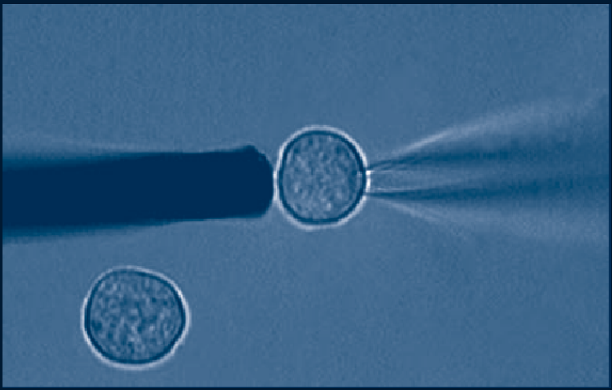
Manual 1.0

EVA 8

Voltammetric Amplifier



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Title Page: Adrenal Chromaffin Cell, contacted by a patch pipette (right) and a carbon fiber microelectrode; Courtesy of Dr. Jakob Soerensen, Max-Planck-Institut for biophysical Chemistry, Gttingen, Germany

COMEVA/1

Contents

1. Safety Guidelines

Please read the instruction manual of the EVA 8 before putting the amplifier into operation to prevent any possible damage to life and equipment. In addition to the reading instruction manual of the EVA 8 amplifier, the regulations for accident prevention applicable to your country (VBG 4 in Germany) should be respected and the relevant safety rules of your work environment applied.

The instruction manual has been designed in such a way that taking the EVA 8 voltammetric amplifier into operation is comprehensible, safe, economical, and helps to prevent dangerous misuse. A safe use of the amplifier, minimal service costs, and no delay in service can be guaranteed only if the instructions given in the operation manual are followed. The instruction manual should always be in proximity to the amplifier. Misuse, neglected inspection of the instrument, or disregarding operating instructions may endanger the user and any third party, and may cause damage to technical equipment.

The EVA 8 voltammetric amplifier is manufactured according to current applicable safety regulations. The amplifier is to be operated only, if they are working properly. The amplifiers should immediately be sent for repair if any technical problem occurs which may endanger the safety of any user. The EVA 8 should only be used for their intended purpose as described in the instruction manual. "Intended purpose" includes regular inspection and service of the amplifier.

Any technical equipment added to the amplifier not defined as an "instrument" according to the European Community (EC) rules should not be used. Thus, equipment can only be added if it is labeled with CE-certification or has an accompanying statement certifying conformity with EC-rules.

Only technical equipment approved by HEKA can be added to the amplifier. Information concerning this matter will be provided on request by our service team. Any further use of the EVA 8 and added equipment, which does not fall within the "intended purpose" of the amplifier, is not in accordance with the liability regulations. HEKA does not accept liability for any misuse or modification to the EVA 8 amplifier.

If you are uncertain regarding the operating interactions, safety rules, or the instruction manual in general please contact HEKA before taking the EVA 8 amplifier into operation.

The EVA 8 instruction manual does not provide instructions for repair. Any necessary repair of the amplifier has to be done by certified specialists. HEKA offers such certified service.

2. Introduction

The Czech Jaroslav Heyrovsky first developed the voltammetry technique from the discovery of polarography in 1922. The early stages of voltammetric measurements experienced a large number of problems, making the technique impractical. However with significant advances in theory, methodology, instrumentation and electronic, the technique is now well establish and practical.

2.1 Introducing the Eva 8

The EVA 8 offers all the capabilities to fulfill the changing demands in electrochemistry laboratories. The EVA8 design and construction is based on Heka's well-known EPC 8 patch clamp amplifier with the same quality of manufacturing.

The versatility of the EVA 8 can best be appreciated by the variety of experiments that can be carried out with them. Besides high-resolution recordings of ion concentration this unit can be used in studies of quantitative determination of organic and inorganic compounds, determination of electron transfer and reaction mechanism, studies of oxidation and reduction process.

Technically, the EVA 8 is noteworthy for three special features: the range-changing capability of the head stage, the extremely wide bandwidth available from the current monitor circuitry, and the computer control possibility. Together these features allow that a single head stage suffices for use with the different size of electrodes to allow a wide range of current measurement.

This manual is designed to provide a general guide for setting up and using the EVA 8 for experiments. It covers general information about the hardware and basic principles of the EVA 8 functions and voltammetry techniques.

It is assumed that the reader has some familiarity with voltammetry techniques.

2.2 References

2.2.1 Voltammetry

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3. Description of the Hardware

The hardware components of the EVA 8 voltammetric system consist of a head stage (or probe) and the amplifier main unit. Specific information about the hardware installation is given elsewhere (see chapter 4 on page 13).

Figure 3.1: The Voltammetric Amplifier - EVA 8

3.1 Probe

The probe, or "head stage" of the EVA 8 is contained in a small enclosure, the enclosure is intended to be mounted on a micromanipulator and directly attached to the recording electrode. It contains a sensitive amplifier that constitutes the current-to-voltage converter, as well as components for injecting test signals into the amplifier. The following connectors are found on the probe:

Input Connector: This is a Teflon-insulated BNC connector. The standard pipette holder plugs directly into this connector; the center pin is the amplifier input, the shield is driven with the command potential Vp.

> ***Note:*** *Avoid touching the probe's input terminal, since the input circuitry of the probe can be damaged by static electricity. When it is necessary to touch the input (e.g., while inserting a pipette into the holder), ground yourself first by touching a grounded metal surface.*

Ref Output: The red 0.04" pin jack carries the command potential Vp.

> ***Note:*** *The metal case of the probe is also connected to this signal, and therefore must be insulated from ground.*

Gnd Connector: The black pin jack carries a high quality ground signal. This signal is useful for grounding the bath electrode and nearby shields without potential errors that could arise from ground loops. This ground is connected directly to the signal ground on the controller through the probe's cable. More details on grounding practices will be provided in chapter 6 on page 19.

3.2 Main Unit

The main unit of the EVA 8 contains the power supply, the signal processing electronics and all controls. The main unit if factory calibrated to account for different properties of components within the probe.

> ***Note:*** *Calibration parameters are unique to each amplifier and head stage combination. Thus, if you exchange the head stage, be sure a new hardware calibration is performed by HEKA.*

The controls on the main unit front panel are divided into four functional groups containing, from left to right, the functions of: current monitoring, capacitance compensation, command signal processing and the power switch.

3.2.1 Current monitor group

GAIN: Sets the scaling of the current monitor output. The range is 0.005 to 1000 mV/pA. The gain setting automatically selects one of the three available current-measuring feedback resistors within the probe. (5 MOhm, 500 MOhm, and 50 GOhm), corresponding to low, medium and high gain ranges. The table below summarizes the main features and limitations of the gain ranges.

Gain Range	**Low**	**Medium**	**High**
Resistor	5 MOhm	500 MOhm	50 GOhm
Gain (mV/pA)	0.005 – 0.2	0.5 – 20	50 – 1000
Bandwidth	100 KHz	100 KHz	60 KHz
I max	+/- 2 μA	+/- 20 nA	+/- 200 pA

Table 3.1: Gain Ranges of the EVA 8

The low gain range of the EVA 8 is useful when large currents are required within an experiment (up to 2uA). In the medium gain range, the background noise is larger than in the high gain, but the full 100 kHz bandwidth is available, and currents of up to approximately 20 nA can be recorded. The high gain range offers lower noise with a current limit of 200 pA. However this is obtained at the expanse of a lower maximum bandwidth of 60 KHz.

CLIPPING: This LED lights whenever the amplifier saturates in the current monitor pathway. This indicator serves as a simple monitor of this condition. It is particularly useful since it can also indicate internal amplifier clipping even in cases where, because of filtering, the output voltage is not saturated.

PROBE: This input accepts the multi-pin connector of the head stage.

SIGNAL GND: This banana jack is a high-quality signal ground connection that can be used to ground other parts of the experimental setup as required.

REMOTE: This LED lights when a computer is remotely controlling the EVA 8. In this case turning the controls on the front panel (Gain, Filter) has no effect.

FILTER switch: The EVA 8 has one filter switch. This switch selects the filter setting of the `Current Monitor` output in 12 steps. 0.1, 0.3, 0.5, 0.7, 1, 3, 5, 7,10, 20, 30 kHz (7-pole Bessel Filter) and FULL (3-pole Bessel Filter).

CURRENT MONITOR: The EVA 8 has a single output to monitor the current. The signal is filtered according to the setting described above.

3.2.2 Capacitance Compensation Group

C-FAST control: This adjusts the size of the rapid transients that cancel the charging current of the electrode and other stray capacitance. Full scale is 10 pF. With nothing connected to the Probe input, cancellation is typically obtained at a setting of 1-2 pF due to the residual input capacitance of the current-measuring amplifier.

T-FAST control: This control adjusts the time constant of the cancelling transient to best match that of the charging currents to be cancelled.

3.2.3 Command signal processing Group

These controls allow a steady voltage (set by VHold) to be added to an external stimulus (scaled by `Stim. Scaling`) to provide the command signal. This command signal determines the electrode voltage.

STIM. INPUT: Pulses from an external stimulus source can be applied here. The input impedance is approximately 8.5 KOhm.

Command Range switch: The stimulus signal is passed through a divide by 5 or divide by 10 internal circuit selected by this switch. This provides a 100mV/Volt or a 200mV/Volt ranges with a maximum command voltage input of +/- 10 Volt at the unit and +/- 2 Volt at the probe.

STIM. SCALING: The stimulus signal is scaled in polarity selected by this switch. Positive and negative factors are provided to allow the user to apply pulses of either polarity to the experiment under study.

VCommand: This control adjusts the constant voltage that is added to the stimulus signal. The range is +/- 2 Volt. A setting of 5.00 on the knob corresponds to 0 mV.

I/V Switch: This 3 position switch select current or voltage to be displayed on the LCD display. The voltage is expressed in milivolt and the current in two ranges; nA and pA.

I/V LEDs: These 3 LEDs represent the position and range of the I/V switch

LCD display: The LCD display when in 'V' mode displays the command potential applied to the probe in millivolts. The command potential is the sum of `VCommand` and the scaled stimulus. In current mode the LCD displays the current at the probe in two different ranges. nA for the low and medium ranges and pA for the high range.

VOLTAGE MONITOR: This output signal provides a monitor of the electrode potential. The output impedance is 50 Ohm.

Vp-OFFSET control: As the final step in the command processing a variable offset voltage is added to compensate for electrode offset potentials. The range of this control is +/-500mV which is adequate for most electrodes.

3.2.4 Power switch and chassis ground

Power switch: Applies power to the unit.

> ***Note:*** *Since the internal calibration of the amplifier have been performed with a warmed-up amplifier; switch on the amplifier about 15 minutes before starting an experiment. This will ensure that the amplifier has warmed up to proper working temperature.*

CHAS connector: The chassis jack is connected to the ground line of the power cord, as is typical of most instruments. The unit and probe `Signal Ground` separated from the chassis to avoid ground loops through a 10 Ohm resistor.

3.2.5 Rear panel

Connector: A 40-pin input/output connector allows the EVA 8 to be connected to a computer for remote control.

Voltage switch: This switch selects between 110 and 220 Volt operation. Make sure that this switch is set in the proper position and that the correct fuse is installed.

Dither Box : This BNC connector provides a TTL-level output.

4. Setting Up

This chapter provides a guideline for setting up the Eva 8.

4.1 Power

A switch on the rear panel of the controller selects between 110 and 220 Volt operation. Make sure that the switch is in the proper position, and that the correct fuse is installed before applying power. Irreversible damage to the circuitry may occur otherwise.

4.2 Static electricity

The input circuitry of the probe can be damaged by static electricity. To avoid this, please observe the following rules:

1. Avoid touching the input terminal unnecessarily.
2. When it is necessary to touch the input (e.g. while inserting a pipette into the holder), ground yourself first by touching a grounded metal surface.

4.3 Initial Checkout

Connect a function generator or stimulator to `Stim. Input`, and connect the `Current Monitor` output to an oscilloscope. Insure VCom and Vp are set to 5.0 and that the Command switch is set to divide by 10. Connect the model cell to the probe and select the 10 MOhm position on the model cell. With the `Gain` set to 0.1 mV/pA, any signal applied to the `Stim. Input` connector should be reproduced with the same amplitude at the `Current Monitor` output. A 100 Hz square wave signal with an amplitude of about 1 volt is appropriate.

> ***Note:*** *The probe input usually needs to be shielded from low-frequency noise. A simple way to do this is to cover the input connector with a cap made from aluminum foil.*

> ***Note:*** *The Test mode current-injection system is intrinsically AC-coupled, which means that long (¿30 ms) pulses may be distorted in shape. Also, large low-frequency inputs can cause the current-injection circuitry to saturate.*

The action of the internal filters on the background noise level and the temporal response can be observed by changing the `Filter` switch setting.

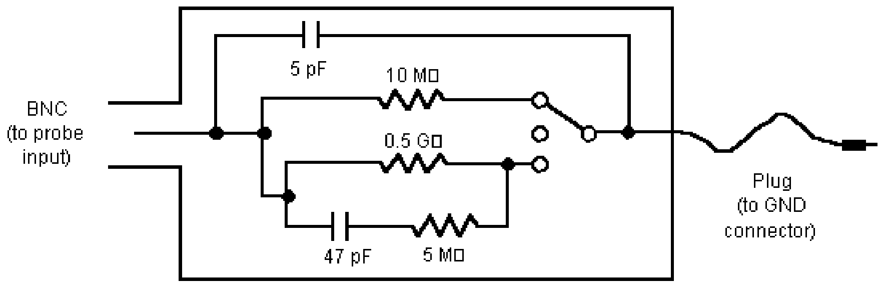

Figure 4.1: MC8 Model Circuit

The model circuit connects to the probe input via a BNC adapter and the plug goes to the black `Gnd` connector on the probe. In the 10 MOhm setting, the model circuit simulates a electrode that is open to the bath solution. Now move the switch of the model circuit to the center position, which leaves only a capacitance of about 5 pF connected. This simulates a gigaseal, and the `C-Fast` controls can be used to cancel the capacitive spikes resulting from stimulus pulses.

Note: *Because of poor dielectric properties in the internal switch, the model circuit introduces excess random noise above the level that can be obtained with a gigaseal.*

5. Operating Modes

The EVA 8 is fundamentally an instruments for measuring small electrical currents. It uses a current-to-voltage (I-V) converter circuit to convert the currents to an analog voltage, which is then made available at the current monitor outputs for display and recording. At the same time that electrode currents are being recorded, the potential must be specified, and the various operating modes of the EVA 8 correspond mainly to different ways of controlling that potential.

5.1 Voltage-Clamp Mode

This is the only mode available with the EVA 8. It is a basic patch-clamp mode, and is implemented by the circuitry shown in the Figure 2 below. The electrode potential is derived from the signal applied to `Stim. Input`, with a variable offset added from the `VCom` control. The sum of these two sources is displayed and monitored as the `VCom` signal. Before being applied to the electrode a further variable offset is added from the `VP-OFFSET` control to allow the user to cancel electrode offsets.

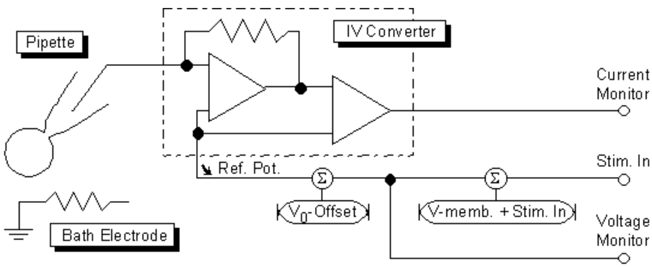

Figure 5.1: Voltage Clamp Mode

5.2 Procedures

5.2.1 Offset Compensation

In any experiment a number of offsets have to be taken into account. These include amplifier offsets (+/- 30 mV), electrode potentials (+/- 500 mV), depending on the concentration of working electrode and reference electrode, liquid junction potentials, and potentials of background electrode/electrolyte. Some of these offsets are fixed during an experiment. like amplifier and electrode offsets, some are variable. It is standard practice to take care of the fixed offsets by performing a reference measurement at the beginning of an experiment. Thereby an adjustable amplifier offset is set for zero electrode current. Thereafter the command potential of the amplifier will be equal in magnitude to the electrode potential if no changes in offset potentials occur.

6. Voltammetry Setup

6.1 Mounting the Probe

For low-noise recording, the pipette holder must be attached directly to the EVA 8 probe. Although the probe amplifier can tolerate the additional capacitance of a short connecting cable without instability or oscillations, we find that the dielectric and electrostrictive properties of coaxial cables introduce excessive noise. In typical setups, the probe is therefore mounted directly on a 3-axis micromanipulator. The EVA 8 probe is supplied with a plastic mounting plate for mounting on a flat surface. Holes can be drilled through the protruding surfaces for attachment to a matching plate or other surface. Please remember, that the metal case of the probe must remain insulated from ground.

Because of the extreme sensitivity of the EVA 8, special care must be taken in grounding all surfaces that will be near the probe input in order to minimize line-frequency interference. Even one millivolt of AC on a nearby surface, which can easily arise from a ground loop, can result in significant 50 or 60 Hz noise. A high-quality ground is available at the `Gnd` terminal of the probe; this is internally connected through the probe's cable directly to the `Signal Gnd` in the main unit. The `Gnd` terminal on the probe is best used for the bath electrode, and perhaps for grounding nearby objects such as the microscope.

6.2 Ground Wires

It is a good idea to run a separate ground wire from the `Signal Ground` jack on the main unit to ground large objects such as the table, Faraday cage, etc. It is best to have the high quality ground wire run parallel to the probe's cable in order to avoid magnetic pickup and ground loop effects. Besides 50 or 60 Hz magnetic pickup, there may be some 35 kHz pickup from the magnetic deflection of the computer monitor. This pickup becomes visible only when the EVA 8 filters are set to high frequencies; it can usually be nulled by changing the orientation or spacing of the ground wire from the probe cable.

6.3 External Shielding

Especially when an unshielded pipette holder is used, some electrostatic shielding of the experimental setup is necessary to avoid line-frequency pickup from lights and power lines in the room. Most experimenters use a table-top Faraday cage with a closable front, and lead all of the cables (e.g., from the microscope lamp, probe, cooling system, ground lines) through a hole in the cage to an equipment rack mounted outside. If the pipette holder is somewhat exposed, or if the Faraday cage has an open front, a small grounded screen placed near the pipette holder may help.

6.4 Connections to other Instruments

Voltage pulses for testing the electrode resistance and for stimuli are provided by a stimulator. (Actually, if voltage pulses are not required for stimulating the species of interest under study, a full-blown stimulator is not required, and a very simple square-wave or pulse generator is sufficient for monitoring the pipette resistance. On the other hand, the stimulus source for applying pulses in cyclic voltammetry recordings should have a low noise level see chapter 8 on page 25.) The monitor signal from the EVA 8 is applied to an oscilloscope for observation during the experiment, and to a data recorder. A computer with suitable analog input and output interfaces can fulfill both the stimulator and data recorder functions.

Filtering beyond that provided in the EVA 8 is sometimes needed in observing channel activity during an experiment. For this reason many users insert a variable filter (typically a 4 or 8-pole Bessel filter) between the `Current Monitor` output and the oscilloscope. Also, it is often convenient to observe the voltage and current monitor signals at the recorder's outputs, rather than directly from the patch clamp. In most data recorders the input signals are passed through the outputs, and observing the signals there can increase the experimenter's confidence that the data are being recorded correctly.

6.5 Pipette Holder

A shielded version of the pipette holder is available; this holder can provide sufficient shielding from 50-60 Hz interference even without the use of a Faraday cage. The shielded holder, however, introduces much more random noise than the unshielded one. This random noise arises from the non-ideal dielectric properties of the plastic in the holder and from thermal voltage fluctuations in films of aqueous solutions; the metal shield allows more of this noise to be coupled capacitively into the amplifier input. For low level recordings, the unshielded holder is strongly recommended. The difference in background noise level between the two holder types is roughly a factor of two.

The unshielded holder is made from polycarbonate, having low dielectric loss. If you make your own holder, you should give some thought to the choice of materials. The insulating parts of the holder should be of a low-loss material, and should have a hydrophobic surface to prevent the formation of conducting water films. Polycarbonate fulfills these criteria better than any material we have tried. You can test the noise level of a holder by mounting it (with the electrode wire installed, but dry) on the probe input, and measuring the noise. The probe should be in a shielded enclosure, so that no line-frequency pickup is visible on an oscilloscope connected to the current monitor output at a bandwidth of 3 kHz or less. A good holder increases the rms noise only by about 10%.

6.6 Working Electrode, Reference Electrode and Samples.

A typical electrochemical cell consist of sample dissolve in solvent, an ionic electrolyte and electrodes. Because the type and amount of cell, the working electrode material and the reference electrode material used depends on the technique, and the analytical data to be obtained, it is beyond this manual to discuss this mater.

7. Using the Voltammetric Amplifier

A brief description of the measurement techniques for recording from a cell is provided here.

7.1 Initial Setup

The object is to apply voltage pulses to the working electrode and observe the `Current Monitor` signal on an oscilloscope to monitor the oxidation current. Different techniques in use today are well known and can be used with the EVA 8.

It may be convenient to pick up a Gain setting close to the expected currents from the experiment. After selecting a proper gain a filter setting should be selected which provides sufficient bandwidth for the experiment. Do not set the filter setting higher than required since this will increase the noise in the output signal. After entering the solution with the electrode the Cfast knob can be used if pulses are used to cancel fast capacitance transients.

7.2 Amperometric Measurement

The EVA 8 is ideal for constant voltage amperometric measurement. The front panel VHold potentiometer can be used to set the required voltage while the current of interest is measured at the current monitor output. The Stim Input can also be used to provide a constant DC input.

7.3 Cyclic Voltammetry

Cyclic voltammetry has become widely used in electrochemical technique. It is used rarely for quantitative analysis, but is widely used for the study of redox process. This technique is base on varying the applied potential at the working electrode in both forward and reverse directions and measuring the current.

Over the years many forms of potential modulation have been tried. The most common ones are normal pulse voltammetry. This technique uses a series of pulses of increasing magnitude. The current measurement is normally done at the end of each pulse. The second method is differential pulse voltammetry. In this technique a voltage scan is added to the pulses of the same magnitude creating a staircase effect. The current in this case is read at two points, before the pulse is applied and towards the end of each pulse. The third method is square wave voltammetry. In this technique the stimulus is a symmetrical square wave superimpose on a staircase waveform. Taking the difference between the forward and reverse currents derives the net current.

8. Low-Noise Recording

The Eva 8 amplifier has a particularly low background noise level. The noise level is in fact low enough that in most experimental situations it can be neglected in view of other background noise sources that make larger contributions to the total. As we consider these other sources, first let us make it clear that in this section we are concerned with random noise, which is fundamentally due to the thermal motion of electrons and ions; we assume that any user who is interested in low-noise recording has shielded and grounded his setup sufficiently well to take care of any synchronous noise due to line-frequency pickup, computer power supplies, TV cameras, etc. Synchronous noise can be readily identified as stationary features on an oscilloscope trace when the oscilloscope is triggered by the appropriate signal source, for example, line-frequency triggering. Grounding and shielding is discussed in chapter 6 on page 19.

Starting from the intrinsic noise with nothing connected to the probe and shielding the probe input, one observes increments in the noise level when the holder and pipette are installed and when an actual recording is made. By analyzing these increments, you can see where there is the most room for improvement in your technique.

As we mentioned in chapter 6 on page 19, the unshielded holder is greatly superior to the shielded one for low background noise. For low noise, the holder must be made from a low-loss, hydrophobic plastic; polycarbonate is one of the best, and Plexiglas one of the worst materials. (For our purposes, low-loss materials are those that show little dielectric relaxation in the frequency range of a few kHz. Dielectric relaxation involves the reorientation of dipoles within the material; since any dipoles will be in thermal motion, thermal reorientation in this frequency range will result in current fluctuations coupled capacitively into the pipette. It is very important that the pipette holder be kept clean and dry. Noise can be coupled into the pipette from the thermal motion of ions in films of aqueous solution, especially on the inside of the pipette. A good practice for low-noise work is to connect a valve to the pipette-suction line, and arrange for dry air or nitrogen to flow into the suction line during the time while you change pipettes.

9. Appendix

9.1 Controlling the Eva 8 with a Computer

The Eva 8 can be controlled by computer (Remote Mode) or manually (Local Mode). In the latter case computer can read the settings. This is done by setting or reading digital lines, which are accessible via the 40 pin connector on the rear panel of the Eva 8 (connector: 'COMPUTER CONTROL'). For pin assignment please refer to table 9.1 . In order to control the Eva 8 digitally the ITC-16 or ITC-18 can be used. The front panel LED when lit reflects the state of the Eva 8 as being controlled externally. When the Eva 8 is switched it defaults "Local Mode". The following controls can be set and read by computer:

- Gain and Gain Range
- Filter

Epc 8 Pin	**Name**	**ITC-16 Pin**	**Epc 8 Pin**	**Name**	**ITC-16 Pin**
1	Range0	IN-0	21	GND	GND
2	———	OUT-14	22	GND	GND
3	———	——-	23	——-	——-
4	EPC8-Mode	OUT-15	24	Strobe	STRout
5	Gain0	IN-2	25	Out0	OUT-0
6	ReadRemote	IN-10	26	——-	OUT-8
7	Gain1	IN-3	27	Out1	OUT-1
8	Filter0	IN-11	28	D1	OUT-9
9	Gain2	IN-4	29	——-	OUT-2
10	Filter1	IN-12	30	D0	OUT-10
11	Mode0	IN-5	31	——-	OUT-3
12	Filter2	IN-13	32	A1	OUT-11
13	Mode1	IN-6	33	——-	OUT-4
14	Filter3	IN-14	34	A0	OUT-12
15	Mode2	IN-7	35	A2	OUT-5
16	———	IN-9	36	SetRemote	OUT-13
17	Range1	IN-1	37	D3	OUT-6
18	———	CLIP	38	——-	IN-8
19	GND	GND	39	D2	OUT-7
20	GND	GND	40	——-	——-

Table 9.1: Pin Assignment Digital I/O. For convenience, the pin names of the ITC-16/ITC-18 boards are also listed.

9.1.1 Setting of Eva 8 functions

To set the functions of the EVA 8 by computer, the EVA 8-Mode line (pin 4) must be kept LOW. Before any other operation the EVA 8 must be set to 'Remote Mode'. To set a function proceed as follows:

1. Set the address lines (A2..A0) (e.g. '010' for 'Mode').
2. Set the data lines (D3..D0) (e.g. '0010' for 'Voltage Clamp Mode').
3. Transfer the setting to the EVA 8 by applying a negative going strobe on the line 'strobe' (pin 24) (high-low-high; Tstrobe ¿ 50 ns).

The following tables show the coding of the lines to set EVA 8 functions.

	D3	D2	D1	D0	A2	A1	A0
0.5	0	0	0	0	0	0	0
1	0	0	0	1	0	0	0
2	0	0	1	0	0	0	0
5	0	0	1	1	0	0	0
10	0	1	0	0	0	0	0
20	0	1	0	1	0	0	0

Table 9.2: Gain

	D3	D2	D1	D0	A2	A1	A0
Low	0	0	1	0	0	0	1
Med	0	0	0	0	0	0	1
High	0	0	0	1	0	0	1

Table 9.3: Gain Range

	D3	D2	D1	D0	A2	A1	A0	SetRemote
On	0	0	0	0	0	1	1	1
Off	0	0	0	0	0	1	1	0

Table 9.4: Set Remote

	D3	D2	D1	D0	A2	A1	A0
100 Hz	0	0	0	1	1	0	0
300 Hz	0	0	1	0	1	0	0
500 Hz	0	0	1	1	1	0	0
700 Hz	0	1	0	0	1	0	0
1 kHz	0	1	0	1	1	0	0
3 kHz	0	1	1	0	1	0	0
5 kHz	0	1	1	1	1	0	0
7 kHz	1	0	0	0	1	0	0
10 kHz	1	0	0	1	1	0	0
20 kHz	1	0	1	0	1	0	0
30 kHz	1	0	1	1	1	0	0
full	1	1	0	0	1	0	0

Table 9.5: Filter

9.1.2 Reading of Eva 8 settings

The settings of the Eva 8 can be read in all modes of operation. The following tables show the coding of the Eva 8 output lines:

	Gain2	**Gain1**	**Gain0**
0.5	0	0	0
1	0	0	1
2	0	1	0
5	0	1	1
10	1	0	0
20	1	0	1

Table 9.6: Gain

	Range1	**Range0**
Low	1	0
Med	0	0
High	0	1

Table 9.7: Gain Range

	Filter3	**Filter2**	**Filter1**	**Filter0**
100Hz	0	0	0	1
300Hz	0	0	1	0
500Hz	0	0	1	1
700Hz	0	1	0	0
1kHz	0	1	0	1
3kHz	0	1	1	0
5kHz	0	1	1	1
7kHz	1	0	0	0
10kHz	1	0	0	1
20kHz	1	0	1	0
30kHz	1	0	1	1
full	1	1	0	0

Table 9.8: Filter

	ReadRemote
On	1
Off	0

Table 9.9: Remote Mode

9.2 Connecting an Eva 8 to an EPC 9

The settings of the two amplifiers have to be performed separately: the computer controls The EPC 9, while the EVA 8 is controlled in "local mode" with the front switches.

9.2.1 Hardware connections:

Connect the EVA 8 with the EPC 9 on the rear panel with the flat cable. On the EPC 9 use the connector labeled "DIGITAL". Connect the voltage monitor of the EPC 9 with AD 0. Connect DA 0, 1 or 2 to the "Stim. In" input of the EVA 8 (DA 3 is internally connected to the "Stim. In" input of the EPC 9). Connect current and voltage monitor of the EVA 8 each to a free AD input of the EPC 9.

9.2.2 Software settings in Pulse:

1. In the configuration window select as AUX-Gain "source EVA 8".
2. Stimulation via EVA 8:
   (a) Select "Absolute voltage" in the Pulse Generator Window. Absolute voltage means that the user has to take care of the "Stim. In" factor of the EVA 8 (e.g., if the "Stim. In" factor is divide by 10 and the user wants to Stimulate with 80 mV, the value written in the PGF must be 800 mV). Also keep in mind that the "Stim. In" signal is **added** to V-hold. (e.g., if V-hold is -80 mV and the above given example is applied to the cell, the summed voltage will be 0 mV)

(b) Select the DA you connected to "Stim. In" of the EVA 8 as "StimDA" in the section "AD/DA Channels" of the Pulse Generator Window c) Select the AD-channels according to the signals you want to record.

3. Stimulation via EPC 9:

   (a) Select "Absolute Stimulus" or "Relative Stimulus" in the Pulse Generator (see PULSE manual)

   (b) Select "Epc9" or "Default" as "StimDA".

   (c) Select the AD-channels according to the signals you want to record.

9.3 Technical Data

Head stage current measuring resistors	50 GOhm 500 MOhm 5 MOhm
Largest measurable currents	200 pA (50 GOhm range) 20 nA (500 MOhm range) 2 μA (5 MOhm range)
Input Connector	Standard BNC
Gain	0.005 – 1000 mV/pA
Bandwidth	100 KHz (med and low ranges) 60 KHz (high range)
Filters	7 pole Bessel filter in 11 steps: 0.1, 0.3, 0.5, 0.7, 1, 3, 5, 7, 10, 20, 30 KHz 3 pole Bessel filter at full bandwidth
Cfast	0 – 10 pF calibrated, 0.5 to 5 μs time constant
Command Range	+/-2.0 Volt command range
Pipette offset range	+/- 0.5 Volt pipette offset range
Power Requirement	100 – 230 VAC, 50 – 60 Hz

Table 9.10: Technical Specifications of the EVA 8

9.4 Support Hotline

If you have any question, suggestion, or improvement, please contact HEKA. The best way is to sent us a fax specifying:

- Your address and your fax number
- The computer, acquisition hardware, and amplifier you are using
- The program name and version number you are using

- If applicable, the serial number of your Eva 8
- The questions, problems, or suggestions you have
- Under which conditions and how often the problem occurs

We will try to solve the problem as best and as soon as possible.

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