginnings several attempts have been made to make such precise extracellular methods accessible to various preparations. A nice overview can be found in the chapter by Roberts & Almers [Roberts & Almers, 1992]. Over the years the method was extended to cultured neurons and brain slice preparations, and also for *in vivo* recordings [Bureau et al, 2004]. The method is particularly well suited for long term recording with little damage to the recorded neuron [Nunemaker et al, 2003]. It can be used both for somatic and axonal recording [Khaliq & Raman 2005]. Even subcellular structures such as synaptic boutons are accessible to loose patch recordings [Auger & Marty, 2000].
Another valuable application of this method is single cell stimulation. The high resistance loose patch makes possible the application of 1-2 V stimuli to one cell only [Babour & Isope, 2000].
In the nineties of the last century the method of juxtacellular dye application (juxtasomal filling) became popular [Pinault, 1996]. This staining method is based on repetitive current pulse trains applied in the close vicinity of cell somata or dendrites and is meanwhile well established in the field of slice and *in vivo* preparations [Klausberger, 2004]. Juxtacellular filling together with extracellular measurements are today often summarized under the term "juxtacellular recording".
In parallel attempts were made towards transfection of single cells by electroporation using patch pipettes. DNA or other large molecules were successfully inserted through a patch pipette into living cells by using an optimized protocol (application of 10 V / 1 ms pulse trains) [Rathenberg et al, 2003].
Far in excess of classical *in vivo* recording methods [Lalley et al, 1999] several new approaches are used for monitoring neuronal activity under natural conditions, using new techniques, e.g. the combination of two photon excitation and patch clamp *in vivo* [Helmchen et al, 2001; Stosiek et al, 2003; Brecht et al, 2002]. Assays have been developed that allow to monitor and manipulate single cells under *in vivo* conditions [Brecht et al, 2004]. Besides sophisticated optics these techniques always require precise recording and stimulation amplifiers, mostly based on the use of patch electrodes.

Today three methods are used to record electrical *in vivo* or *in vitro*:

- Recordings using patch (suction) electrodes from single neurons
  - Whole cell patch clamp technique (tight seal recording, intracellular)
  - Loose patch technique (loose seal recording, extracellular)
- Intracellular recordings with sharp microelectrodes
- Extracellular recordings with glass or metal electrodes

The amplifiers used for such recordings are specialized on the recording of the potentials or currents generated by the neurons under investigation. If these recording methods are combined with dye injection, electroporation, stimulation protocols etc. through the recording electrode, serious constraints occur and several additional devices have to be added to the experimental set-up.

The ELC series of amplifiers fills this gap. It allows intracellular, extracellular, voltage –clamp or current clamp recordings both with sharp or patch electrodes as well as additional protocols like electroporation or juxtasomal recordings. Even iontophoresis and voltammetry/amperometry can be performed.

The ELC amplifier is the “Swiss Army Knife” of modern electrophysiology. It is easy to use, versatile, and makes possible a lot of sophisticated experiments with only one instrument.

3. ELC-03XS amplifier

3.1. ELC-03XS Components

The following items are shipped with the system:

- ELC-03XS amplifier
- GND (2.4 mm banana jack) connector and (optional) REF. connector for headstage
- Headstage
- User manual

3.1. Optional Accessories

- Differential headstage
- Miniature headstage
- Cell model
- Pipette holder

3.2. System Description

The ELC-03XS was designed for intra- and extracellular recording, precise (single cell) electrical stimulation as well as juxtasomal filling with patch electrodes. The system consists of an amplifier in a rackmount cabinet and a small headstage with mounting plate or a holding bar. It can be used in slices or in *in vivo* preparations using the optional headstage with a differential input. It has separate capacity compensation controls for VC and CC mode, all controls (Bridge balance, CC cap comp, offset, holding controls) are calibrated 10-turn potentiometers.

The ELC-03XS is capable to record extracellulary DC or AC coupled, to stimulate with current or voltage and to perform non-invasive juxtasomal filling of cells with dyes or DNA. The amplifier can also be used to record and stimulate intracellulary in current clamp (CC) with sharp or patch electrodes, or voltage (patch) clamp (VC) with patch electrodes. It is also suitable for amperometric or voltammetric investigations with carbon fiber electrodes.

Operation modes of the amplifier
The operation modes of the amplifier are selected by a rotary switch with six positions:
The selected mode is indicated by LEDs above the MODE OF OPERATION switch.

EXT:	All MODEs OF OPERATION can be selected by TTL signals connected to the rear panel (see chapter 0).
CC:	CURRENT CLAMP MODE: used to inject predefined current signals
CCx10:	CURRENT CLAMP MODE: for stimulation and electroporation
OFF:	CC Mode with all input signals turned off
VC:	VOLTAGE CLAMP mode: potential commands are applied to the electrode
VCx10:	High range VC mode, for stimulation and electroporation

In addition, using a toggle switch a bridge balance circuit can be activated, to compensate for the electrode artifact (BRIDGE mode, only in CC mode). An ELECTRODE RESISTANCE test mode can be activated with a push button. The electrode resistance is measured directly in MΩ and displayed on the POTENTIAL display.

Input configuration:

The amplifier has two inputs, both for VC and CC mode. The signal applied to the analog input BNCs is converted either into a voltage command signal (x1 or x10 scaling) for the VC or VCx10 voltage clamp modes, or to a current in the CC, CCx10 and BRIDGE mode. Besides this, a signal generated from the 10-turn HOLD potentiometer can be transferred into a pulse using the GATE TTL input BNC. This control can be also used as HOLDING potentiometer if the switch in the GATE BNC is turned off.

Computer control of the mode of operation

In the EXT position of the MODE SELECT switch all MODEs OF OPERATION can be selected by TTL signals connected to the rear panel (see chapter 0).

Output configuration

The ELC-03XS amplifier has two output BNCs for POTENTIAL and one output BNC for the CURRENT signal. The POTENTIAL OUTPUT x1 is a pure DC output that monitors the electrode potential directly from the headstage. The signal at the POTENTIAL OUTPUT can be high and low-pass filtered and amplified.
The current output signal is amplified and can be filtered by a low-pass filter.

Digital displays

All ELC amplifiers are equipped with two digital displays, one for CURRENT (nA) and one for POTENTIAL (mV) or ELECTRODE RESISTANCE (MΩ). The mode of operation is indicated by LEDs located close to the digital displays.

Oscillation shut-off unit

All ELC amplifiers are equipped with an oscillation shut-off unit to protect the preparation. If it is active all outputs of the amplifier were disconnected if the system begins to oscillate.

Penetration unit

All ELC amplifiers are also equipped with a penetration unit (BUZZ) to facilitate the penetration of the cell membrane or to clean clogged electrodes.

3.3. Front Panel View of the ELC-03XS Amplifier

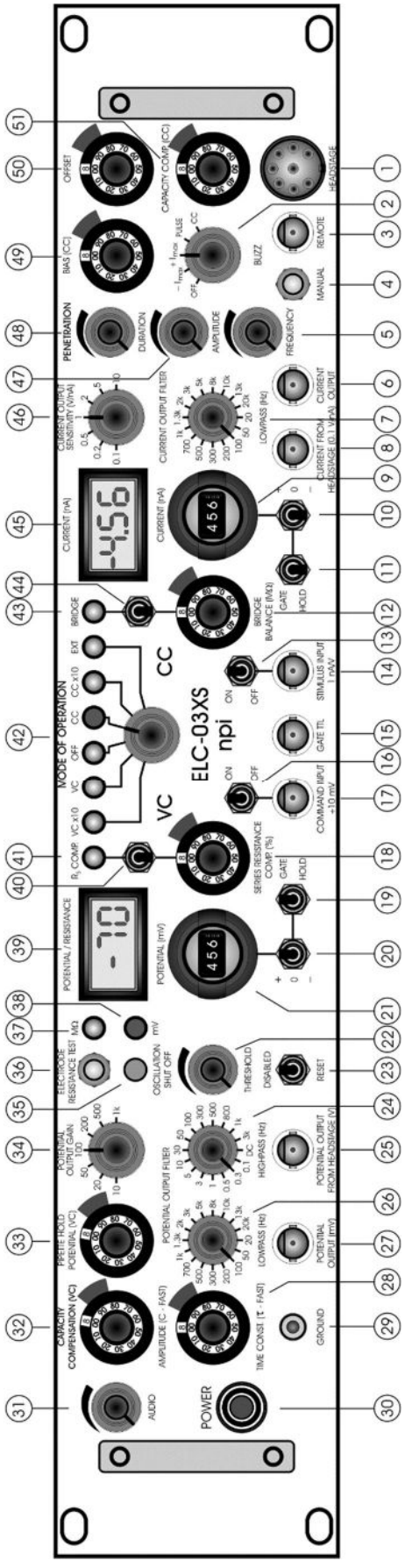

Figure 1: ELC-03XS front panel view

3.4. Description of the Front Panel

In the following description of the front panel elements each element has a number (in bold) 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. Some elements are grouped in functional units (e.g. PENETRATION / BUZZ unit) and are described as units regardless of the order of numbers.

(**1**) HEADSTAGE connector

Connector for the headstage (optionally with differential input). REF of the headstage must be connected to ground (single-ended measurement) or to a reference electrode in the bath (differential measurement), see also Figure 16.

PENETRATION / BUZZ unit

The PENETRATION / BUZZ unit consist of (**2**) mode switch, (**3**) REMOTE connector, (**4**) MANUAL push button, (**5**) FREQUENCY potentiometer, (**47**) AMPLITUDE potentiometer and (**48**) DURATION potentiometer.

(**2**) mode switch

5-position switch to set the mode of PENETRATION

CC: The BUZZ circuit is activated (duration set by **48**). To facilitate the penetration of the cell membrane the BUZZ circuit is provided which is based on oscillations caused by overcompensating the capacitance compensation system. The overcompensation of capacitance compensation yields to very powerful high-frequency (approx. 2 kHz) oscillations (see Figure 2).

PULSE: A pulse train is applied to the electrode. The pulses are positive and rectangular. The AMPLITUDE is set by **47**, the DURATION of the train is set by **48** and the FREQUENCY of pulses within the train is set by **5**.

$+I_{max}$: The maximum positive current is applied to the electrode. The DURATION is set by **48**.

$-I_{max}$: The maximum negative current is applied to the electrode. The DURATION is set by **48**.

$+I_{max}$ or $-I_{max}$ can also be used to clean the tip of the electrode by passing large amounts of positive or negative current. The maximum current is dependent on the setting of the current range, see also (**42**).

OFF: The PENETRATION / BUZZ unit is disabled. When in OFF position the preparation is protected from unintentional use of the unit.

(**3**) REMOTE connector

BNC connector to attach a remote switch in active-low configuration for activating the PENETRATION / BUZZ unit.

(**4**) MANUAL push button

Push button for activating the PENETRATION / BUZZ unit manually. The DURATION is set by **48** and the mode by **2**.

Note: The duration is dependent on the setting of the DURATION potentiometer, but independent from how long the button is pushed or a REMOTE switch is pressed.

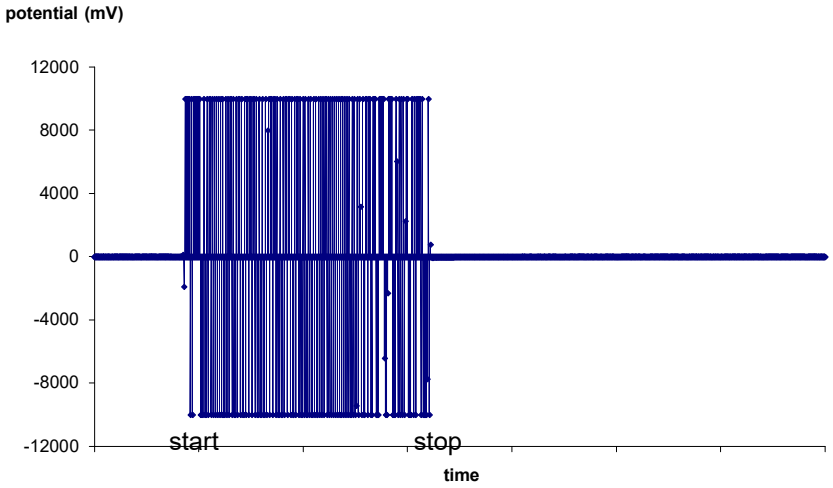

Figure 2: BUZZ function of the ELC-03XS (CC Mode)

(**5**) FREQUENCY potentiometer

Potentiometer to set the FREQUENCY of pulses within a train in PULSE mode (see also **2**). Range: 100 Hz to 2 kHz.

(**47**) AMPLITUDE potentiometer

Potentiometer for setting the AMPLITUDE of pulses in PULSE mode (see also **2**).

(**48**) DURATION potentiometer

Control to set the duration of the BUZZ (potentiometer, clockwise, range: ~1 ms to ~100 ms). It is effective in all modes; REMOTE controlled and when pushing the MANUAL button.

(**6**) CURRENT OUTPUT connector

BNC connector providing the current output signal; scaling is set by CURRENT OUTPUT SENSITIVITY (V/nA) switch **46**, filter is set by CURRENT OUTPUT FILTER LOWPASS (Hz) switch **7**.

(**7**) CURRENT OUTPUT FILTER LOWPASS (Hz) switch

16-position rotary switch to select the corner frequency of the LOWPASS FILTER (range: 20 Hz to 20 kHz) for the CURRENT OUTPUT connector (**6**).

(**8**) CURRENT FROM HEADSTAGE (0.1V/nA) connector

BNC connector providing a voltage proportional to the current at the electrode with a fixed scaling of 100 mV / nA. This current signal is not filtered.

CURRENT set unit

The CURRENT set unit consist of (**9**) CURRENT nA potentiometer, (**10**) +/0/- switch and (**11**) GATE / HOLD switch.

(**9**) CURRENT nA potentiometer

Ten-turn potentiometer for generating a holding current (switch **11** in HOLD position) or a gated stimulus (switch **11** in GATE position) in CC / BRIDGE or CCx10 mode, range: ±10 nA (100 = 1 nA) or ±100 nA (100 = 10 nA), respectively. Polarity is set by the switch **10**.

(**10**) +/0/- switch

Switch for setting the polarity of the holding current or gated current stimulus, respectively (see also **9**). In zero position the holding current or gated current stimulus is disabled.

(**11**) GATE / HOLD switch

Switch that determines the function of the CURRENT nA potentiometer **9**

(**12**) BRIDGE BALANCE MΩ potentiometer

Potentiometer for balancing the BRIDGE circuit; 10 MΩ / turn, range: 0 to 100 MΩ. The BRIDGE must be balanced correctly before using the series resistance compensation in VC mode, because this measure is taken as value for the series resistance compensation (see also **43** and **44**).

(**13**) STIMULUS INPUT switch

Switch for grounding BNC connector **14**. If **14** is not in use, please switch the INPUT off by setting this switch to OFF. This prevents noise pick-up through an open input.

(**14**) STIMULUS INPUT 1 nA/V connector

BNC connector for the current stimulus in CC / BR or in CCx10 mode; scaling 1 nA / V or 10 nA / V, respectively.

Very Important: It is possible to apply 12 V to this input. This is the only way to achieve a stimulus amplitude of ±120 nA!!

(**15**) GATE TTL connector

BNC connector for gating the potential step in VC / VCx10 mode, or the current step in CC / CCx10 mode.

VC modes: As long as the voltage linked to this BNC is HIGH, i.e. +5 V, a voltage stimulus with the amplitude set by the POTENTIAL (mV) potentiometer **21** is generated by the amplifier. Switch **19** has to be in GATE position.

CC modes: As long as the voltage linked to this BNC is HIGH, i.e. +5 V, a current stimulus with the amplitude set by the CURRENT (nA) potentiometer **9** is generated by the amplifier. Switch **11** has to be in GATE position.

(**16**) COMMAND INPUT switch

Switch for grounding BNC connector **17**. If **17** is not in use, please switch the INPUT off by setting this switch to OFF. This prevents noise pick-up through an open input.

(**17**) COMMAND INPUT ÷10 connector

BNC connector for the command potential in VC mode or VCx10 mode; scaling: ÷10 mV or ÷1 mV, respectively.

(**18**) SERIES RESISTANCE COMP. (%) potentiometer

Potentiometer for setting the compensation for the SERIES RESISTANCE (VC mode) in percent of the resistance value that is set at the BRIDGE BALANCE potentiometer **12**. Therefore, it is important to set the BRIDGE BALANCE correctly before using the series resistance compensation (see also **40** and **41**).

Very Important: When using the SERIES RESISTANCE compensation **do not use** the potential values recorded from the POTENTIAL OUTPUT (mV) BNC connector (**27**) or the value shown at the POTENTIAL display (**39**) as a measure for the membrane potential, since these values include also the additional voltage generated by SERIES RESISTANCE COMP. for compensation of the voltage drop at the SERIES RESISTANCE.
For instance, when for calculating I/V curves **do not use** the potential values recorded from the POTENTIAL OUTPUT (mV) BNC connector (**27**). Use the COMMAND potential instead.

COMMAND set unit

The COMMAND set unit consist of (**19**) GATE / HOLD switch, (**20**) +/0/- switch and (**21**) POTENTIAL (mV) potentiometer

(**19**) GATE / HOLD switch

Switch that determines the function of the POTENTIAL (mV) potentiometer **21** (see also **21**).

(**20**) +/0/- switch

Switch for setting the polarity of the holding potential or gated potential stimulus in VC mode, respectively (see also **21**). In zero position the holding potential or gated potential stimulus is disabled.

(**21**) POTENTIAL (mV) potentiometer

Ten-turn potentiometer for generating a holding potential (switch **19** in HOLD position) or a gated stimulus (switch **19** in GATE position) in VC or VCx10 mode; range: ±1 V (100 = 100 mV) or ±10 V (100 = 1 V), respectively. Polarity is set by the switch **20**.

OSCILLATION SHUT OFF unit

The OSCILLATION SHUT OFF unit consist of (**22**) THRESHOLD potentiometer, (**23**) DISABLED / RESET switch and of (**35**) OSCILLATION SHUT OFF LED.

(**22**) THRESHOLD potentiometer

Control to set the activation THRESHOLD of the OSCILLATION SHUT-OFF circuit (potentiometer, linear clockwise, range: 0-1200 mV).

(**23**) DISABLED/RESET switch

Switch to DISABLE the OSCILLATION SHUT-OFF unit or RESET the circuit. RESET is done if one wants to reset the circuit after previous activation. After resetting the OSCILLATION SHUT-OFF unit is active again.

(**35**) OSCILLATION SHUT OFF LED

Indicates whether the OSCILLATION SHUT OFF circuit is active (LED: red) or not (LED: green).

Note: If the OSCILLATION SHUT OFF unit is active and the SHUT OFF was triggered, the output of the amplifier to the headstage is disabled. The amplifier is automatically switched to CC OFF mode and only potential measurement works.

POTENTIAL OUTPUT FILTER

The POTENTIAL OUTPUT FILTER consist of (**24**) HIGHPASS (Hz) and of (**26**) LOWPASS (Hz) and is used mainly for filtering extracellular signals.

(**24**) HIGHPASS (Hz) switch

16-position rotary switch to select the corner frequency of the HIGHPASS filter (range: DC to 3 kHz) for the POTENTIAL OUTPUT (mV) connector **27**. In DC position the HIGHPASS is disabled.

(**26**) LOWPASS (Hz) switch

16-position rotary switch to select the corner frequency of the LOWPASS filter (range: 20 Hz to 20 kHz) for the POTENTIAL OUTPUT (mV) connector **27**.

(**25**) POTENTIAL OUTPUT FROM HEADSTAGE (V) connector

BNC connector providing the POTENTIAL at the electrode from the headstage. This POTENTIAL OUTPUT is not filtered and fixed scaled (x1 V).

(**27**) POTENTIAL OUTPUT (mV) connector

BNC connector providing the POTENTIAL at the electrode. The scaling is set by POTENTIAL OUTPUT GAIN switch **34**. This POTENTIAL OUTPUT is filtered by (**26**) LOWPASS and (**24**) HIGHPASS FILTER.

CAPACITY COMPENSATION (VC) unit

The CAPACITY COMPENSATION (VC) unit consist of (**28**) TIME CONSTANT (τ-FAST) potentiometer and of (**32**) AMPLITUDE (C-FAST) potentiometer. It compensates for the electrode capacity and functions only in VC mode. Tuning is done by application of voltage pulses to the electrode and trying to get the resulting current pulses as fast as possible using potentiometers **28** and **32**.

(**28**) TIME CONSTANT (τ-FAST) potentiometer

Potentiometer for setting τ-FAST in the electrode capacity compensation circuit.

(**32**) AMPLITUDE (C-FAST) potentiometer

Potentiometer for setting C-FAST in the electrode capacity compensation circuit.

Note: CAPACITY COMPENSATION works only for the electrode capacity, not for the capacity of the cell membrane. Therefore, capacitive transients are always present when square shaped pulses are applied in VC mode.

(**29**) GROUND connector

Banana jack providing system GROUND. System GROUND is not connected to the chassis or to protective earth, respectively.

(**30**) POWER switch

Push button to switch the amplifier ON (pushed) or OFF (released).

(**31**) AUDIO volume potentiometer

Potentiometer for setting the volume of the AUDIO monitor for POTENTIAL. Turning the potentiometer clockwise increases the volume.

(**33**) PIPETTE HOLD POTENTIAL (VC)

Ten-turn potentiometer setting the current through the pipette to zero when approaching a cell in VC mode, i.e. the pipette is clamped to its OFFSET potential resulting in zero current flow through the pipette. In VCx10 mode the pipette hold potential does not change. Range is ±100 mV and zero position is 5, i.e. the same as the OFFSET potentiometer **50**. This is very convenient since this adjustment can be done starting with the same value as in **50**.

(**34**) POTENTIAL OUTPUT GAIN switch

7-position switch for selecting the amplification for the signal at (**27**) POTENTIAL OUTPUT (mV); range: x10 to x1000.

(**36**) ELECTRODE RESISTANCE TEST push button

Push button for activating the ELECTRODE RESISTANCE TEST. The ELECTRODE RESISTANCE is determined by application of ±1 nA square pulses to the electrode and is shown in MΩ at display **39**. Function is indicated by the MΩ LED **37**. The ELECTRODE RESISTANCE TEST works in OFF mode as well.

Hints: This function can also be used to adjust (**51**) CAPACITY COMP. (CC). The POTENTIAL OUTPUT FILTER LOWPASS (**26**) should be set to 20 kHz and the HIGHPASS (**24**) to DC. With an amplifier with switchable headstage (ELC-SWI) the electrode resistance test works also in enhanced (x100) current mode.

Note: If the ELC-03XS is equipped with the optional SEAL TEST circuit, the push button is replaced by a three position switch. In the upper position the ELECTRODE RESISTANCE TEST is carried out and in the lower position the SEAL resistance test is accomplished. The value of the SEAL resistance is shown at the CURRENT display (**45**).

(**37**) MΩ LED

LED indicating that the unit of the POTENTIAL/RESISTANCE display (**39**) is MΩ.

(**38**) mV LED

LED indicating that the unit of the POTENTIAL/RESISTANCE display (**39**) is mV.

(**39**) POTENTIAL / RESISTANCE display

Display for the potential at the electrode in ±XXXX mV (±1999 mV max.) or the electrode resistance in XXX MΩ (999 MΩ max.). The unit is indicated by **37** or **38**.

SERIES RESISTANCE COMPENSATION unit

The SERIES RESISTANCE COMPENSATION unit consist of (**18**) SERIES RESISTANCE COMP. (%) potentiometer, (**40**) R_S COMP. switch and (**41**) R_S COMP. LED

(**18**) SERIES RESISTANCE COMP. (%) potentiometer

Potentiometer for setting the compensation for the SERIES RESISTANCE in percent of the value that is set at the BRIDGE BALANCE potentiometer **12**. Therefore, it is important to set the BRIDGE BALANCE correctly before using the series resistance compensation to avoid oscillations (see also **40** and **41**).

(**40**) R_S COMP. switch

Switch for activating the SERIES RESISTANCE COMPENSATION circuit. The active circuit is indicated by **41**.

(**41**) R_S COMP. LED

LED indicating that the SERIES RESISTANCE COMPENSATION circuit is active. The amount of SERIES RESISTANCE COMPENSATION is set by **18**.

Very Important: When using the SERIES RESISTANCE compensation **do not use** the potential values recorded from the POTENTIAL OUTPUT (mV) BNC connector (**27**) or the value shown at the POTENTIAL display (**39**) as a measure for the membrane potential, since these values include also the additional voltage generated by SERIES RESISTANCE COMP. for compensation of the voltage drop at the SERIES RESISTANCE.
For instance, when for calculating I/V curves **do not use** the potential values recorded from the POTENTIAL OUTPUT (mV) BNC connector (**27**). Use the COMMAND potential instead.

(**42**) MODE OF OPERATION switch and LEDs

Switch for selecting the MODE OF OPERATION

VC x10: the amplifier operates in Voltage (Patch) Clamp mode. The COMMAND POTENTIAL is enhanced by a factor of ten.
VC: the amplifier operates in Voltage (Patch) Clamp mode

Important: VC modes do not function properly with sharp microelectrodes, i.e. electrodes with more than 10 MΩ resistance.

OFF: all inputs of the amplifier are switched OFF, and the amplifier is set to CC mode. R_{EL} test and potential measurement work
CC: the amplifier operates in Current Clamp mode
CC x10: the amplifier operates in Current Clamp mode. The STIMULUS amplitude is enhanced by a factor of ten.
EXT: the amplifier is set to CC mode. R_S, R_{EL} test, BR, x10 or VC / CC modes can be selected by application of a TTL HIGH (+5 V) signal to the respective BNC at the rear panel (see also chapter 0).

The MODE OF OPERATION that is currently activated, is indicated by the respective LED above the switch.

BRIDGE unit

The BRIDGE unit consist of (**12**) BRIDGE BALANCE MΩ potentiometer, (**43**) BRIDGE MODE LED and (**44**) BRIDGE MODE switch.

(**12**) BRIDGE BALANCE MΩ potentiometer

Potentiometer for balancing the BRIDGE circuit; 10 MΩ / turn, range: 0 to 100 MΩ. The BRIDGE must be balanced correctly before using the series resistance compensation in VC mode, because this measure is taken as value for the series resistance compensation (see also **43** and **44**).

(**43**) BRIDGE MODE LED

LED indicating that the BRIDGE circuit is active.

(**44**) BRIDGE MODE switch

Switch for activating the BRIDGE circuit. The active circuit is indicated by **43**.

(**45**) CURRENT (nA) display

Display for the current at the electrode in ±XX.XX nA, i.e. 10.00 is 10 nA (19.99 nA max.)

Note: If the ELC-03XS is equipped with the optional SEAL TEST circuit, the value of the SEAL resistance is also shown at the CURRENT display in XX.XX GΩ (19.99 GΩ max.).

(**46**) CURRENT OUTPUT SENSITIVITY (V/nA) switch

7-position switch for selecting the amplification of the current output signal in V/nA; range 0.1 V/nA to 10 V/nA.

(**49**) BIAS (CC) potentiometer

Ten-turn potentiometer for BIAS current cancellation of the headstage; (ten-turn potentiometer, symmetrical, i.e. 0 pA = 5 on the dial), range: ±100 pA.

(**50**) OFFSET potentiometer

Ten-turn potentiometer for OFFSET cancellation of the electrode; (ten-turn potentiometer, symmetrical, i.e. 0 mV = 5 on the dial), range: ±100 mV.

(**51**) CAPACITY COMP. (CC) potentiometer

Ten-turn potentiometer for the capacity compensation of the electrode in CC mode (ten turn potentiometer, clockwise, range: 0-30 pF).

4. Description of the Rear Panel

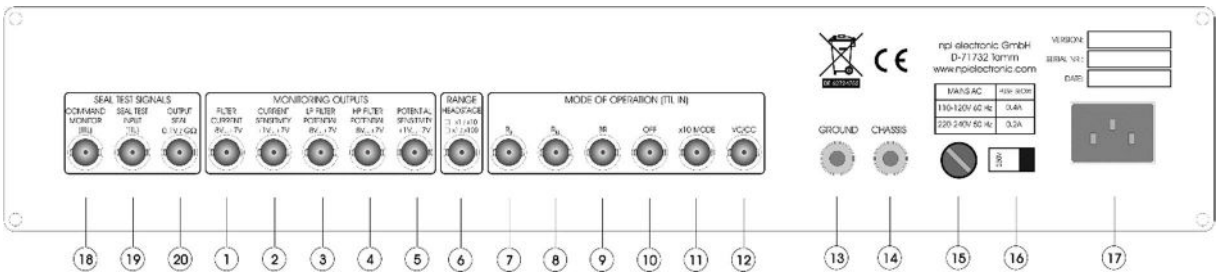

Figure 3: ELC-03XS rear panel view

MONITORING OUTPUT connectors

(**1**) FILTER CURRENT connector

BNC connector providing a voltage monitoring the position of the CURRENT FILTER switch (-7 V to +8 V, 1V/STEP).

(**2**) CURRENT SENSITIVITY connector

BNC connector providing a voltage monitoring the position of the CURRENT OUTPUT SENSITIVITY switch (+1 V to +7 V, 1V/STEP).

(**3**) LP FILTER POTENTIAL connector

BNC connector providing a voltage monitoring the position of the POTENTIAL LOWPASS FILTER switch (-8 V to +7 V, 1V/STEP).

(**4**) HP FILTER POTENTIAL connector

BNC connector providing a voltage monitoring the position of the POTENTIAL HIGHPASS FILTER switch (-8 V to +7 V, 1V/STEP).

(**5**) POTENTIAL SENSITIVITY connector

BNC connector providing a voltage monitoring the position of the POTENTIAL OUTPUT GAIN switch (+1 V to +7 V, 1V/STEP).

RANGE connector

(**6**) HEADSTAGE connector

BNC connector for remote control of a switchable headstage (ELC-SWI, optional). A TTL HI (+5 V) signal switches the feedback resistance in the switchable headstage from the standard feedback resistor to the alternative feedback resistor. The value of the resistor is indicated on the headstage.

MODE SELECT connectors (see also chapter 3.4)

All MODEs OF OPERATION can be selected by TTL signals connected to the rear panel (see below), if the MODE OF OPERATION switch (**42**, Figure 1**)** is in EXT position. This is very convenient when switching often between electroporation and recording, because this can be done automatically by the data acquisition system using TTL signals.

(**7**) R_S connector

BNC connector for remote control of the series resistance compensation circuit. A TTL HI (+5 V) signal is connected here to activate the series resistance compensation circuit remotely.

(**8**) R_{EL} connector

BNC connector for remote control of the electrode resistance test. A TTL HI (+5 V) signal can be connected here to select the electrode resistance test remotely.

(**9**) BR connector

BNC connector for remote control of the bridge mode. A TTL HI (+5 V) signal can be connected here to select the bridge mode remotely.

(**10**) OFF connector

BNC connector to switch the ELC-03XS in OFF mode remotely with a TTL HI (+5 V) signal.

(**11**) x10 MODE connector

BNC connector to switch the ELC-03XS to x10 mode of operation remotely (TTL HI signal). Dependent on the signal level at **12**, this is CCx10 (signal at **12** = LOW) or VCx10 (signal at **12** = HI).

(**12**) VC / CC connector

BNC connector for remote control of the VC / CC mode of operation. A TTL signal can be connected here to select the mode of operation remotely (HI = VC, LO = CC).

(**13**) GROUND connector

Banana plug providing internal ground (see below).

(**14**) CHASSIS connector

Banana plug providing mains ground (see below).

(**15**) FUSE holder

Holder for the line fuse. For changing the fuse rotate the holder counterclockwise using a screw driver.

(**16**) LINE SELECT switch

Switch for selecting the line voltage. Switch to the right for 230 V AC, to the left for 115 V AC. The selected voltage is indicated on the switch.

Caution: Before turning on the instrument, make sure that the correct line voltage is selected.

(**17**) Mains connector

Plug socket for the mains power-plug.

Important: Check line voltage before connecting the ELC amplifier to power. Always use a three-wire line cord and a mains power-plug with a protection contact connected to ground. Disconnect mains power-plug when replacing the fuse or changing line voltage. Replace fuse only by appropriate specified type (one spare fuse is supplied). Before opening the cabinet unplug the instrument.

SEAL TEST SIGNALS connectors (optional)

The SEAL resistance is determined similar to the R_{EL} test.
±10 mV square pulses with 15 Hz are applied to the pipette and the resulting current is measured. The resistance is calculated according to Ohm's law and is indicated on the CURRENT DISPLAY. The maximum SEAL resistance that can be displayed is 19.99 GΩ. The value of the SEAL resistance is also monitored at **20** (see below).

(**18**) COMMAND MONITOR (TTL) connector

BNC connector providing a TTL (+5V) signal synchronous to the ±10 mV test pulses.

(**19**) SEAL TEST INPUT (TTL) connector

Starts seal test remotely (see also **36**, Figure 1).

(**20**) OUTPUT SEAL 0.1V / GΩ connector

BNC connector monitoring the value of the SEAL resistance; scaling 100 mV / GΩ.

Grounding

ELC instruments have two ground systems:

1. the internal ground (called internal GROUND) represents the zero level for the recording electronics and is connected to the recording chamber and the BNC input/output sockets
2. mains ground (CHASSIS) is connected to the 19" cabinet and through the power cable to the protection contact of the power outlet.

Both grounds are provided at the rear panel:

GROUND (black socket):	internal system ground
CHASSIS (green/yellow socket):	mains ground, 19" cabinet

All ELC systems have a high quality toroid transformer in order to minimize stray fields. In spite of this, noise problems could occur if other mains-operated instruments are used in the same setup. The internal system ground (GROUND sockets) should be connected to only one point on the measuring ground. Multiple grounding should be avoided, i.e. all ground points should originate from a central point to avoid ground loops.

5. Setting up the ELC-03XS

The following steps should help you set up the ELC-03XS correctly. Always adhere to the appropriate safety measures (see chapter 1).

After unpacking, the ELC-03XS is attached to the setup by assembling the electrical connections. It is assumed that first a cell model will be attached.

א Electrical connections

- o Turn POWER off.
- o Plug the power cord of the instrument into a grounded outlet.
- o Connect the headstage to the HEADSTAGE connector (#**1**, Figure 1) at the ELC-03XS.
- o Connect a cell model (see chapter 6). Connect a digital/analog timing unit or a stimulation device to STIMULUS INPUT or to GATE TTL if you intend to use a gated stimulus.
- o Connect a store oscilloscope or a data acquisition system to the POTENTIAL OUTPUT and to the CURRENT OUTPUT triggered from the stimulation device. Set the desired gain at the POTENTIAL OUTPUT GAIN switch (#**34**, Figure 1) and the CURRENT OUTPUT SENSITIVITY switch (#**46**, Figure 1).

Before using the ELC-03XS always make the basic settings to avoid oscillations.

ℑ Basic settings

- o Turn all controls to low values (less than 1) and the OFFSET and BIAS controls in the range of 5 (zero position, see chapter 3.4).
- o Set the MODE OF OPERATION switch (#**42**, Figure 1) to CC.
- o Turn POWER switch on.

Now the ELC-03XS is ready for an initial check with the cell model.

6. Passive Cell Model

The ELC-03XS can be ordered with a passive cell model as an optional accessory. An active cell model is also available on request (for ref. see Draguhn et al. (1997)).
The passive cell model is designed for use with single electrode amplifiers (BA series, ELC series) to check the function of the instrument in the following circumstances:

1. just after unpacking to see whether the instrument has been damaged during transport or
2. to train personnel using the instrument or
3. in case of trouble to check which part of the setup does not work correctly, e.g. to find out whether the amplifier or headstage is damaged, or something is wrong with the electrodes or holders etc.

The passive cell model consists only of passive elements, i.e. resistors that simulate the resistance of the cell membrane and the electrodes, and capacitances that simulate the capacitance of the cell membrane. A switch allows simulation of two different cell types: a cell with 50 MΩ and 22 pF (CELL 1, represents an astrocyte like cell) or a “small” cell with 200 MΩ membrane resistance and 100 pF membrane capacitance (CELL 1, represents a neuron like cell). Electrode immersed into the bath or SEAL formation can be mimicked as well. The headstage of the amplifier can be connected to one of two different types of electrodes (see below).

6.1. Cell Model Description

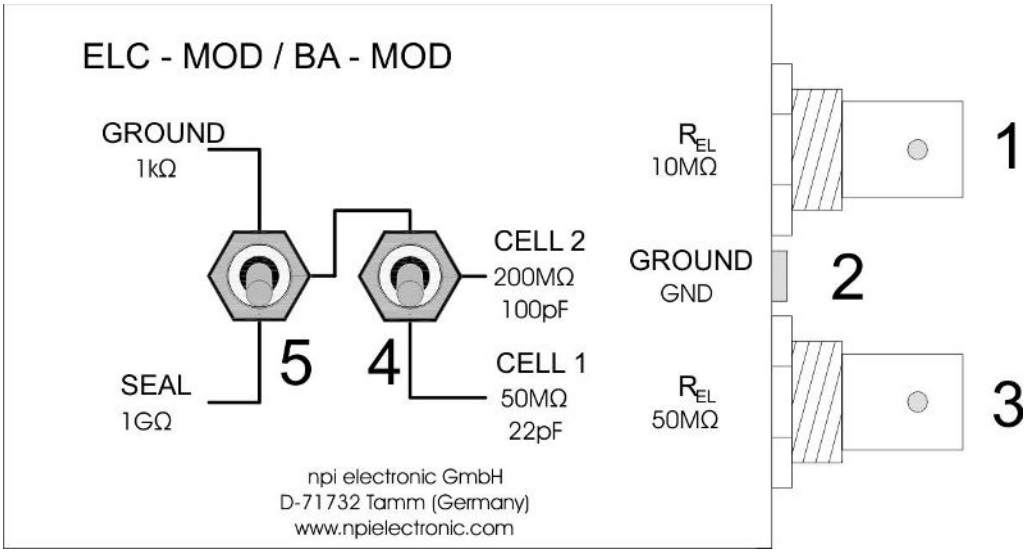

Figure 4: passive cell model

1, 3: connectors for the headstage, **1**: electrode resistance: 50 MΩ, **3**: electrode resistance: 10 MΩ

2: GND ground connector, to be connected to GND jack of the headstage

4: CELL: switch for cell membrane representing a membrane of either 50 MΩ and 22 pF (CELL 1) or 200 MΩ and 100 pF (CELL 2). In upper position electrodes are connected to ground or to a 1 GΩ resistor (see below)

5: In GROUND (upper) position the electrodes are connected to ground via a 1 kΩ resistor. In SEAL (lower) position are connected to a 1 GΩ resistor simulating the formation of a GIGASEAL with a patch electrode.

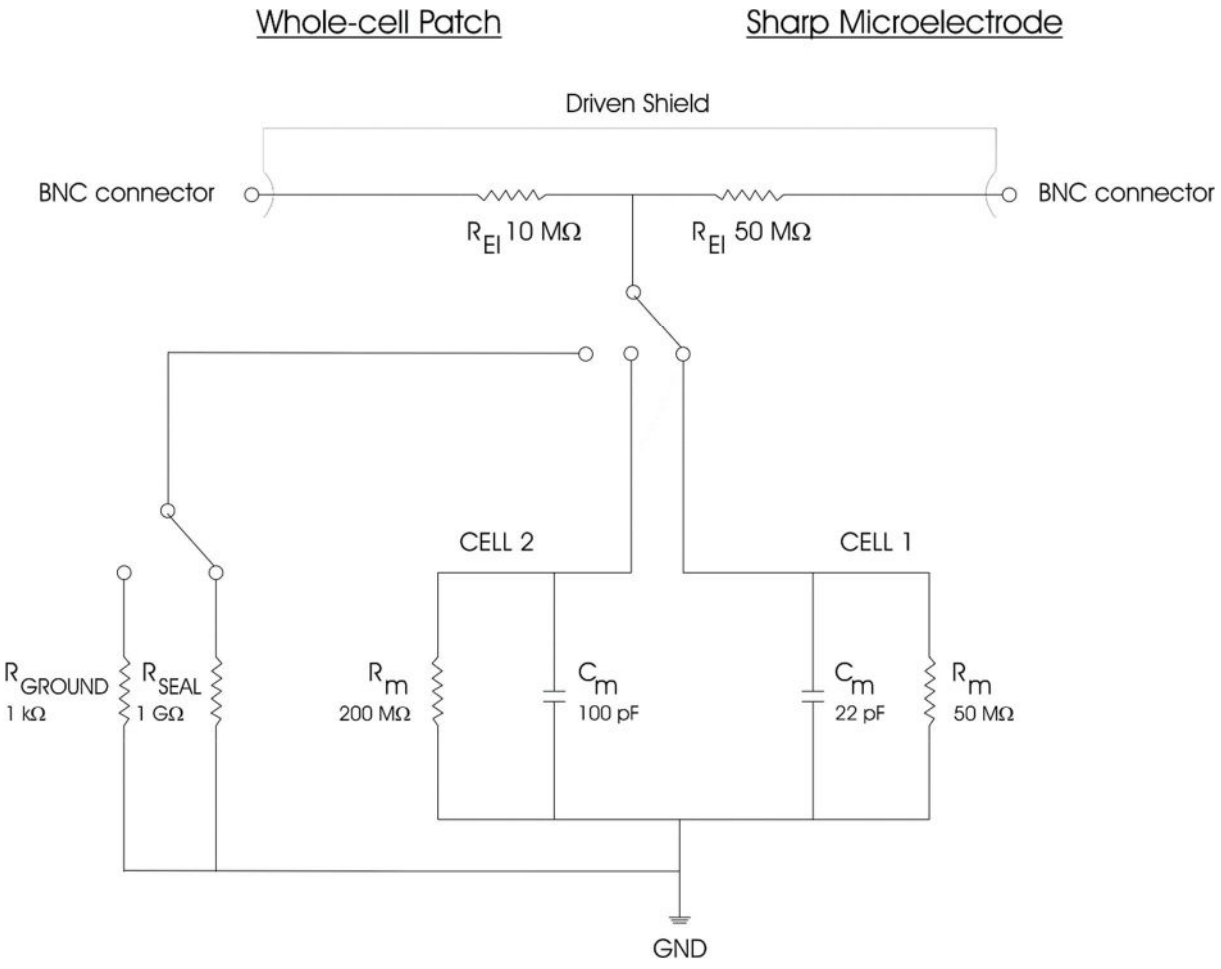

Figure 5: Schematic diagram of the passive cell model

6.2. Connections and Operation

It is assumed that all connections are built as described in chapter 5.

Checking the configuration

o Turn POWER switch of the amplifier off.

a) For simulation of an experiment using a suction electrode

- Connect the BNC jack labeled 10MΩ of the cell model to the BNC connector P_{EL} of the headstage.

b) For simulation of an experiment using a sharp electrode

- Connect the BNC jack labeled 50MΩ of the cell model to the BNC connector P_{EL} at the headstage. For headstages with SMB connector use the supplied SMB to BNC adapter.

For a) and b)

- Connect GND of the cell model to GND of the headstage.

Important: When using the **differential headstage (optional) the REF connector** must not be left open. It must be connected to ground.

Simulation of electrode in the bath

- Set switch #**4**, Figure 4 to the upper position.
- Set switch #**5**, Figure 4 to GROUND position. The 1 kΩ resistor simulates the resistance of the bath solution. This can be used to train cancellation of offsets, using the bridge balance and using the capacity compensation.

Simulation of SEAL formation

- Set switch #**4**, Figure 4 to the upper position.
- Set switch #**5**, Figure 4 to SEAL position. The 1 GΩ resistor simulates the SEAL resistance when forming a GIGASEAL in patch clamp experiments.

Simulation of intracellular recording

Intracellular recordings can be mimicked with one of two cells with different properties. Use the 50 MΩ electrode connector (#**3**, Figure 4) for an experiment with sharp electrodes or the 10 MΩ electrode connector (#**1**, Figure 4) for simulating an experiment with patch electrodes.

- Switch the CELL membrane switch (see #**4**, Figure 4) to the desired position (CELL 1 or CELL 2).
- Turn all controls at the amplifier to low values (less than 1) and the OFFSET in the range of 5 (zero position) and the OSCILLATION SHUTOFF in the DISABLED position.
- Turn POWER switch of the amplifier on.

Now you can adjust the amplifier (see below) and apply test pulses to the cell model. The lower position of the CELL membrane switch (CELL 1) simulates a cell with a resistance of 50 MΩ and a capacitance of 22 pF. In the middle position (CELL 2) a cell membrane with 200 MΩ and 100 pF is simulated.

7. Headstage

The ELC-03XS comes with a headstage for connecting suction electrodes for loose-patch clamp or whole cell recordings and / or stimulation or electroporation, respectively or sharp electrodes for intracellular or extracellular recordings. The use of metal electrodes is possible as well.
A differential (miniature) headstage (see **Optional accessories** in chapter 3.1) for measurements *in vivo* is also available (see also chapter 10.1 and contact npi for details).

7.1. Headstage Elements

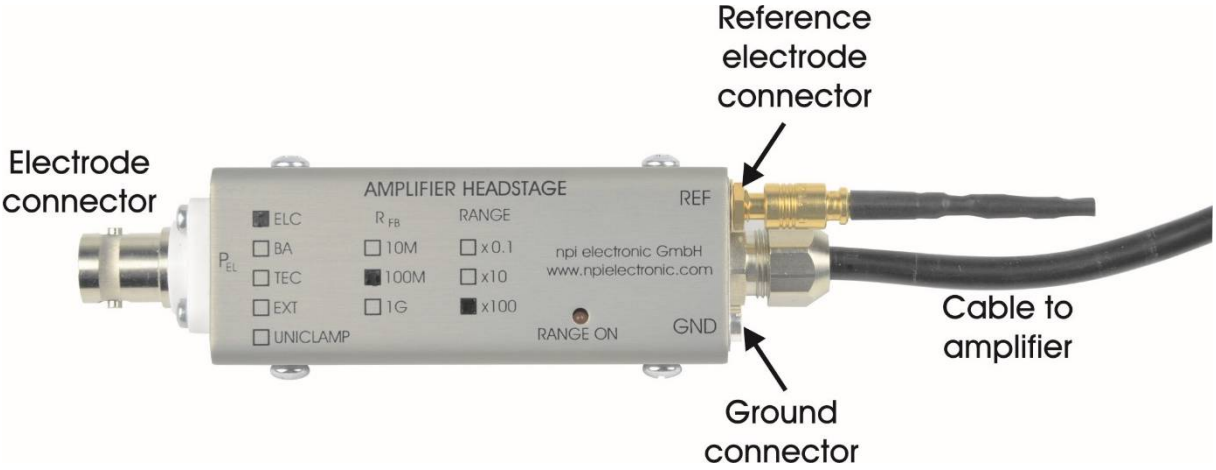

Figure 6: ELC-03XS headstage

- P_{EL} BNC connector for the electrode holder
- REF Connector for the reference electrode (differential headstage only)
- GND Ground connector
- TYPE Type of amplifier, BA → Bridge amplifier, ELC → ELC amplifier, etc.
- R_{FB} Value of standard feedback resistor (RANGE: OFF); 10M: 10 MΩ, 100M: 100 MΩ, 1G: 1 GΩ
- RANGE (optional) current range in switchable headstage (ELC-SWI)
- RANGE-ON LED indicating that the current range switch is active (see chapter 7.1)

The electrode filled with electrolyte is inserted into an electrode holder (optional) that fits into the BNC connector of the headstage or into an electrode holder adapter. The electrical connection between the electrolyte and the headstage is established using a carefully chlorinated silver wire. Chlorinating of the silver wire is very important since contact of silver to the electrolyte leads to electrochemical potentials causing varying offset potentials at the electrode, deterioration of the voltage measurement etc. (for details see Kettenmann and Grantyn (1992)). For optimal chlorinating of sliver wires an automated chlorinating apparatus (ACl-01) is available (contact npi for details).
GND provides system ground and is linked to the bath via an agar-bridge or an Ag/AgCl pellet. The headstage is attached to the amplifier with the headstage cable and a 12-pole connector. The headstage can be mounted directly to a micromanipulator using a mounting plate, a dove tail or a holding bar.

Important: The shield of the BNC connector is linked to the driven shield output and must not be connected to ground. The headstage enclosure is grounded.

Caution: Please always adhere to the appropriate safety precautions (see chapter 1). Please turn power off when connecting or disconnecting the headstage from the HEADSTAGE connector!

7.1. Headstage with switchable current ranges (ELC-SWI, optional)

As an option, the ELC-03XS amplifier can be ordered with switchable current ranges. This is realized by changing the headstage feedback resistance from standard 100 MΩ (= x1) to a different value. This enables the amplifier to generate either higher currents or voltages with low resistance electrodes, e.g. for electroporation, or to record smaller currents in a more sensitive resolution. This works both in current clamp and in voltage clamp mode.
The ELC-03XS automatically recognizes the type headstage which is connected. The displays and scaling factors will be adapted internally (see also Table 1).

Figure 7: ELC-03XS with switchable headstage, front panel view

The extended current range can be set at the front panel of the ELC-03XS with an additional switch labeled x1 / RANGE or with a TTL signal applied to the RANGE HEADSTAGE INPUT (TTL) BNC connector at the rear panel of the amplifier (see also chapter 0). If the switch is set to RANGE the feedback resistor in the headstage is changed and the current range changes accordingly (see Table 1). This mode is indicated at the headstage by an additional LED (RANGE ON, see Figure 8).

Important: In the VC x10 range which is usually used for generating high amplitude voltage pulses suitable for electroporation of large molecules the x10 or x100 current range is automatically activated allowing for instance 10 V pulses with 1 MΩ electrodes. Note that the current output scaling changes accordingly!

Note: All basic operations and settings (e.g. bridge balance or BIAS adjustment) should be performed in standard x1 mode, i.e. RANGE switch set to x1, because these are also affected by the different RANGE modes.

If the ELC-03XS operates in the RANGE mode with alternative feedback resistor, all current related signals change accordingly. An overview is shown in Table 1. The CURRENT DISPLAY shows always nA, scaling is adapted automatically. Also BRIDGE BALANCE and RESISTANCE TEST are automatically adapted to show the correct values.

Important: Scaling of CURRENT OUTPUT SENSITIVITY and CURRENT FROM HEADSTAGE applies also to operation in VCx10 mode since the headstage is switched automatically into the respective RANGE mode!

Figure 8: Current RANGE switch (left) and ELC switchable headstage (right).

Table 1: Scaling overview with different switchable headstages.

Headstage range	**x 0.1**	**x 1**
Feedback resistor	1 GΩ	100 MΩ
Current Output	1, 2, 5, 10, 20, 50, 100 V/nA	0.1, 0.2, 0.5, 1, 2, 5, 10 V/nA
Current Output from Headstage	1 V/nA	0.1 V/nA
Potential Output	10, 20, 50, 100, 200, 500, 1k V/V	10, 20, 50, 100, 200, 500, 1k V/V
Potential Output from Headstage	1 V/V	1 V/V
(Current) Stimulus Input	0.1 nA/V (1 nA/V in CCx10)	1 nA/V (10 nA/V in CCx10)
(Voltage) Command Input	÷10 (÷1 in VCx10)	÷10 (÷1 in VCx10)
Current Potentiometer (max.)	± 1 nA (±10 nA in CCx10)	± 10 nA (±100 nA in CCx10)
Voltage Potentiometer (max.)	± 1 V (± 10 V in VCx10)	± 1 V (± 10 V in VCx10)

Headstage range	**x 10**	**x 100**
Feedback resistor	10 MΩ	1 MΩ
Current Output	0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1 V/nA	0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1 V/nA
Current Output from Headstage	0.01 V/nA	0.001 V/nA
Potential Output	10, 20, 50, 100, 200, 500, 1k V/V	10, 20, 50, 100, 200, 500, 1k V/V
Potential Output from Headstage	1 V/V	1 V/V
(Current) Stimulus Input	10 nA/V (100 nA/V in CCx10)	100 nA/V (1 µA/V in CCx10)
(Voltage) Command Input	÷10 (÷1 in VCx10)	÷10 (÷1 in VCx10)
Current Potentiometer (max.)	± 100 nA (±1 µA in CCx10)	± 1 µA (±10 µA in CCx10)
Voltage Potentiometer (max.)	± 1 V (± 10 V in VCx10)	± 1 V (± 10 V in VCx10)

8. Test and Tuning Procedures

Important: The ELC-03XS 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 to first connect a cell model to the amplifier to perform some basic adjustments and to get familiar with these procedures. It is assumed that all connections are built as described in chapter 5.

Important: Except for *Headstage bias current adjustment* (see 8.1) all adjustments described below should be carried out every time before starting an experiment or after changing the electrode.

8.1. 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 ELC-03XS is equipped with a voltage-to-current converter with a very high output impedance which is connected to the recording electrode. The zero current of this unit is tuned with the BIAS current potentiometer (#**49**, Figure 1).
The tuning procedure should be performed regularly (at least once a month) since the BIAS current changes over time.

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

- Disconnected **all** input signals (except the headstage). Put the CURRENT (nA) switch (**10**, Figure 1) to position 0. Set the operation mode to CC.
- Set the BIAS control potentiometer to 5 (zero position).
- Connect the P_{EL} connector of the headstage to ground.

  Note: This cannot be done with the cell model. Please use a wire to connect the input of the BNC connector on the headstage to GND of the headstage. Do not use the shield of the BNC connector since it is connected to driven shield.
- Tune the OFFSET to zero using the OFFSET control.

  Remember: The zero position of the OFFSET control is at 5!!
- Remove the wire and attach the cell model or a resistor with a value of about 5 MΩ across the same connection.
- The value displayed at the POTENTIAL DISPLAY is related to the BIAS current of the headstage according to Ohm's Law. Cancel this voltage by tuning the headstage BIAS current potentiometer until the POTENTIAL DISPLAY shows 000.

8.2. 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 zero carefully with the OFFSET control (#**16**, Figure 1) before recording from a cell. When adjusting the OFFSET make sure that no current flows through the electrode. Thus, it is recommended to disconnect CURRENT INPUT and to disable GATE (TTL) STEP SIZE and the HOLDING CURRENT unit (see chapter 3.3).

If a cell model is connected the OFFSET control should read a value around 5, otherwise it is likely that the headstage or the amplifier is damaged.

8.3. Capacitance Compensation

High resistances of electrodes and stray capacitances (C_{stray}) form a low-pass filter which deteriorates the shape of recorded intracellular signals (see also Figure 11). The frequency response (bandwidth) of the amplifier is improved considerably by using the capacitance compensation function. This function is based on positive feedback ("negative capacitance") circuit. The tuning of the capacitance compensation control is performed using pulses applied to the CURRENT INPUT or pulses provided by the electrode resistance test circuit.

With the cell model connected or the electrode in the bath the CAPACITY COMP. control is turned clockwise until there is no artifact on the POTENTIAL OUTPUT (see Figure 9).

- Make the basic settings at the amplifier (see chapter 5).
- Connect a cell model or immerse the electrode into the bath as deep as necessary during the experiment.
- Tune the OFFSET to zero (see chapter 0)
- Push the ELECTRODE RESISTANCE TEST button (#**36**, Figure 1) or apply pulses to the CURRENT STIMULUS INPUT and watch the POTENTIAL OUTPUT.
- Compensate the input capacitance as shown in Figure 9 using the CAPACITY COMP. potentiometer (#5**1**, Figure 1).

Figure 9 illustrates the capacitance compensation procedure using a 100 MΩ resistor that represents the electrode. The pulses were generated using the automated electrode resistance test circuit of the ELC-03XS. The upper diagram shows an undercompensated capacitance. In the diagram in the middle the capacitance is slightly overcompensated and in the lower diagram it is well compensated.

Important: The capacity compensation for the electrode does NOT work if the oscillation shut-off circuit is activated. This may lead to an incorrect reading of the electrode resistance (see also **36**) especially when electrodes with high resistances are used.

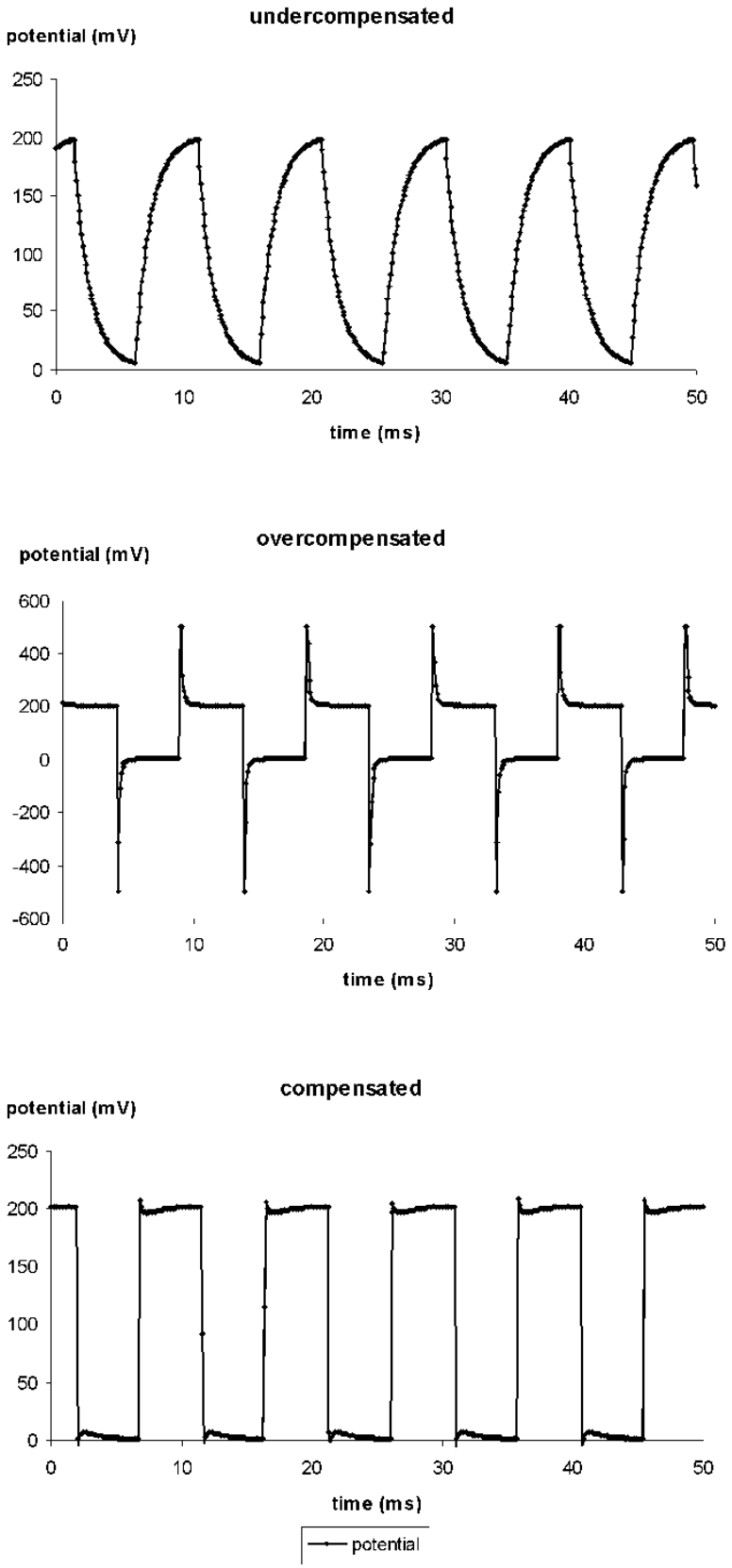

Figure 9: Tuning of the capacitance compensation using a 100 MΩ resistor

8.4. Bridge Balance

If current is passed through an electrode the occurring voltage deflection (potential drop at R_{EL}) affects the intracellular recording of membrane potentials in CC mode or CCx10 mode. This deflection must be compensated carefully by means of the BRIDGE BALANCE control (#**12**, Figure 1). This control is activated by the BRIDGE switch (#**44**, Figure 1) and calibrated in MΩ. An LED (#**43**, Figure 1) indicates that the BRIDGE BALANCE is active.

With the cell model connected or the electrode in the bath the BRIDGE BALANCE control is turned on clockwise until there is no artifact on the POTENTIAL OUTPUT (see Figure 10).

- Make the basic settings at the amplifier (see chapter 5).
- Connect a cell model or immerse the electrode into the bath as deep as necessary during the experiment.
- Tune the OFFSET to zero (see chapter 0) and compensate the input capacitance (see chapter 8.3). This is very important since a badly compensated input capacitance prevents setting the BRIDGE BALANCE to correct values.
- Determine the electrode resistance using the ELECTRODE RESISTANCE switch (#**36**, Figure 1) and set the BRIDGE BALANCE RANGE switch (#**12**, Figure 1) accordingly.
- Apply current pulses to the electrode either using an external stimulator (via the STIMULUS INPUT connector (#**14**, Figure 1) or by using the gated stimulus unit.
- Watch the POTENTIAL OUTPUT (#**27**, Figure 1) at the oscilloscope and adjust the BRIDGE BALANCE as shown in Figure 10 using the BRIDGE BALANCE potentiometer (#**12**,Figure 1). After adjustment you should see a straight voltage trace without artifacts caused by the potential drop at R_{EL}.

Figure 10 illustrates the BRIDGE BALANCE procedure using a 100 MΩ resistor that represents the electrode. The current stimuli were generated using the gated stimulus unit gated by two TTL pulses. The amplitude was set to 0.5 nA. In the upper diagram the bridge is slightly undercompensated and in the diagram in the middle it is slightly overcompensated. The lower diagram shows a well balanced bridge (compensated).

Important: BRIDGE BALANCE and CAP. COMP must be tuned several times during an experiment since most parameters change during a recording session. Figure 13 shows artifacts caused by uncompensated stray capacitance and bridge during recording from a cell. It also shows how to cancel these artifacts by tuning with CAP. COMP and BRIDGE BALANCE.
OFFSET deviations can be detected by comparing the readout on the potential display before and after an experiment (with the electrode in the tissue, but not in a cell).

8.5. Electrode Selection

Electrodes need to be tested before use. This is done by application of positive and negative current pulses and by compensating with the BRIDGE BALANCE control. Electrodes which show significant changes in resistance (rectification) cannot be used for intracellular recordings. By increasing the current amplitude the capability of the electrode to apply currents can be estimated. The test current has to cover the full range of currents used in the experiment. Sometimes the performance of electrodes can be improved by breaking the tip or by using the PENETRATION / BUZZ facilities of the amplifier.

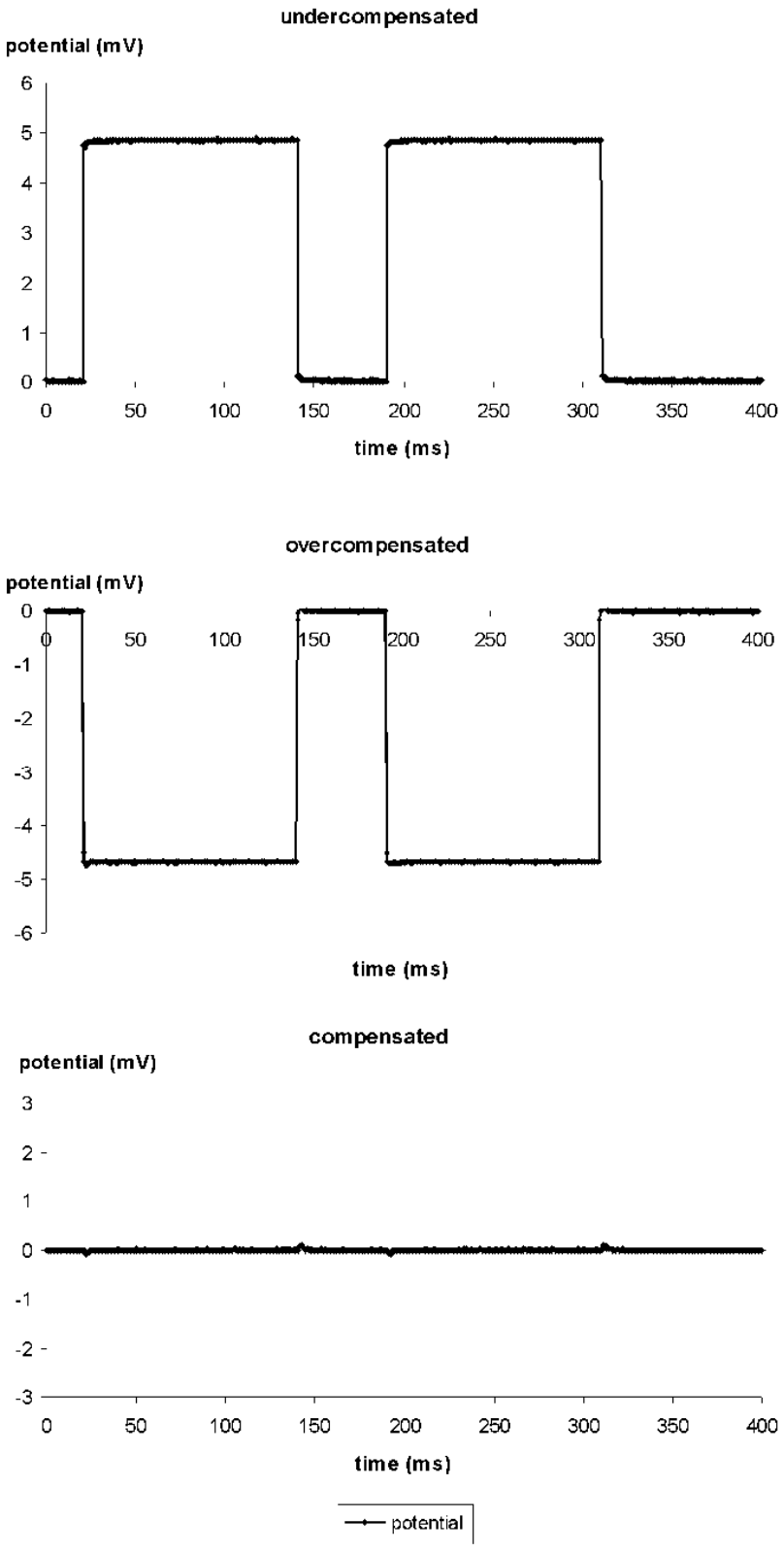

Figure 10: Tuning of the BRIDGE BALANCE using 100 MΩ resistor

9. Sample Experiments (intracellular in CC mode)

In the following the basics of a simple intracellular experiment in CC or CCx10 are described either using a sharp or a suction electrode.
It is assumed that all connections are built as described in chapter 5. Before starting remove the cell model.

9.1. Sample Experiment using a Sharp Electrode

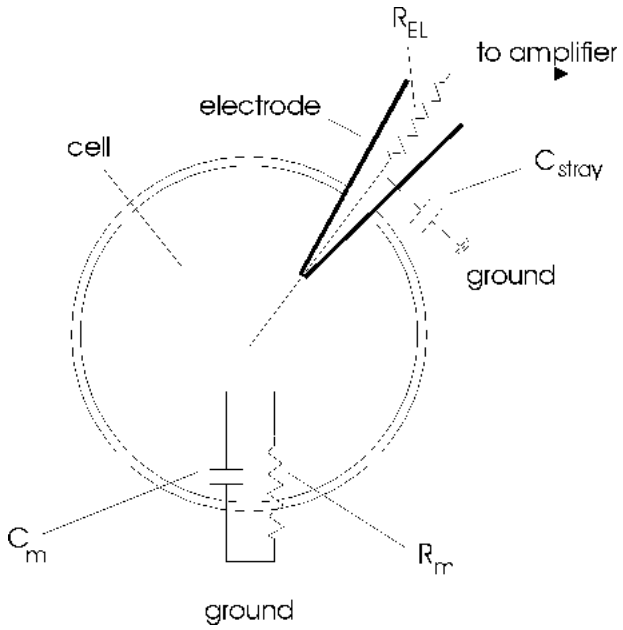

Figure 11: Model circuit for intracellular recording using a sharp electrode
C_m: membrane capacitance, C_{stray}: electrode stray capacitance, R_{EL}: electrode resistance, R_m: membrane resistance

- Connect the electrode cable / holder to the BNC connector and the Ag-AgCl pellet or the agar-bridge for grounding the bath with GND at the headstage.
- Make the basic settings (see chapter 5).

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

- Adjust BIAS CURRENT to zero if necessary (see chapter 8.1)
- Reconnect the STIMULUS INPUT and/or the GATE (TTL) STEP SIZE and put an electrode into the electrode holder.
- Immerse the electrode into the bath (not in a cell) as deep as necessary during the experiment. Test the capability of the electrode to carry current (see chapter 8.4), compensate the potential offset (see chapter 0), measure the electrode resistance (see #**10**, chapter 3.4) and compensate the input capacitance (see chapter 8.3 and Figure 9).

- Enable the OSCILLATION SHUT-OFF unit and set the THRESHOLD so that the OSCILLATION SHUT-OFF unit activates if the system begins to oscillate. Test this by overcompensating the electrode capacitance in several positions of the THRESHOLD potentiometer.
- Now the system is pre-adjusted for measurements. Find a cell!
- Approach the desired cell. There are several indications that the electrode is very close to the cell membrane:
  - the electrode resistance increases (the bridge balance appears undercompensated)
  - extracellular action potentials (APs) are recorded
  - the acoustic monitor signal changes
- Set the PENTRATION / BUZZ DURATION potentiometer to one fourth and apply a BUZZ to the electrode.
- If you are lucky the tip of the electrode is now inside the cell.
- If necessary readjust the BRIDGE BALANCE as shown in Figure 12.
- You read the membrane potential and can apply current pulses to the cell. After penetration the voltage responses of the cell to the test pulses should reflect the cell membrane resistance and time constant.
- Start the experiment.

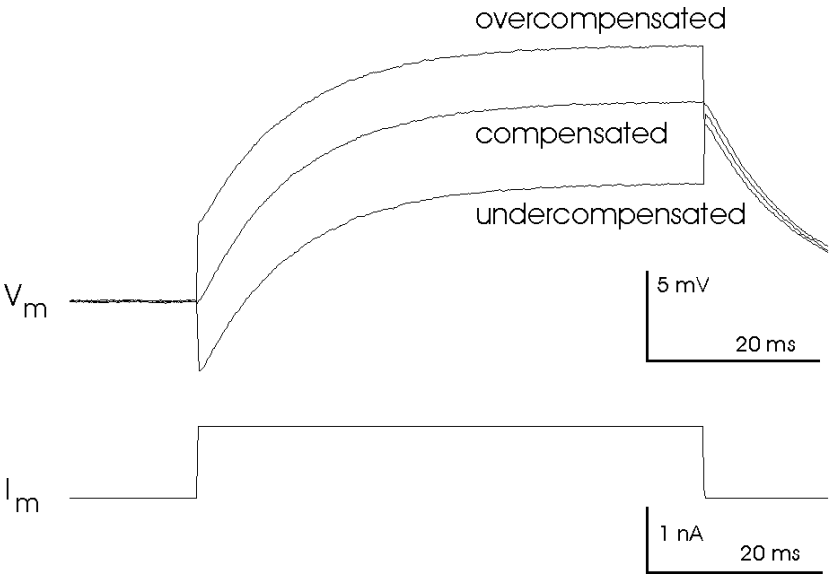

Figure 12: Adjustment of the bridge balance after penetrating a cell

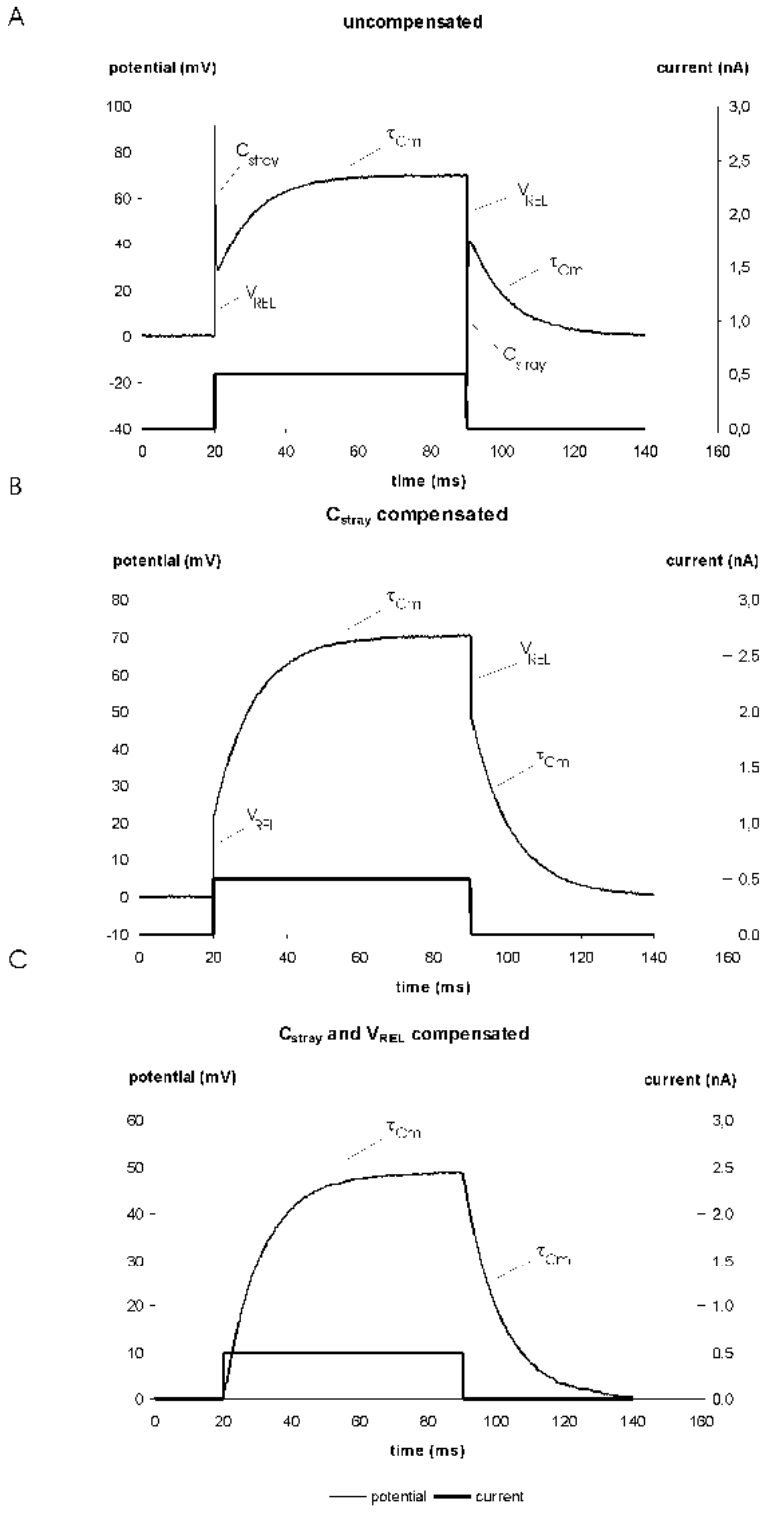

Figure 13: Artifacts caused by the recording electrode. The measurements were done using a cell model with 100 MΩ membrane resistance, 100 pF membrane capacitance and 100 MΩ electrode resistance.
A: C_{stray} and V_{REL} not compensated (bridge not balanced)
B: C_{stray}: compensated and V_{REL} not compensated
C: C_{stray} and V_{REL} compensated (bridge balanced)
C_m: membrane capacitance, C_{stray}: electrode stray capacitance, R_{EL}: electrode resistance, R_m: membrane resistance, τ_{Cm}: time constant of the cell membrane, V_{REL}: potential drop at R_{EL} (see also Figure 11)

9.2. Sample Experiment using a Suction (Patch) Electrode

If suction (patch) electrodes are used for whole cell recordings they are usually called “pipettes”. Thus, in this subchapter “pipette” means “suction electrode”.

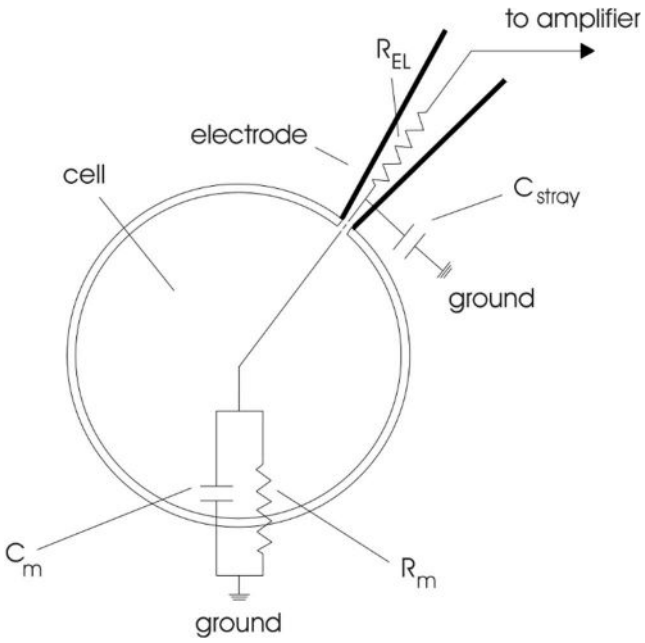

Figure 14: Model circuit for whole cell patch clamp recording using a suction electrode C_m: membrane capacitance, C_{stray}: electrode stray capacitance, R_{EL}: electrode resistance, R_m: membrane resistance

- Prepare the setup and proceed as described in the previous subchapter (9.1) until you have selected a cell. Before immersing the pipette into the bath apply slight positive pressure to the pipette to prevent settling of particles at the tip.
- Apply test pulses to the pipette (about 10 pA). The resulting voltage signals at the pipette are very small (50 µV with a 5 MΩ electrode).
- Approach the cell until the voltage signal changes (**a**, Figure 15). Often you can observe a slight dent in the cell membrane.
- Release pressure from the pipette. Now forming of the seal is indicated by the voltage deflections getting much larger.
- If the seal does not form apply gentle suction to the pipette until a gigaseal is established (**b**, Figure 15).
- Apply stronger suction to the pipette or use the BUZZ unit to break the cell membrane under the pipette and establish the whole cell configuration. The whole cell configuration is established if you see the voltage signal getting smaller again (**c**, Figure 15) and you read the expected membrane potential.
- Read the membrane potential and if necessary, readjust the BRIDGE BALANCE as shown in 8.4 and Figure 13.
- Start the experiment.

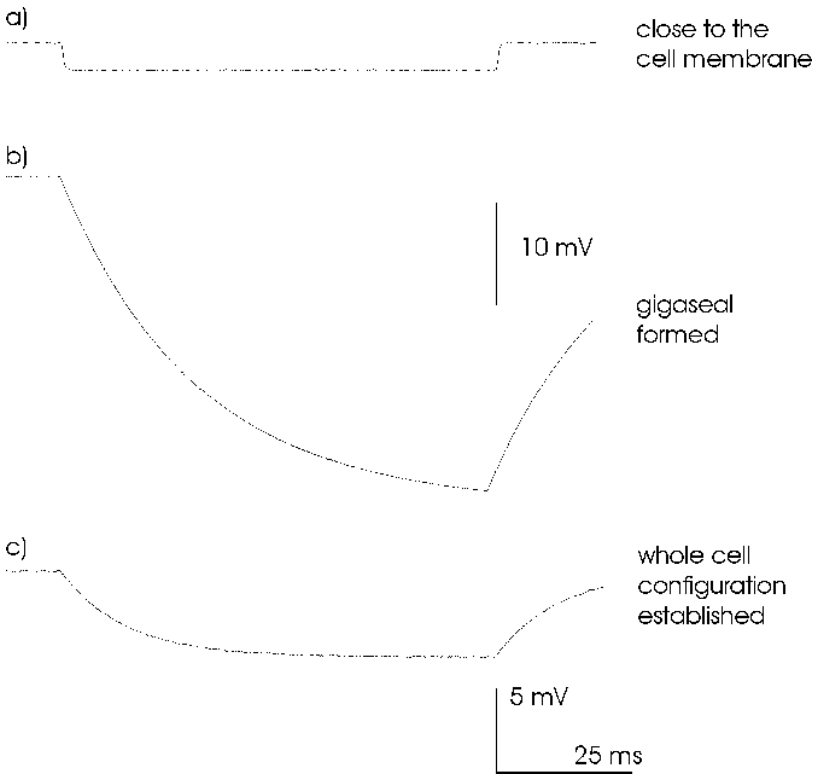

Figure 15: Approaching the cell, forming a gigaseal and establishing the whole cell configuration

10. Introduction into Experiments

The ELC-03XS is capable to perform several types of experiments that are briefly introduced in the following with special focus on loose-patch stimulation and recording. It is assumed that the capacity of the electrode is compensated, the offset of the electrode is cancelled and, for intracellular recordings in BRIDGE mode, electrode artifact is eliminated using the bridge balance circuit. Particularly, when working with charged substances and approaching the cell in VC mode, the current offset is cancelled using the PIPETTE HOLD POTENTIAL potentiometer (**33**, Figure 1).

10.1. Recordings with the Differential Headstage (optional)

Extracellular measurements are mostly done in slices or *in vivo*, in noisy environments, where distortions of the recorded signal caused by other instruments and the animal itself are very common. Additionally, extracellular signals are very small and have to be amplified enormously. The drawback is that noise is amplified as well. Therefore, the headstage of the ELC 03XS can be equipped with a differential input that minimizes noise pick-up. Differential means, that the signal for the amplifier is the difference between the positive (+) (P_{EL}) and negative (-) (REF.) input of the headstage. This results in canceling of all common mode signals (i.e. which both electrodes record, e.g. noise). P_{EL} is connected to the measuring electrode and REF. to the reference electrode. The experimental chamber is grounded by an Ag-AgCl pellet (or an AGAR bridge) connected to GND of the headstage (see Figure 16).

Important: For reliable measurements the experimental chamber or animal, respectively must be grounded.

If differential measurement is not required (single-ended measurement configuration, see Figure 16), the REF input must be connected to ground (GND). The amplifier is in an undefined state, if the REF is left open, and can go into saturation making reliable measurements impossible (for more details see Lalley et al., 1999).

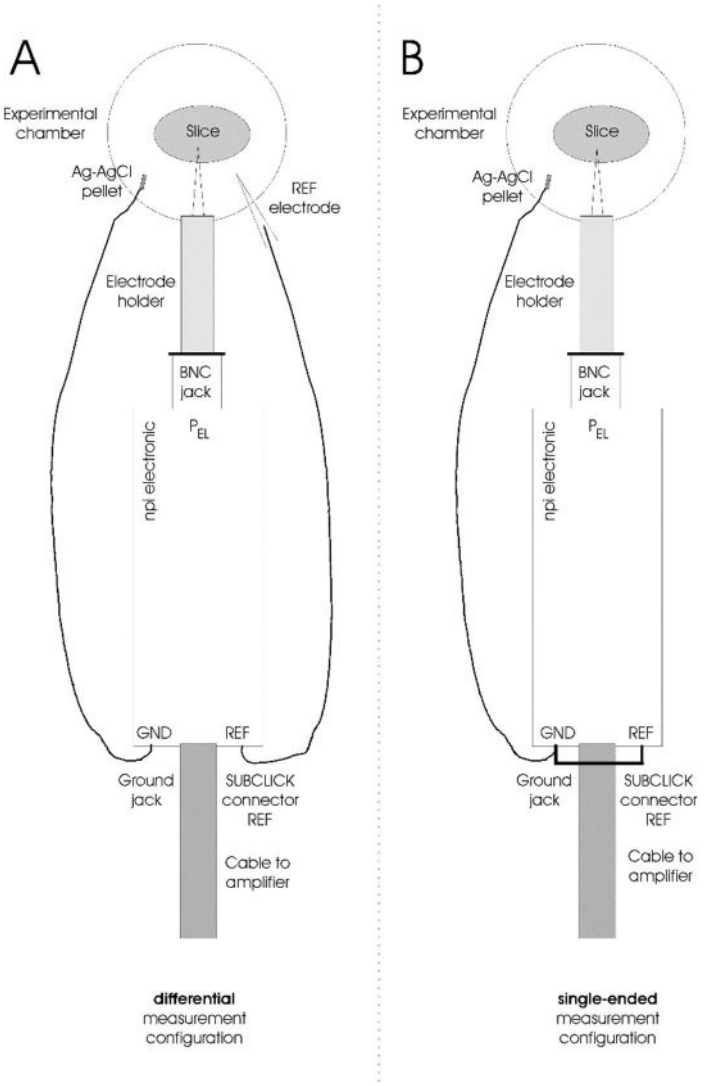

Figure 16: headstage connections, **A**: differential measurement, **B**: single-ended measurement

10.2. Extracellular Voltage Measurement

Extracellular measurements are usually done in the loose-patch configuration or with special metal microelectrodes. Recordings with extracellular metal electrodes is simple. The electrode is advanced into the region where the recordings will be made using a micromanipulator and the signals are filtered and amplified (see chapter 5 in Lalley et al., 1999 for details) as required. For loose patch recording the basic procedure is the following (Barbour & Isope, 2000, Nunemaker et al, 2003):

- Approach the cell in VC mode and apply square voltage pulses to the electrode.
- Contact the cell and establish the loose-patch.
- Set the MODE OF OPERATION switch to OFF.
- Set the required amplification of the POTENTIAL OUTPUT.
- Set the HIGHPASS FILTER to the desired corner frequency, e.g. 0.3 Hz.
- Set the LOWPASS FILTER to the desired corner frequency, e.g. 3 kHz.

10.3. Extracellular Stimulation and Electroporation

Cells can be stimulated using current or voltage signals.

Stimulation with Current

- Approach the cell in VC mode and apply square voltage pulses to the electrode.
- Contact the cell, establish the loose-patch and disconnect the voltage signal from the COMMAND INPUT :10 mV connector.
- Set the MODE OF OPERATION switch to CC or CCx10.
- Set the HOLDING CURRENT to zero.
- For stimulation:

  Apply the stimulus signal to the STIMULUS INPUT 1 nA/V connector.

  or

  Adjust the stimulus amplitude with the HOLDING CURRENT potentiometer and set the stimulus polarity using the switch aside. Gate the preset stimulus with a TTL signal linked to the GATE TTL BNC connector.

Electroporation with Current

Electroporation can be done using the stimulation procedure, but usually the applied current is much higher and the stimulus duration is shorter. Therefore, most electroporation experiments are done in CCx10 mode.

Stimulation with Voltage

- Approach the cell in VC mode and apply square voltage pulses to the electrode.
- Contact the cell and establish the loose-patch.
- For stimulation apply a voltage signal of the required amplitude and duration to the COMMAND INPUT :10 mV connector.

  or

  Adjust the stimulus amplitude with the HOLDING POTENTIAL potentiometer and set the stimulus polarity using the switch aside. Gate the preset stimulus with a TTL signal linked to the GATE TTL BNC connector.

Electroporation with Voltage

Electroporation can be done using the stimulation procedure, but usually the applied voltage is much higher and the stimulus duration is shorter. For stimuli with large amplitudes set the MODE OF OPERATION switch to VCx10 enabling electroporation with up to 10 V.

10.4. Intracellular Recording

Intracellular recordings can be performed in the whole-cell patch configuration and with sharp microelectrodes.

Note: VC mode does not function properly with sharp microelectrodes, i.e. electrodes with more than 10 MΩ resistance.

Current Clamp Recording

The ELC-03XS can be used like a standard bridge amplifier.

- Set the MODE OF OPERATION switch to CC and the BRIDGE MODE switch to the upper position. The BRIDGE MODE LED lights up.
- Compensate the electrode artifact using the BRIDGE BALANCE potentiometer.
- After impaling the cell readjust the bridge.
- If needed set an appropriate holding current using the HOLDING CURRENT potentiometer and the HOLDING CURRENT polarity switch.
- Apply stimuli to the cell using the STIMULUS INPUT 1 nA/V BNC connector.

Voltage Clamp Recording

The ELC-03XS can also be used like a whole-cell patch-clamp amplifier.

- Approach the cell in VC mode and apply square voltage pulses to the electrode.
- Contact the cell, set a holding potential using the HOLDING POTENTIAL potentiometer and establish the whole-cell patch clamp configuration.
- Set the amplifier to BR mode and adjust the BRIDGE. Only if the BRIDGE is adjusted correctly the SERIES RESISTANCE COMPENSATION works with the correct value.
- Switch to VC mode.
- If necessary, adjust the shape of the voltage signal using the CAPACITY COMPENSATION (VC) potentiometer (#**22** and #**27**, Figure 1) and compensate for the SERIES RESISTANCE.
- Apply stimuli to the cell using the COMMAND INPUT :10 mV BNC connector.

Note: CAPACITY COMPENSATION works only for the electrode capacity, not for the capacity of the cell membrane. Therefore, capacitive transients are always present when square shaped pulses are applied in VC mode.

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12. Technical Data

Headstage:	
Input voltage range:	±12 V
Operating voltage:	±15 V
Enclosure:	Size: 23 x 70 x 26 mm, grounded
Dovetail:	Size: 70 mm x 17 mm x 3 mm
on request	
Holding bar:	length 150 mm, ∅ 8 mm
Mounting plate:	Size: 68 mm x 56 mm x 3 mm
Electrode connector:	BNC with driven shield
Ground connector:	2.4 mm connector
Input resistance (CC):	$>10^{13}$ Ω (internally adjustable)
Current range:	±100 nA max. (100 MΩ feedback, x1 range)
	±1.0 µA max. (10 MΩ feedback, x10 range)
	±10 µA max. (1 MΩ feedback, x100 range)
Electrode parameter controls:	
OFFSET:	range ±100 mV, ten-turn control
PIPETTE HOLD POTENTIAL (in VC):	range ±100 mV, ten-turn control
CAPACITY COMPENSATION:	range 0 – 30 pF, ten-turn control
BIAS:	range ±100 pA, ten-turn control
Bridge balance:	
0-100 MΩ	adjustable with ten-turn control
Electrode resistance test:	
Sensitivity 1 mV / MΩ	application of square current pulses ±1 nA
Display:	3 ½ digit, XXX MΩ, activated by key switch (same as POTENTIAL display)
Bandwidth and speed response (CC mode, optimal capacity compensation):	
Full power bandwidth (R_{EL} = 0 MΩ):	>30 kHz, rise time (10% - 90%)
	<10 µs (R_{EL} = 100 MΩ)
	<5 µs (R_{EL} = 10 MΩ)
Outputs:	
Output impedance:	50 Ω
Max. voltage:	±12 V
Current output:	BNC connector, sensitivity 0.1…10 V/nA,
Current output sensitivity:	Rotary switch, 0.1, 0.2, 0.5, 1, 2, 5, 10 V/nA
Current display:	3 ½ digits, XX.XX nA, resolution 10 pA
Current LP filter:	4-pole BESSEL filter (other options available)
attenuation:	-24 dB/octave,
corner frequencies (Hz):	20, 50, 100, 200, 300, 500, 700, 1k, 1,3k, 2k, 3k, 5k, 8k, 10k, 13k, 20k
Potential output x1:	BNC connector, sensitivity 1 V/V
Potential output:	BNC connector, sensitivity 10…1k V/V
Potential output gain:	Rotary switch, 10, 20, 50, 100, 200, 500, 1k
Potential output resolution in AC:	50 µV

Potential LP filter:	4-pole BESSEL filter (other options available)
attenuation:	-24 dB/octave,
corner frequencies (Hz):	20, 50, 100, 200, 300, 500, 700, 1k, 1,3k, 2k, 3k, 5k, 8k, 10k, 13k, 20k
Potential HP filter:	1-pole filter, (other options available)
attenuation:	-6 dB/octave
corner frequencies (Hz):	DC, 0.1, 0.3, 0.5, 1, 3, 5, 10, 30, 50, 100, 300, 500, 800, 1k, 3k
Telegraph potential LP filter	-8…+7 V, 1V/step
Telegraph potential HP filter	-8…+7 V, 1V/step
Telegraph current filter	-8…+7 V, 1V/step
Telegraph potential output sensitivity	+1…+7 V, 1V/step
Telegraph current output sensitivity	+1…+7 V, 1V/step

Digital displays:

Display mV/MΩ	3 ½ digits, XXXX mV or XXX MΩ
Display current	3 ½ digits, XX.XX nA

Inputs:

Input impedance analog	100 kΩ
Input range	±12 V
Input impedance digital (TTL)	10 kΩ
Input range TTL	0-5 V
Current stimulus input CC	via BNC connectors, sensitivity 1 nA / V
Current stimulus input CCx10	via BNC connectors, sensitivity 10 nA / V
Step gate input	via BNC connector (TTL)
Gated stimulus CC	with ten-turn control of holding current resolution: 10 pA, range: ±10 nA
Gated stimulus CCx10	with ten-turn control of holding current resolution: 100 pA, range: ±100 nA
Polarity	selectable with toggle switch
Voltage command input VC	via BNC connectors, sensitivity: ÷10 mV
Voltage command input VCx10	via BNC connectors, sensitivity: ÷1 mV
Step gate input	via BNC connector (TTL)
Gated stimulus VC	with ten-turn control of holding potential resolution: 1 mV, range: ±1 V
Gated stimulus VCx10	with ten-turn control of holding potential resolution: 10 mV, range: ±10 V
Polarity	selectable with toggle switch

Dimensions:
19” rackmount cabinet
19” (483 mm) wide, 10” (250 mm) deep, 3.5” (88 mm) high

Power requirements:
115/230 V AC, 60/50 Hz, fuse 0.4/0.2 A, slow, 25 W

Weight: 5.0 kg

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