OPERATING INSTRUCTIONS AND SYSTEM DESCRIPTION FOR THE

ELC-01X

AMPLIFIER FOR EXTRACELLULAR RECORDING AND ELECTROPORATION

VERSION 2.3
npi 2014

npi electronic GmbH, Bauhofring 16, D-71732 Tamm, Germany
Phone +49 (0)7141-9730230; Fax: +49 (0)7141-9730240
support@npielectronic.com; http://www.npielectronic.com

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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].

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, 2002; 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 for electrical recordings *in vivo* or *in vitro*:

- ❑ Recordings using patch (suction) electrodes from single neurons
  - o Whole cell patch clamp technique (tight seal recording, intracellular)
  - o 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 filling.

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

3. ELC-01X amplifier

3.1. ELC-01X Components

The following items are shipped with the system:

- ✓ ELC-01X amplifier
- ✓ GND and (optional) REF. connectors, (2.6 mm banana plug) for headstage
- ✓ Headstage
- ✓ User manual

3.2. Optional Accessories

- o Differential headstage
- o Cell model
- o Pipette holder
- o Cable set

3.3. System Description

The ELC-01X was optimized for extracellular recording, precise (single cell) electrical stimulation and juxtasomal filling with patch electrodes. It can be used also for intracellular recordings in CC mode. The system consists of a 19” housing and a small headstage with a mounting plate or holding bar. It can be used in slices or in *in vivo* preparations using the optional headstage with a differential input.

Operation modes of the amplifier

The operation modes of the amplifier are selected by a rotary switch with four positions. The selected mode is indicated by LEDs above:

EXT:	VC or CC are selected by a TTL pulse applied to the EXT BNC
CC	CURRENT CLAMP MODE: used to inject current signals
OFF:	CC Mode with all output signals turned off
VC	VOLTAGE CLAMP mode: command potentials are applied to the electrode

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). The ELECTRODE RESISTANCE test mode is activated with a push button, measured directly in MΩ and displayed on the POTENTIAL/RESISTANCE 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 for the VC mode, or to a current in the CC and BRIDGE mode. Besides this, a signal generated from the 10-turn HOLD potentiometer can be transferred into a pulse using the STEP 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.

Output configuration

The ELC-01X amplifier has two output BNCs for POTENTIAL and two output BNCs for the CURRENT signal. The POTENTIAL OUTPUT FROM HEADSTAGE 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 FROM HEADSTAGE monitors the current directly from the headstage with a scaling of 0.1V/nA. The current output signal at CURRENT OUTPUT can be scaled from 0.1V/Na up to 10V/nA.

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 a row of LEDs located close to the digital displays.

3.4. Front Panel View of the ELC-01X Amplifier

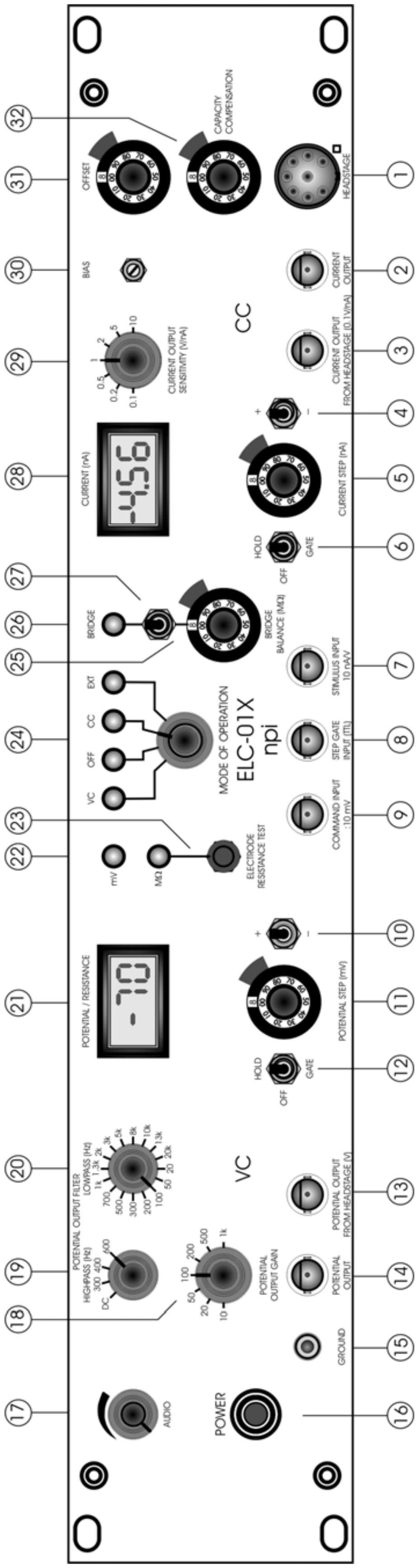


Figure 1: ELC-01X front panel view

3.5. Description of the Front Panel

Basically, the front panel is functionally divided into two halves: the right half has controls for CC and BR modes and the left half for VC mode and extracellular recording. 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.

(**1**) HEADSTAGE connector

Connector for the headstage with optional differential input. REF of the headstage must be connected to ground (single-ended measurement) or to the bath (differential measurement)

(**2**) CURRENT OUTPUT connector

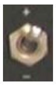
BNC connector providing the CURRENT OUTPUT signal; scaling is set by CURRENT OUTPUT SENSITIVITY switch (#**29**)

Note: The current output is filtered by a one-pole filter with a corner frequency of 5 kHz. Other corner frequencies are possible on request. Please contact npi electronic.

(**3**) CURRENT OUTPUT FROM HEADSTAGE (0.1V/nA) connector

BNC connector providing the CURRENT OUTPUT signal directly from the headstage; scaling is 0.1V/nA

(**4**) current polarity +/- switch



Switch for setting the polarity of the holding current or the gated current stimulus, respectively

(**5**) CURRENT STEP (nA) potentiometer

Potentiometer for setting the amplitude of the holding current or the gated current stimulus; 100 = 10 nA, range: ±100 nA. The polarity of the current stimulus is set by #**4**

(**6**) HOLD / GATE switch

Switch for setting the function of the CURRENT STEP

GATE: Potentiometer #**5** sets a gated stimulus current
HOLD: Potentiometer #**5** sets a holding current in CC mode
OFF: Potentiometer #**5** is disabled

(**7**) STIMULUS INPUT 10 nA/V connector

BNC connector for the current stimulus in CC mode; scaling 10 nA / V

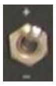
(**8**) STEP GATE INPUT (TTL) connector

BNC connector for gating a CURRENT STEP in CC mode or a VOLTAGE STEP in VC mode. As long as the voltage linked to this BNC is HIGH, i.e. >2.5 V, a current stimulus or command potential is generated by the amplifier. Amplitudes of the stimuli are set by potentiometers #**5** or #**11**, respectively

(**9**) COMMAND INPUT :10 mV connector

BNC connector for the COMMAND potential in VC mode; scaling :10 mV

(**10**) potential polarity +/- switch



Switch for setting the polarity of the holding potential or the gated COMMAND potential, respectively

(**11**) POTENTIAL STEP (mV) potentiometer

Potentiometer for setting the amplitude of the holding potential or the gated command potential; 100 = 100 mV, range: ±1000 mV. The polarity of the current stimulus is set by #**10**

(**12**) HOLD / GATE switch

Switch for setting the function of the POTENTIAL STEP

GATE: Potentiometer #**11** sets a gated command potential
HOLD: Potentiometer #**11** sets a holding potential in VC mode
OFF: Potentiometer #**11** is disabled

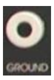
(**13**) POTENTIAL OUTPUT FROM HEADSTAGE (V) connector

BNC connector providing the potential output signal directly from the headstage in Volt.

(**14**) POTENTIAL OUTPUT connector

BNC connector providing the potential output signal; scaling is set by POTENTIAL OUTPUT GAIN switch (#**18**)

(**15**) GROUND connector



Connector providing system GROUND which is not connected to PE (Protective Earth)

(**16**) POWER switch

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

(**17**) AUDIO monitor volume control

Control for setting the volume of the internal speaker connected to POTENTIAL

POTENTIAL OUTPUT FILTER unit

The POTENTIAL OUTPUT FILTER unit consists of (**19**) HIGHPASS (Hz) filter switch and (**20**) LOWPASS (Hz) filter switch

(**18**) HIGHPASS (Hz) filter switch

4-position switch for setting the corner frequency of the one-pole POTENTIAL HIGHPASS filter (300 to 600 Hz, in DC position the HIGHPASS filter is disabled)

(**19**) LOWPASS (Hz) filter switch

16-position switch for setting the corner frequency of the four-pole POTENTIAL LOWPASS filter (20 Hz to 20 kHz)

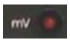
(**20**) POTENTIAL OUTPUT GAIN

Switch for setting the amplification of the POTENTIAL OUTPUT at #**14**

(**21**) POTENTIAL / RESISTANCE display

Display for the potential at the electrode in XXXX mV (1999 mV max.) or the electrode resistance in XXXX MΩ (1999 MΩ max.)

(**22**) mV LED



The unit of the display #**19** is indicated by the mV LED

(**23**) ELECTRODE RESISTANCE TEST button and MΩ LED

Push button activating the R_{EL} test circuit. The unit of the display #**19** is indicated by the MΩ LED

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

Switch for selecting the MODE OF OPERATION

VC: the amplifier operates in Voltage Clamp mode
OFF: all outputs of the amplifier are switched OFF, and the amplifier is set to CC mode, R_{EL} test works
CC: the amplifier operates in Current Clamp mode
EXT: the amplifier is set to CC mode. VC, BR or R_{EL} test modes can be selected by application of a TTL HIGH (>2.5 V) signal to the respective BNC at the rear panel

The MODE OF OPERATION that is currently activated, is indicated by the LEDs above the switch

BRIDGE unit

The BRIDGE unit consists of (**25**) BRIDGE BALANCE potentiometer, (**26**) BRIDGE MODE ON LED and (**27**) BRIDGE MODE ON switch.

(**25**) BRIDGE BALANCE potentiometer

Potentiometer for balancing the BRIDGE circuit that eliminates electrode artifacts in CC mode; 10 MΩ / turn, range: 100 MΩ

(**26)** BRIDGE MODE ON LED

LED that indicates that the amplifier operates in BRIDGE mode

(**27)** BRIDGE MODE ON switch

Switch for activating the BRIDGE mode

(**28**) CURRENT (nA) display

Display for the current at the electrode in ±XXX.X nA, i.e. 10.0 is 10 nA (199.9 nA max.)

(**29**) CURRENT OUTPUT SENSITIVITY switch

Switch for selecting the amplification of the current output signal in V / nA

(**30**) BIAS trim pot

Trim pot for cancellation of the BIAS current; range: ±100 pA

(**31**) OFFSET potentiometer

Control to compensate for the electrode potential OFFSET (ten-turn potentiometer, symmetrical, i.e. 0 mV = 5 on the dial) in CC mode (range: ±100 mV), or to zero the pipette current in VC mode.

In CC mode any offset caused by electrode (tip potential, liquid junction potential etc.) is cancelled by subtracting this potential value from the electrode, i.e. an offset of –10 mV is cancelled by subtracting -10 mV using this potentiometer.

In VC mode an offset at the electrode would lead to current flow through the electrode because without a COMMAND the electrode will be clamped to 0 mV, and the VC circuit generates a current to achieve this. In order to avoid this current flow the electrode has to be clamped to it’s offset potential, i.e. a potential has to be added to the COMMAND. In the scenario mentioned above the electrode has to be clamped to –10 mV. Then, the electrode is clamped to it’s offset potential and no current will flow.

If the OFFSET is correctly compensated in CC mode, there is automatically no current flow when approaching the cell in VC mode. However, liquid junction potentials occurring after establishing the whole-cell configuration are not automatically cancelled.

Important: Command potentials are not affected. Potential out shows zero!

Note: This procedure has to be done at the beginning of the experiment as soon as the pipette has contact to the bath solution, i.e. before approaching a cell.

(**32**) CAP. COMP. potentiometer

Potentiometer for the capacity compensation of the electrode

4. Description of the Rear Panel

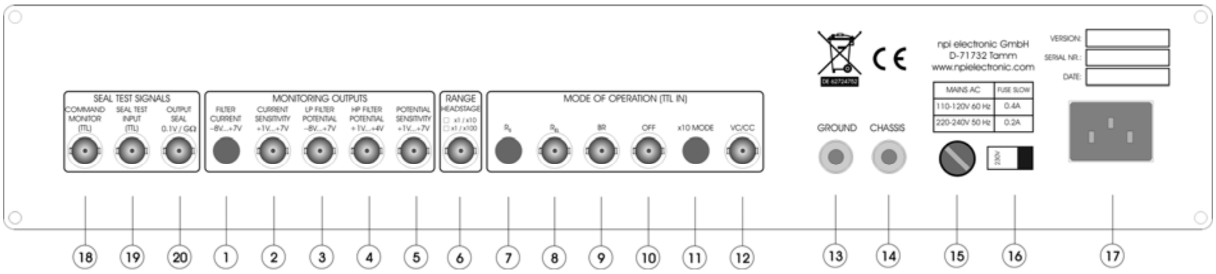


Figure 2: ELC-01X rear panel view

MONITORING OUTPUTS connectors

(**1**) FILTER CURRENT connector

Not installed.

(**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 (+1 V to +4 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 1 MΩ to 10 MΩ or from 1 MΩ to 100 MΩ. To which resistor is indicated by a box to the left.

MODE SELECT connectors

All MODEs OF OPERATION can be selected by TTL signal connected to the rear panel (see below), if the MODE OF OPERATION switch (#**34**, 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

Not installed.

(**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-01X in OFF mode remotely with a TTL HI (+5 V) signal.

(**11**) x10 MODE connector

Not installed.

(**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 counter clockwise using a screw driver.

(**16**) LINE SELECT switch

Switch for selecting the line voltage. Switch to the right for 230 V, to the left for 115 V. 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. 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.

For both grounds there is an outlet on 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 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 and all ground points should originate from a central point to avoid ground loops.

5. Setting up the ELC-01X

The following steps should help you set up the ELC-01X correctly. Always adhere to the appropriate safety measures (see chapter 1).

After unpacking, the ELC-01X is attached to the setup by assembling the electrical connections. It is assumed that first a cell model will be attached.

① **Electrical connections**

- ❑ Turn POWER off.
- ❑ Plug the power cord of the instrument into a grounded outlet.
- ❑ Connect the headstage to the HEADSTAGE connector (#**1**, Figure 1) at the ELC-01X.
- ❑ 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.
- ❑ 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 (#**18**, Figure 1) and the CURRENT OUTPUT SENSITIVITY switch (#**29**, Figure 1).

Before using the ELC-01X always make the basic settings to avoid oscillations.

② **Basic settings**

- ❑ 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).
- ❑ Set the MODE OF OPERATION switch (#**24**, Figure 1) to CC.
- ❑ Turn POWER switch on.

Now the ELC-01X is ready for an initial check with the cell model.

6. Passive Cell Model

The ELC-01X 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

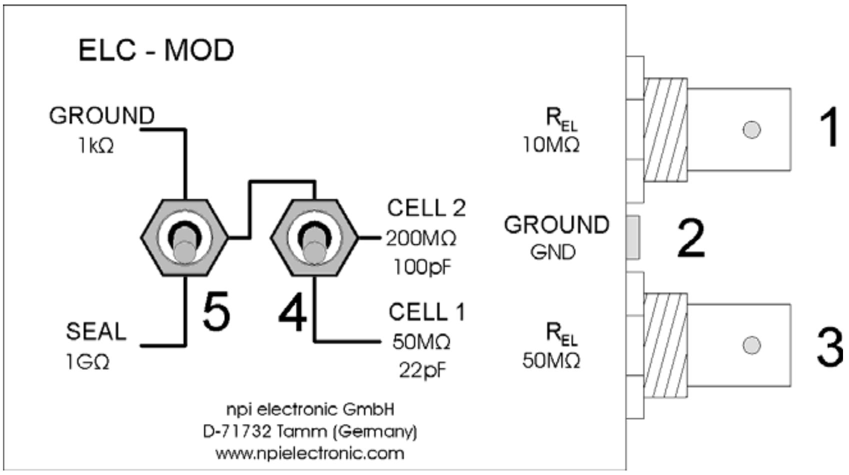


Figure 3: passive cell model

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).

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.

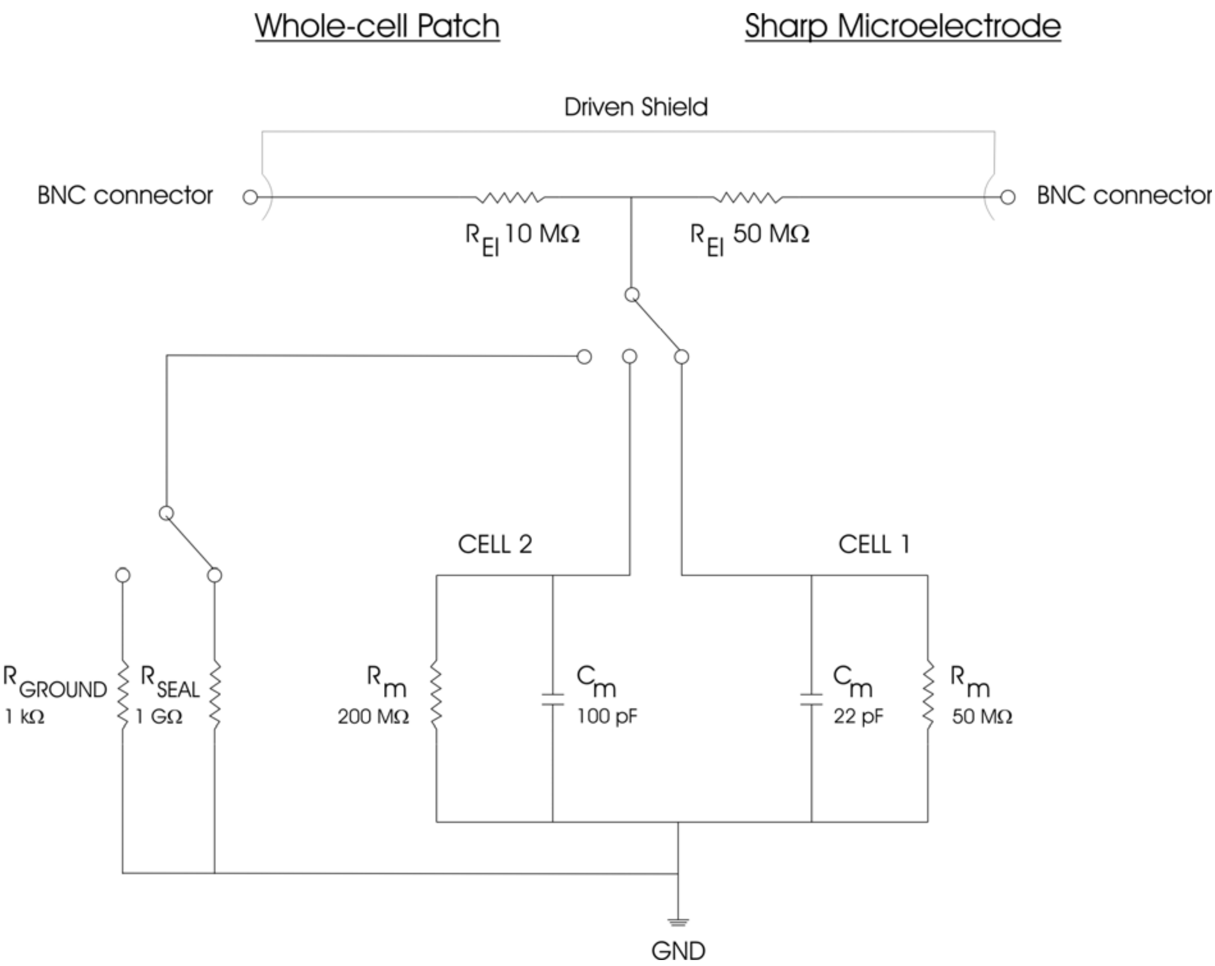


Figure 4: Schematic diagram of the passive cell model

6.2. Connections and Operation

<u>Checking the configuration</u>

❑ 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 3 to the upper position.
- ❑ Set switch #**5**, Figure 3 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 3 to the upper position.
- ❑ Set switch #**5**, Figure 3 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 3) for an experiment with sharp electrodes or the 10 MΩ electrode connector (#**1**, Figure 3) for simulating an experiment with patch electrodes.

- ❑ Switch the CELL membrane switch (see #**4**, Figure 3) 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-01X comes with a headstage for connecting suction electrodes for loose-patch clamp and / or stimulation or electroporation, respectively. The headstage is also capable of intracellular recordings with sharp electrodes in CC mode or extracellular recordings. The use of metal electrodes is possible as well.
A differential headstage (see **Optional accessories** in chapter 3.2) for measurements *in vivo* is also available. For details contact npi.

7.1. Headstage Elements

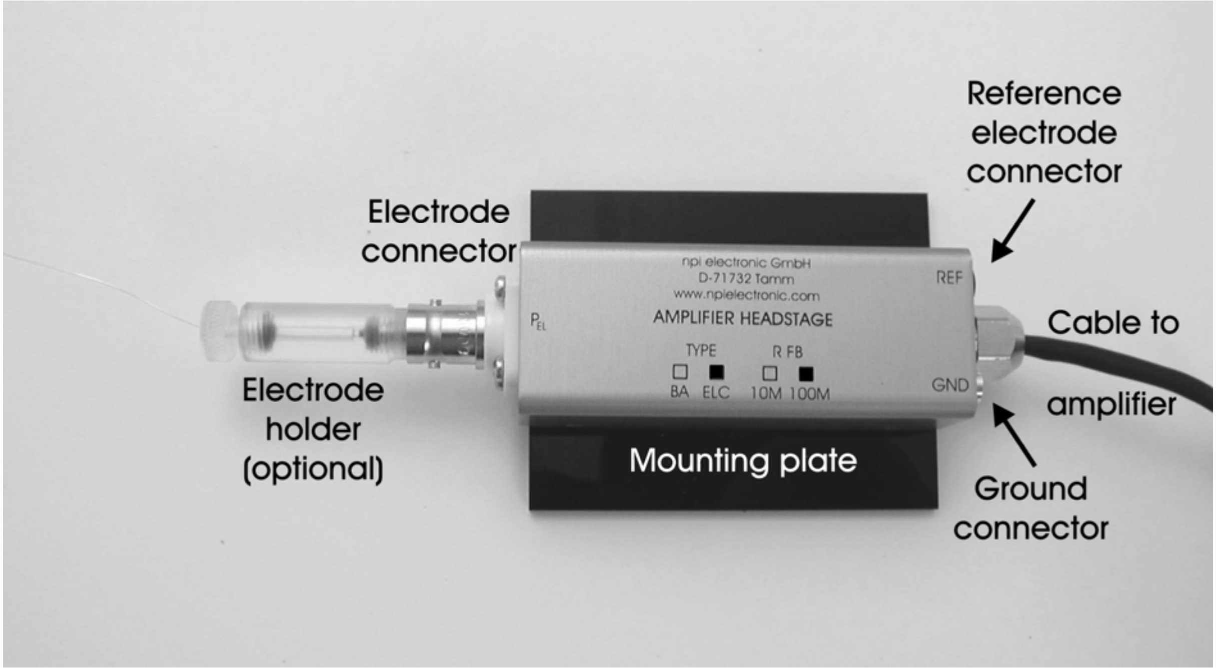


Figure 5: ELC-01X headstage

- 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
- R FB Value of feedback resistor, 10M: 10 MΩ, 100 M: 100 MΩ
- Headstage cable to amplifier
- Mounting plate

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 a Ag-AgCl pellet. The headstage is attached to the amplifier with the headstage cable and an 8-pole connector. The headstage can be mounted directly to a micromanipulator using the mounting plate 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!

7.2. 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-01X 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.
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 HOLD / OFF / GATE switch (**#6**, Figure 1) to position OFF.
- ❑ 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.
- ❑ Remove the wire and attach the cell model or a resistor with a value of about 5 to 10 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. Introduction into Experiments

The ELC-01X 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.

8.1. 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-01X 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). For differential measurements, both inputs of the headstage (REF. and P_{EL}) are connected to microelectrodes using cables with grounded enclosure or electrode holders. 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 6).
If differential measurement is not required (single-ended measurement configuration, see Figure 6), 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).

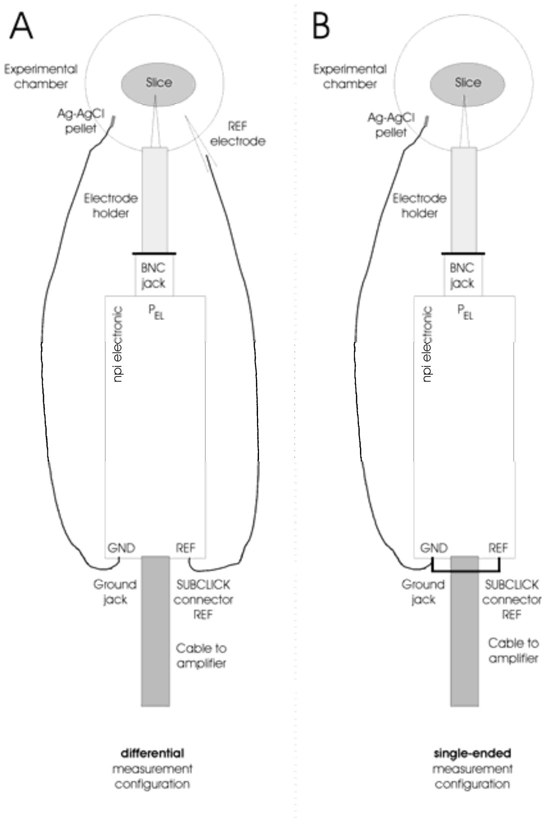


Figure 6: headstage connections, **A**: differential measurement, **B**: single-ended measurement

8.2. Extracellular Voltage Measurement

Extracellular measurements are usually done in the loose patch configuration or with special metal microelectrodes. Recording 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 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 seal.
- ❑ 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.
- ❑ Set the LOWPASS FILTER to the desired corner frequency.

8.3. 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.
- ❑ Set the HOLDING CURRENT to zero.
- ❑ For stimulation:

  Apply the stimulus signal to the STIMULUS INPUT 10 nA/V connector.

  or

  Adjust the stimulus amplitude with the CURRENT STEP potentiometer and set the stimulus polarity using the +/- switch aside. Gate the preset stimulus with a TTL signal linked to the STEP GATE INPUT (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.

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 POTENTIAL STEP potentiometer and set the stimulus polarity using the switch aside. Gate the preset stimulus with a TTL signal linked to the STEP GATE INPUT (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.

8.4. Intracellular Recording

Intracellular current clamp (CC) recordings can be performed with patch or sharp microelectrodes.

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

Although VC mode experiments can be performed in whole cell configuration with patch electrodes, npi do not recommend this, because of the missing series resistance compensation and capacity compensation in VC mode. The VC mode of the ELC-01X amplifier is intended to be used primarily for approaching the cell and forming the loose seal.

Current Clamp Recording

The ELC-01X 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 10 nA/V BNC connector.

9. Literature

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10. Technical Data

Headstage:	
Input voltage range:	±12 V
Operating voltage:	±15 V
Enclosure:	Size: 23 x 70 x 26 mm, grounded
Mounting plate:	Size: 70 mm x 50 mm
on request	
Holding bar:	length 150 mm, ∅ 9 mm
Dovetail:	Size: 70 mm x 17 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:	±120 nA max. (100 MΩ feedback), standard (±1.2 µA max. (10 MΩ feedback)) ±12 µA max. (1 MΩ feedback)
Electrode parameter controls:	
OFFSET:	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, XXXX 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, XXX.X nA, resolution 100 pA
Current filter:	1-pole, corner frequency: internally set to 5 kHz
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, 300, 400, 600
Telegraph potential LP filter	-8…+7 V, 1V/step
Telegraph potential HP filter	+1…+4 V, 1V/step
Telegraph potential output sensitivity	+1…+7 V, 1 V/ step
Telegraph current output sensitivity	+1…+7 V, 1 V/ step

<u>Digital displays</u>:

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

<u>Inputs</u>:

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 10 nA / V
Gated stimulus	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
Gated stimulus VC	with ten-turn control of holding potential resolution: 1 mV, range: ±1 V
Polarity	selectable with toggle switch
Step gate input	via BNC connector (TTL)

<u>Dimensions</u>:
19” rackmount cabinet
19” (483 mm), 10” (250 mm), 3.5” (88 mm)

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

<u>Weight</u>: 4.0 kg