

OPERATING INSTRUCTIONS AND SYSTEM DESCRIPTION OF THE

VA-10X

VOLTAMMETRIC AND AMPEROMETRIC AMPLIFIER



VERSION 8.0
npi 2024

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>

Table of Contents

1. Safety Regulations	3
2. Introduction.....	4
3. VA-10X Components	5
4. VA-10X System.....	5
4.1. System Description.....	5
4.2. Description of the Front Panel.....	5
4.3. Description of the Rear Panel.....	9
5. Headstage.....	11
5.1. Headstage Elements.....	11
5.2. 3-Electrode Headstage (optional)	12
5.3. Headstage with customized feedback resistor	13
6. Operation	14
6.1. Setting up the VA-10X Voltammeter.....	14
6.2. Testing Basic Functions of the VA-10X	16
Open Circuit Test.....	16
DC Accuracy	17
Dynamic Test / Frequency Response	17
Setting the booster	18
Testing gain and filters	18
6.3. Carbon-Fiber Electrodes.....	18
6.4. Counter Electrode	19
6.5. Amperometric Measurements.....	19
6.6. Cyclic Voltammetry	19
7. Literature.....	20
8. Technical Data	24

1. Safety Regulations

VERY IMPORTANT: Instruments and components supplied by npí 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. npí electronic disclaims any warranties for such purpose. Equipment supplied by npí 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 npí electronic, D-71732 Tamm, Germany.

- 1) **GENERAL:** This system is designed for use in scientific laboratories and must be operated by trained staff only. General safety regulations for operating electrical devices are to be followed.
- 2) **AC MAINS CONNECTION:** While working with the npí systems, always adhere to the appropriate safety measures for handling electronic devices. Before using any device, please read manuals and instructions carefully.
The device is to be operated only at 115/230 Volt 60/50 Hz AC. Please check for appropriate line voltage before connecting any system to mains.
Always use a three-wire line cord and a mains power-plug with a protection contact connected to ground (protective earth).
Before opening the cabinet unplug the instrument.
Unplug the instrument when replacing the fuse or changing line voltage. Replace fuse only with an appropriate specified type.
- 3) **STATIC ELECTRICITY:** Electronic equipment is sensitive to static discharges. Some devices such as sensor inputs are equipped with very sensitive FET amplifiers, which can be damaged with electrostatic charge and must therefore be handled with care. Electrostatic discharge can be avoided by touching a grounded metal surface when changing or adjusting sensors. **Always turn power off when adding or removing modules, connecting or disconnecting sensors, headstages or other components from the instrument or 19" cabinet.**
- 4) **TEMPERATURE DRIFT / WARM-UP TIME:** All analog electronic systems are sensitive to temperature changes. Therefore, all electronic instruments containing analog circuits should be used only in a warmed-up condition (i.e. after internal temperature has reached steady-state values). In most cases a warm-up period of 20-30 minutes is sufficient.
- 5) **HANDLING:** Please protect the device from moisture, heat, radiation and corrosive chemicals.

2. Introduction

Recently, electrochemical methods using carbon-fiber microelectrodes have been applied to measure the release of oxidizable transmitter from *single cells*, and, even more impressively, from *single exocytotic vesicles*. Transmitters that are oxidizable and which, therefore, can be measured with this approach, include serotonin, dopamine, adrenaline, and noradrenaline. In addition, some peptides or proteins such as insulin may be oxidizable owing to the presence of oxidizable amino acids such as cysteine or tyrosine.

Cells that have been studied successfully with this technique include adrenal chromaffin cells, sympathetic neurons, mast cells, pancreatic beta cells, carotid glomus cells and melanotrophs, but the list is growing. In addition, in brain slices simultaneous intracellular and voltammetric studies have been made to correlate intracellular electric signals with transmitter release (10).

Two useful electrochemical approaches are **amperometry** and **cyclic voltammetry**. In **amperometry**, a DC potential is applied to a carbon-fiber microelectrode. The applied potential appears at the interface between the carbon and the mammalian ringer solution. If the potential is much greater than the redox potential for a given transmitter, then molecules of transmitter diffusing to the carbon surface are oxidized rapidly yielding a current that can be measured. The sensitivity of the **amperometric** approach, in particular, has provided an unprecedented look at the time course of transmitter release revealing distinct phases of release. On the other hand, the amperometric approach provides little information about the substance being oxidized or reduced.

Cyclic voltammetry provides a limited amount of information about the substance being studied, at some expense to the time resolution. In this approach a cyclically repeating voltage waveform, typically consisting of voltage ramps, is applied to the carbon-fiber electrode and the resulting current is plotted as a function of the applied voltage (after subtraction of a "background" record obtained in the absence of the redox species). Since different substances have different potentials for oxidation and for reduction one can distinguish transmitters from each other.

For more detailed information on the principles of electrochemical measurements at single cells and the fabrication of carbon-fiber microelectrodes refer to several recent reviews (see also chapter 7).

3. VA-10X Components

The following items are shipped with the VA-10X system:

- VA-10X amplifier
- Headstage
- GND connector for headstage (1 mm)
- COMMAND connector for headstage (1 mm)
- REF connector for headstage (1 mm)
- Mains cord
- Ground bridge
- User manual

Optional accessories:

- Electrode holder
- Carbon-fiber electrodes, \varnothing 5 μ m
- 3-electrode headstage with differential input
- Headstage with customized feedback resistor
- Test adapter

4. VA-10X System

4.1. System Description

The VA-10X is a sensitive (picoampere range) current amplifier that is intended for voltammetric measurements with carbon-fiber microelectrodes in biological systems, where the total currents do not exceed a ten to twenty of nA. It can be used for either DC amperometry using the built-in voltage source, or it can be operated with user-supplied external voltage waveforms (e.g. for cyclic voltammetry).

The VA-10X is ideally suited for measurements from single cells plated onto glass cover slips and with carbon-fiber disk electrodes having diameters of 10 μ m or less. However, it can also be used for measurements made on superficially located cells in tissue slices. The VA-10X is not recommended for use in *in vivo* recordings with carbon-fiber electrodes having long cylindrical measuring surfaces, because in this case currents approach the μ A range and a third electrode is required to compensate for the IR drop as currents flow through the extracellular fluid.

4.2. Description of the Front Panel

In the following description of the front panel elements each element has a number that is related to that in Figure 1. The number is followed by the name (in uppercase letters) written on the front panel and the type of the element (in lowercase letters). Then, a short description of the element is given. Some elements are grouped in functional units (e.g. OUTPUT unit) and are described as units regardless of the order of numbers.

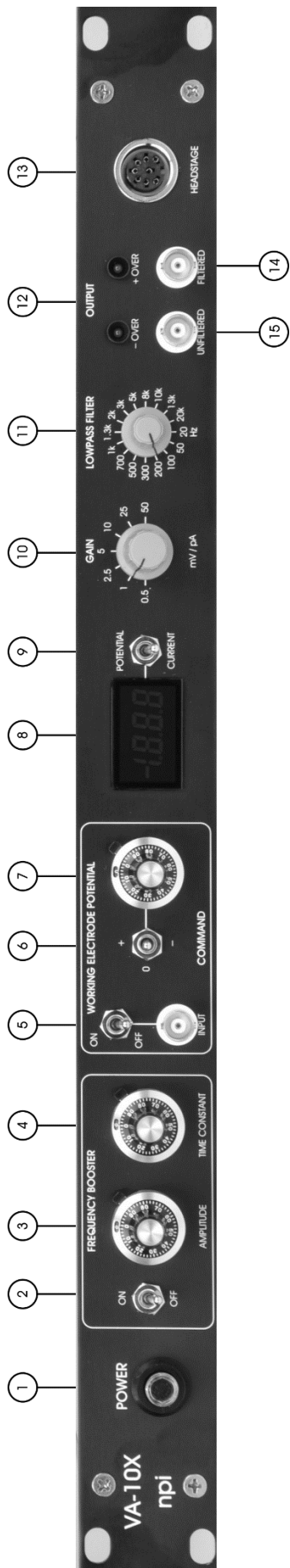


Figure 1: VA-10X Front panel view

(1) POWER switch

POWER switch to turn power on/off.

FREQUENCY BOOSTER unit

The FREQUENCY BOOSTER unit consists of **(2)** ON/OFF switch, **(3)** AMPLITUDE potentiometer and **(4)** TIME CONSTANT potentiometer.

(2) ON/OFF switch

Switch to enable (ON) or disable (OFF) the FREQUENCY BOOSTER.

(3) AMPLITUDE potentiometer

10-turn potentiometer for adjusting AMPLITUDE of the FREQUENCY BOOSTER.

(4) TIME CONSTANT potentiometer

10-turn potentiometer for adjusting TIME CONSTANT of the FREQUENCY BOOSTER.

Note: The BOOSTER is best adjusted by following the procedure described in chapter 6.2

WORKING ELECTRODE POTENTIAL - COMMAND unit

The WORKING ELECTRODE POTENTIAL - COMMAND unit consists of **(5)** INPUT ON/OFF switch with INPUT BNC connector, **(6)** WORKING ELECTRODE POTENTIAL - COMMAND polarity switch and **(8)** WORKING ELECTRODE POTENTIAL - COMMAND potentiometer

(5) WORKING ELECTRODE POTENTIAL - COMMAND INPUT ON/OFF switch

Switch to enable (ON) or disable (OFF) the INPUT BNC connector. The switch must be set to ON, if an external waveform is used. If no external waveform is used, the switch should be set to OFF to avoid coupling of noise

Important: If an external voltage source is used, the 3-position toggle switch controlling the internal voltage source (#6) should be set to "0". If the switch is set to "-" or "+", the voltage at the electrode is the sum of the external voltage and the internal voltage source.

Note: The input voltage will be scaled down by a factor of 10 (for maximum input voltage range of ± 1 V) or by a factor of 5 (for maximum input voltage range of ± 2 V).

(6) WORKING ELECTRODE POTENTIAL - COMMAND polarity switch

This switch selects the polarity of the WORKING ELECTRODE POTENTIAL - COMMAND voltage. “+” corresponds to a range of 0 - +1000 mV, “-” to a range of 0 - -1000 mV and “0” disables the internal voltage source.

(7) WORKING ELECTRODE POTENTIAL - COMMAND potentiometer

10-turn potentiometer for dialing the COMMAND voltage for DC amperometry if the internal voltage source is used. The polarity is set by switch #6 and the amplitude is displayed at #8.

(8, 9) POTENTIAL or CURRENT display and switch



Display (8) that shows the POTENTIAL at the working electrode or the CURRENT flowing through the working electrode. The switch (9) selects whether POTENTIAL or CURRENT is shown.

Note: The display is rather sluggish and will not show changes in the sub-second range.

(10) GAIN switch



The GAIN of the output signals is controlled by a 7-position rotary switch that selects gains of 0.5, 1, 2.5, 5, 10, 25 and 50 mV/pA.

(11) LOWPASS FILTER switch



16-position switch to select corner frequencies of the Bessel low pass filter. Corner frequencies: 20, 50, 100, 200, 300, 500, 700, 1k, 1.3k, 2k, 3k, 5k, 8k, 10k, 13k, 20k Hz.

OUTPUT unit



The OUTPUT unit consists of (12) - OVER + OVER LED, (14) OUTPUT FILTERED connector and (15) OUTPUT UNFILTERED connector

(3) - OVER + OVER LED

The VA-10X has an over voltage indicator with two LEDs, which light up if the amplifier is near it's positive or negative limit (± 10 V).

(14) OUTPUT FILTERED connector

The OUTPUT FILTERED BNC connector provides a voltage that is proportional to the current passed through the electrode (mV/pA). This voltage is FILTERED by a Bessel filter (see #11) and scaled by the GAIN factor (see #10).

(15) OUTPUT UNFILTERED connector

The OUTPUT UNFILTERED BNC connector provides a voltage that is proportional to the current passed through the electrode (mV/pA with GAIN 1). This voltage is scaled by the GAIN factor and not filtered.

(13) HEADSTAGE connector

The HEADSTAGE is connected via a flexible cable and an 8-pole connector to the mainframe.

Important: Always turn power off when connecting or disconnecting the headstage.

4.3. Description of the Rear Panel

Figure 2 shows the rear panel of the VA-10X. The rear panel elements are described below.

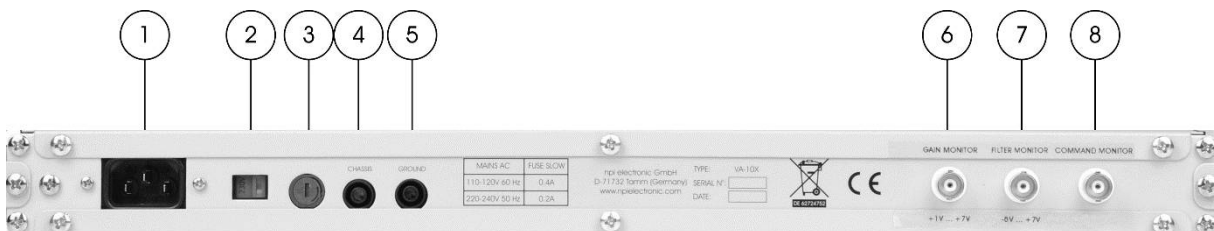


Figure 2: VA-10X rear panel view

(1) Mains connector

Plug for connecting the VA-10X to mains.

(2) Mains voltage selector

Turning knob for selecting the mains voltage, 115 V or 230 V.

Important: Switch always to the appropriate mains voltage before connecting the filter to mains.

(3) Fuse holder

Turning knob for the fuse holder. Turn counterclockwise to open.

Important: Use always the appropriate fuse for the present mains voltage before connecting the filter to mains (230 V / 0.2 A; 115 V / 0.4 A, slow).

(4) Cassis connector

This banana jack is linked to the chassis. It can be used for grounding the housing of VA-10X.

(5) Ground connector

This banana jack is linked to the internal system ground which has no connection to the 19" cabinet and to mains ground to avoid ground loops.

MONITORING unit

The MONITORING unit consists of **(6) GAIN MONITOR** connector, **(7) FILTER MONITOR** connector and **(8) COMMAND MONITOR** connector.

(6) GAIN MONITOR connector

BNC connector that monitors the setting of the GAIN switch. The GAIN is monitored in steps of 1 V as the gain knob is dialed clockwise (+1 V...+7 V).

(7) FILTER MONITOR connector

BNC connector that monitors the setting of the FILTER switch. The FILTER is monitored in steps of 1 V as the FILTER knob is dialed clockwise (-8 V...+7 V).

(8) COMMAND MONITOR connector

The COMMAND MONITOR voltage provided at this BNC connector is the voltage at the electrode, i.e. it is the sum of the setting at the COMMAND potentiometer (#7) and the INPUT voltage at the INPUT (#5).

5. Headstage

The VA-10X comes with the standard headstage (range: ± 1000 mV) for connecting carbon-fiber electrodes via an electrode holder (optional).

A 3-electrode headstage with differential input (see also **Optional accessories** in chapter 3, and chapter 5.2) is also available. For details contact npj.



Figure 3: Headstage of the VA-10X

5.1. Headstage Elements

- 1 BNC connector for the electrode holder
- 2 GROUND: ground
- 3 COMMAND: command potential output
- 4 headstage cable to amplifier
- 5 REFERENCE: not installed

In the 2-electrode headstage the REFERENCE is not installed. The table indicates whether the headstage is equipped with the standard feedback resistor (500 M Ω) or with a different one. It is also marked whether the headstage is in 2-electrode or in 3-electrode configuration.

The electrical connections are made like in a conventional patch-clamp headstage (e.g. the headstage of the EPC-7).

The carbon-fiber electrode fits into the BNC connector of the headstage (#1, Figure 3). An electrode holder (optional) gives additional mechanical stability. Ask npj for details. GROUND provides the ground and is linked to the bath, e.g. via an Ag-AgCl pellet. COMMAND provides the command potential at the electrode and remains usually open, but it can be used to optimize the measurements by connecting it to an electrode shield (see Ogden (1994) for setting up a

driven shield configuration). The headstage is attached to the amplifier with the headstage cable (see #4, Figure 3) and an 8-pole connector. For maximal flexibility the headstage is mounted on a plastic plate by customized screws. Thus, the user can modify the mounting plate according to his needs, e.g. to mount the headstage to a micromanipulator.

Note: The shield of the BNC connector and the enclosure of the headstage are linked to the command potential output (driven shield configuration) and must not be connected to ground.

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!

5.2.3-Electrode Headstage (optional)

The 3-electrode headstage differs from the standard headstage in having an additional 1 mm electrode connector (REFERENCE) between the GROUND and COMMAND connectors for measuring the bath potential. This signal is processed electronically, so that the command potential is floating with respect to the bath potential. Therefore, the command potential is independent from any bath potential that may occur. Usually an Ag-AgCl silver electrode or pellet is used for measuring the bath potential.

Important: If REF is not used, REF must be connected to GROUND.



Figure 4: VA-10X 3 electrode headstage with CFE electrode holder (optional)

Reference for typical application:

Marinesco, S. and Carew, T. J. (2002). Serotonin Release Evoked by Tail Nerve Stimulation in the CNS of *Aplysia*: Characterization and Relationship to Heterosynaptic Plasticity. *J. Neurosci.* **22**(6), 2299–2312.

5.3. Headstage with customized feedback resistor

The amplifier can be equipped with headstages that have a different feedback resistor compared to the standard headstage. Therefore, the scaling at the OUTPUT BNC connectors (#14 and #15, Figure 1) is different. The table below will give the scaling set at the GAIN rotary switch (#2, Figure 1) for the different feedback resistors. The resistor of your headstage will be indicated by a label on the headstage's housing.

Front Panel Scaling	Feedback							
	1 M Ω		5 M Ω		10 M Ω		50 M Ω	
	mV/pA	mV/nA	mV/pA	mV/nA	mV/pA	mV/nA	mV/pA	mV/nA
50	0.1	100	0.5	500	1	1000	5	5000
25	0.05	50	0.25	250	0.5	500	2.5	2500
10	0.02	20	0.1	100	0.2	200	1.0	1000
5	0.01	10	0.05	50	0.1	100	0.5	500
2.5	0.005	5	0.025	25	0.05	50	0.25	250
1	0.002	2	0.01	10	0.02	20	0.1	100
0.5	0.001	1	0.005	5	0.01	10	0.05	50

Front Panel Scaling	Feedback				
	100 M Ω	500 M Ω	1 G Ω	5 G Ω	10 G Ω
	mV/pA	mV/pA	mV/pA	mV/pA	mV/pA
50	10	50	100	500	1000
25	5	25	50	250	500
10	2	10	20	100	200
5	1	5	10	50	100
2.5	0.5	2.5	5	25	50
1	0.2	1	2	10	20
0.5	0.1	0.5	1	5	10

6. Operation

6.1. Setting up the VA-10X Voltammeter

The VA-10X amplifier is shipped as a stand-alone system and equipped with a small headstage with a BNC connector. When the system arrives the headstage will not be connected to the cabinet.

For biological voltammetric measurements the experimental setup typically consists of a microscope located within a Faraday cage to minimize noise pickup. A manipulator is used for positioning the voltammeter headstage with the attached electrode so that the electrode tip is near the cell(s) to be investigated.

- o Install the VA-10X unit in a convenient location near the experimental setup
- o Mount the headstage to the manipulator. Be careful and avoid static discharge during the handling of the headstage. Once the headstage has been mounted it can be connected to the VA-10X via the 8-pole connector.

Caution: Please always adhere to the appropriate safety regulations (see chapter 1). Please turn power off when connecting or disconnecting the headstage from the HEADSTAGE connector! Remember that the shield of the BNC connector and the enclosure of the headstage are linked to the command potential output (driven shield configuration) and must not be connected to ground.

To improve noise reduction of the setup, the faraday cage and the microscope may be connected to the INTERNAL GROUND located on the back of the VA-10X. Needless to say, grounding for low noise is an art. If you are not familiar with the principles of low noise connections, you should consult the local electrophysiology expert or electrical engineer.

- Set the switches at the front panel to the following positions:

Gain:	1 mV / pA
LP Filter:	20 kHz
3-position command polarity switch:	0
- Turn on the power. The reading of the display in the module is an indicator for a present power supply. The LCD should read 0.

As mentioned above the VA-10X can be used for DC amperometry, taking advantage of the internal voltage source, or it can be used with user-supplied external waveforms, e.g. for cyclic voltammetry.

- For use of the internal voltage source the 3-position command toggle has to be switched to “+” or “-” depending on the polarity of the desired command potential. The command voltage is displayed at the LCD in mV. This voltage is applied to the electrode mounted on the headstage.

If you intend to read the signal from the VA-10X into a data acquisition system:

- connect a BNC cable from the data acquisition system to the FILTERED or UNFILTERED connector (#14 or #15, Figure 1). Additionally, you can monitor the GAIN setting by connecting a BNC cable from the data acquisition system to GAIN MONITOR (#13, Figure 1) and / or the FILTER setting by connecting a BNC cable from the data acquisition system to FILTER MONITOR (#7, Figure 2: VA-10X rear panel view)

If you intend to use an external voltage source (e.g. for cyclic voltammetry):

- connect your external voltage source to the INPUT connector (#5, Figure 1). Remember that the input voltage will be scaled down by a factor of 10 at the headstage (for maximum input voltage range of ± 1 V) or by a factor of 5 at the headstage (for maximum input voltage range of ± 2 V). Note that, when an external voltage source is used, the 3-position toggle switch controlling the internal voltage source should be set to “0”, unless you want to sum the external voltage with the internal voltage source.

The VA-10X is now ready for measurements.

6.2. Testing Basic Functions of the VA-10X



Figure 5: VA-10X headstage with connected TEST ADAPTER.

All tests should be made in a noise free environment (e.g. Faraday cage or metal box connected to GROUND). Please be careful, the headstage is sensitive to electrostatic discharges. Please note that the headstage enclosure is NOT connected to GROUND, it is connected to the COMMAND signal applied to the microelectrode.

Special notice for 3-electrode headstage: The 3-electrode headstage differs from the standard headstage in having an additional 1 mm electrode connector (REFERENCE) between the GROUND and COMMAND connectors for measuring the bath potential. This signal is processed electronically, so that the command potential is floating with respect to the bath potential.

The REFERENCE input must not be open. It has to be connected to GROUND for these tests.

Before starting the tests, check that if everything at the amplifier is set to zero, that there is no offset at the output BNC connectors or digital meter. Also please check that the headstage enclosure (driven shield) is also at zero, e.g. with a digital meter. Then you are ready to start.

Open Circuit Test

- o Do not connect anything to the electrode BNC. With no command signals, the current should be zero.
- o Connect a pulse to the command input BNC connector. You should observe only capacitive transients and NO current during the pulse.

DC Accuracy

- Connect a 100 MΩ or another high value resistor from the electrode BNC to ground, or use our test adapter (see Figure 5).

Caution: Do not use the BNC shield or the headstage enclosure for grounding since they are connected to COMMAND!

- Apply a command signal of 100 mV DC to the headstage from the COMMAND setting of the voltmeter. Alternatively, connect a DC signal of 1 V to the INPUT BNC connector.

Important: If an external voltage source is used, the 3-position toggle switch controlling the internal voltage source (#6) should be set to “0”. If the switch is set to “-“ or “+”, the voltage at the electrode is the sum of the external voltage and the internal voltage source.

- Check with a digital meter that the headstage enclosure and the shield of the headstage BNC connector are at the COMMAND potential of 100 mV.
- The COMMAND MONITOR output BNC should provide the correct signal of 1 V.
- At the current output BNC should be a signal corresponding to Ohm’s Law and multiplied by the selected gain factor.
- Changing the polarity or magnitude of the command signal must lead to corresponding output signals, especially at the CURRENT OUT BNC connectors (according to Ohm’s Law).

Dynamic Test / Frequency Response

For this test a good signal generator with a ramp (triangle / sawtooth) output and an oscilloscope is required.

- Connect a 1 pF capacitor to the electrode BNC at the headstage or use our test adapter. To this capacitor connect a triangle wave generator with approx. 0.5 V pp and 20-100 Hz. Alternatively, you can use the INPUT connector and use a 5 V pp signal.
- This ramp is transferred into a small current following the formula:

$$I_c = C * \frac{dU}{dt}$$

where dU/dt is the slope of the triangle signal (V/sec).

- Observe the current at the UNFILTERED output using an oscilloscope.

Note: The observed current is always double since you change from a positive (+) slope to a negative (-) slope [x- (-x) = 2x].

Note The observed values might be slightly differ from the expectation. The amplitude of the current is also influenced by the accuracy of the capacitor and the connecting wires.

Setting the booster

- Start with AMPLITUDE and TIME CONSTANT turned into the left most position (counter-clockwise) and increase first AMPLITUDE and then TIME CONSTANT by turning the trim pots clockwise. By changing the amplitude and/or frequency you change the dU/dt , and so you can evaluate the range of linearity of the amplifier and also the frequency response.
- The BOOSTER is set correctly, if the current output is as square as possible. This also depends on the quality of the triangle wave at the 1 pF capacitor.

Testing gain and filters

- The effect of the gain stage and filters can be tested easily, if these tests work.
Gain stage: When testing the DC accuracy change the setting of the GAIN and observe the correct signal magnitude at the output BNC.
Filter: If the booster is set correctly connect the oscilloscope to the FILTERED output and change the filter corner frequency. You should see the changes on the shape of the pulses.

6.3. Carbon-Fiber Electrodes

Most voltammetric measurements in today's biological investigations involve the use of carbon-fiber electrodes. These electrodes can be purchased, or you can make your own. For use with the VA-10X voltammeter the electrodes must fit to the BNC connector at the input of the headstage. Two types of connection are commonly used:

- 1) direct connection via a BNC pin that is soldered onto the end of the electrode or
- 2) connection via a metal/liquid junction, for example using a 3 M KCl solution to interface the end of a carbon fiber to a Ag/AgCl wire.

For the first type of connection no special holder is required. For the metal/liquid junction type a special electrode holder must be used. For some electrodes a patch-pipette holder is adequate. Carbon fiber disk microelectrodes with small diameter (5-10 μm range) can be obtained from npj or ALA Scientific Instruments. The electrodes are manufactured using an anodic electrophoretic insulation method (see Schulte, A. and R. Chow, 1996, Anal. Chem. 68, 3054-3058).

6.4. Counter Electrode

The counter electrode used for biological measurements is typically an Ag/AgCl pellet (a sodium-saturated calomel electrode is sometimes used). The pellet should be immersed into the recording chamber and connected via a thin wire to the ground input of the headstage.

6.5. Amperometric Measurements

For high time resolution measurements of transmitter release from single vesicles DC amperometry is the appropriate approach. In this approach, the carbon-fiber electrode is energized with a command potential that exceeds the redox potential of the transmitter being studied. In practice, a command potential of equal to or greater than +650 mV is sufficient for measurements of all major oxidizable transmitters that have been studied so far.

To generate a command potential for DC amperometry, there should be no control voltage at the INPUT, and the INPUT coupling switch should be set to "OFF". The 3-position command toggle switch should be set for example to the "+" position. Then, the desired potential can be dialed in with the 10-turn potentiometer. As indicated above +650 mV is sufficient for most measurements. The amperometric signal is diffusion based. Thus, the distance between the carbon-fiber electrode detecting face and the cell surface must be kept to a minimum. For maximum signal size and most rapid kinetics, try to touch the cell membrane with the electrode.

6.6. Cyclic Voltammetry

In order to facilitate the identification of the transmitter being released, it is possible to use various voltage waveforms. One common approach is to apply fast voltage ramp potentials, i.e. to perform fast cyclic voltammetry.

For this application it is necessary to connect an external voltage source to the INPUT connector (#5, Figure 1) at the front panel of the VA-10X. Because one has to relate the measured current to the applied instantaneous voltage, the current and the applied voltage should be recorded simultaneously with a data acquisition system.

7. Literature

VA-10 Typical recordings

- Bai, J., Wang, C. T., Richards, D. A., Jackson, M. B., & Chapman, E. R. (2004). Fusion pore dynamics are regulated by synaptotagmin**t*-SNARE interactions. *Neuron* **41**, 929-942.
- Barclay, J. W., Craig, T. J., Fisher, R. J., Ciufu, L. F., Evans, G. J., Morgan, A., & Burgoyne, R. D. (2003). Phosphorylation of Munc18 by protein kinase C regulates the kinetics of exocytosis. *J Biol.Chem.* **278**, 10538-10545.
- Bertrand, P. P. (2006). Real-time measurement of serotonin release and motility in guinea pig ileum. *J Physiol.* **577**, 689-704.
- Bristol, A. S., Sutton, M. A., & Carew, T. J. (2004). Neural circuit of tail-elicited siphon withdrawal in aplysia. I. Differential lateralization of sensitization and dishabituation. *Journal of Neurophysiology* **91**, 666-677.
- Bristol, A. S., Marinesco, S., & Carew, T. J. (2004). Neural Circuit of Tail-Elicited Siphon Withdrawal in Aplysia. II. Role of Gated Inhibition in Differential Lateralization of Sensitization and Dishabituation. *Journal of Neurophysiology* **91**, 678-692.
- Britt, J. P. & McGehee, D. S. (2008). Presynaptic opioid and nicotinic receptor modulation of dopamine overflow in the nucleus accumbens. *Journal of Neuroscience* **28**, 1672-1681.
- Chan, S. A., Chow, R., & Smith, C. (2003). Calcium dependence of action potential-induced endocytosis in chromaffin cells. *Pflugers Arch.* **445**, 540-546.
- Chan, S. A., Polo-Parada, L., Landmesser, L. T., & Smith, C. (2005). Adrenal chromaffin cells exhibit impaired granule trafficking in NCAM knockout mice. *J Neurophysiol.* **94**, 1037-1047.
- Ciufu, L. F., Barclay, J. W., Burgoyne, R. D., & Morgan, A. (2005). Munc18-1 Regulates Early and Late Stages of Exocytosis via Syntaxin-independent Protein Interactions. *Molecular Biology of the Cell* **16**, 470-482.
- Evans, G. J., Barclay, J. W., Prescott, G. R., Jo, S. R., Burgoyne, R. D., Birnbaum, M. J., & Morgan, A. (2006). Protein kinase B/Akt is a novel cysteine string protein kinase that regulates exocytosis release kinetics and quantal size. *J Biol.Chem.* **281**, 1564-1572.
- Fischer, R. J., Pevsner, J., & Burgoyne, R. D. (2001). Control of Fusion Pore Dynamics During Exocytosis by Munc18. *Science* **291**, 875-878.
- Fulop, T., Radabaugh, S., & Smith, C. (2005). Activity-dependent differential transmitter release in mouse adrenal chromaffin cells. *J Neurosci.* **25**, 7324-7332.
- Graham, M. E., & Burgoyne, R. D. (2000). Comparison of Cysteine String Protein (Csp) and Mutant α -SNAP Overexpression Reveals a Role for Csp in Late Steps of Membrane Fusion in Dense-Core Granule Exocytosis in Adrenal Chromaffin Cells. *J.Neurosci.* **20**, 1281-1289.
- Graham, M. E., Barclay, J. W., & Burgoyne, R. D. (2004). Syntaxin/Munc18 interactions in the late events during vesicle fusion and release in exocytosis. *Journal of Biological Chemistry* M400827200.
- Han, X., Wang, C. T., Bai, J., Chapman, E. R., & Jackson, M. B. (2004). Transmembrane segments of syntaxin line the fusion pore of Ca^{2+} -triggered exocytosis. *Science* **304**, 289-292.
- Han, X. & Jackson, M. B. (2005). Electrostatic Interactions between the Syntaxin Membrane Anchor and Neurotransmitter Passing through the Fusion Pore. *Biophys.J.* **88**, L20-L22.
- Jaffe, E. H, Marty, A., Schulte, A. and Chow, R.H. (1998). Extrasynaptic vesicular transmitter release from the somata of substantia nigra neurons in rat midbrain slices. *J.Neurosci.* **18**, 3548-3553.

- Lerner, I., Trus, M., Cohen, R., Yizhar, O., Nussinovitch, I., & Atlas, D. (2006). Ion interaction at the pore of Lc-type Ca²⁺ channel is sufficient to mediate depolarization-induced exocytosis. *J Neurochem.* **97**, 116-127.
- Moore, J. M., Papke, J. B., Cahill, A. L., & Harkins, A. B. (2006). Stable gene silencing of synaptotagmin I in rat PC12 cells inhibits Ca²⁺-evoked release of catecholamine. *Am.J Physiol Cell Physiol.* **291**, C270-C281.
- Petzinger, G. M., Walsh, J. P., Akopian, G., Hogg, E., Abernathy, A., Arevalo, P., Turnquist, P., Vuckovic, M., Fisher, B. E., Togasaki, D. M., & Jakowec, M. W. (2007). Effects of treadmill exercise on dopaminergic transmission in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse model of basal ganglia injury. *J Neurosci.* **27**, 5291-5300.
- Wang, P., Wang, C. T., Bai, J., Jackson, M. B., & Chapman, E. R. (2003). Mutations in the effector binding loops in the C2A and C2B domains of synaptotagmin I disrupt exocytosis in a nonadditive manner. *J Biol.Chem.* **278**, 47030-47037.
- Wang, C. T., Bai, J., Chang, P. Y., Chapman, E. R., & Jackson, M. B. (2006). Synaptotagmin-Ca²⁺ triggers two sequential steps in regulated exocytosis in rat PC12 cells: fusion pore opening and fusion pore dilation. *J Physiol.* **570**, 295-307.
- Wang, H., Chan, S. A., Ogier, M., Hellard, D., Wang, Q., Smith, C., & Katz, D. M. (2006). Dysregulation of brain-derived neurotrophic factor expression and neurosecretory function in *Mecp2* null mice. *J Neurosci.* **26**, 10911-10915.
- Xie, Z., Herring, B. E., & Fox, A. P. (2006). Excitatory and Inhibitory Actions of Isoflurane in Bovine Chromaffin Cells. *Journal of Neurophysiology*
- Xu, J., Xu, Y., Ellis-Davies, G. C. R., Augustine, G. J. & Tse, F. W. (2002). Differential Regulation of Exocytosis by α - and β -SNAPs. *J.Neurosci.* **22**, 53–61.
- Zhang, Z. & Jackson, M. B. (2008). Temperature dependence of fusion kinetics and fusion pores in Ca²⁺-triggered exocytosis from PC12 cells. *J.Gen.Physiol.* **131**, 117-124.
- Zhuge, R., Decrescenzo, V., Sorrentino, V., Lai, F. A., Tuft, R. A., Lifshitz, L. M., Lemos, J. R., Smith, C., Fogarty, K. E., & Walsh, J. V., Jr. (2006). Syntillas release Ca²⁺ at a site different from the microdomain where exocytosis occurs in mouse chromaffin cells. *Biophys.J.* **90**, 2027-2037.

VA-10 USED FOR RECORDINGS WITH 3 ELECTRODES

- Marinesco, S., & Carew, T. J. (2002). Serotonin Release Evoked by Tail Nerve Stimulation in the CNS of *Aplysia*: Characterization and Relationship to Heterosynaptic Plasticity. *J.Neurosci.* **22**, 2299–2312.
- Marinesco, S., & Carew, T. J. (2002). Improved electrochemical detection of biogenic amines in *Aplysia* using base-hydrolyzed cellulose-coated carbon fiber microelectrodes. *J.Neurosci.Meth.* **117**, 87-97.
- Marinesco, S., Kolkman, K. E., & Carew, T. J. (2004). Serotonergic modulation in *Aplysia*. I. Distributed serotonergic network persistently activated by sensitizing stimuli. *J Neurophysiol.* **92**, 2468-2486.
- Marinesco, S., Wickremasinghe, N., Kolkman, K. E., & Carew, T. J. (2004). Serotonergic modulation in *Aplysia*. II. Cellular and behavioral consequences of increased serotonergic tone. *J Neurophysiol.* **92**, 2487-2496.
- Marinesco, S., Wickremasinghe, N., & Carew, T. J. (2006). Regulation of behavioral and synaptic plasticity by serotonin release within local modulatory fields in the CNS of *Aplysia*. *J Neurosci.* **26**, 12682-12693.

VA-10 USED FOR RECORDINGS WITH ELECTRODE ARRAYS

- Dias, A. F., Dernick, G., Valero, V., Yong, M. G., James, C. D., Craighead, H. G., & Lindau, M. (2002). An electrochemical detector array to study cell biology on the nanoscale. *Nanotechnology* **13**, 285-289.
- Hafez, I., Kislser, K., Berberian, K., Dernick, G., Valero, V., Yong, M. G., Craighead, H. G., & Lindau, M. (2005). Electrochemical imaging of fusion pore openings by electrochemical detector arrays. *Proc.Natl.Acad.Sci.U.S.A* **102**, 13879-13884.
- Spéjel, C., Heiskanen, A., Acklid, J., Wolff, A., Taboryski, R., Emnéus, J., & Ruzgas, T. (2007). On-Chip Determination of Dopamine Exocytosis Using Mercaptopropionic Acid Modified Microelectrodes. *Electroanalysis* **19**, 263-271.

VA-10 USED FOR SCANNING ELECTROCHEMICAL MICROSCOPY

- Etienne, M., Schulte, A., & Schuhmann, W. (2004). High resolution constant-distance mode alternating current scanning electrochemical microscopy (AC-SECM). *Electrochem.Commun.* **6**, 288–293.
- Hengstenberg, A., Dietzel, I. D., & Schuhmann, W. (1999). Visualization of biological activities using the scanning electrochemical microscope. In: *Monitoring Molecules in Neuroscience*. ed.: Rollema, H., Abercombie, E., Sulzer, D., & Zackheim, J., Proceedings of the 8th international conference on *in vivo* methods, 19-23 June 1999, S.U.N.Y. at Stony Brook, New York, USA, 47-48.
- Hengstenberg, A., Dietzel, I. D., Blöchl, A., & Schuhmann, W. (1999). Zell-Zell-Kommunikationsprozesse mittels elektrochemischer Rastermikroskopie. *BioForum* **10**, 595-599, GIT Verlag, Darmstadt, Germany.
- Turcu, F., Schulte, A., Hartwich, G., & Schuhmann, W. (2004). Label-Free Electrochemical Recognition of DNA Hybridization by Means of Modulation of the Feedback Current in SECM. *Angew.Chem.Int.Ed Engl.* **43**, 3482-3485.

REFERENCES (METHODS)

- Alvarez de Toledo, G., Fernandez-Chacon, R., & Fernandez, J. M. (1993). Release of secretory products during transient vesicle fusion. *Nature* **363**, 554-557.
- Britt, J. P. & McGehee, D. S. (2008). Presynaptic opioid and nicotinic receptor modulation of dopamine overflow in the nucleus accumbens. *Journal of Neuroscience* **28**, 1672-1681.
- Chow, R. H., von Rüden, L., & Neher, E. (1992). Delay in vesicle fusion revealed by electrochemical monitoring of single secretory events in adrenal chromaffin cells. *Nature* **356**, 60-63.
- Chow, R. H., & von Rüden, L. (1995). Electrochemical Detection of Secretion from Single Cells, in: Sakmann, B., & Neher, E. (eds.). *Single Channel Recording*. Plenum Press, New York and London.
- Doreian, B. W., Fulop, T. G., & Smith, C. B. (2008). Myosin II activation and actin reorganization regulate the mode of quantal exocytosis in mouse adrenal chromaffin cells. *Journal of Neuroscience* **28**, 4470-4478.
- Huang, L., Shen, H., Atkinson, M. A., & Kennedy, R. T. (1995). Detection of exocytosis at individual pancreatic beta-cells by amperometry at a chemically-modified microelectrode. *Proc.Natl.Acad.Sci.* **92**, 9608-9612.
- Kudernatsch, M., & Sutor, B. (1994). Cholinergic modulation of dopamine overflow in the rat neo-striatum: a fast cyclic voltammetric study in vitro. *Neurosci.Lett.* **181**, 107-112.
- Paras, C. D., & Kennedy, R. T., (1995). Electrochemical detection of exocytosis at single-rat melanotrophs. *Anal.Chem.* **67**, 3633-3637.
- Smith, P. A., Duchen, M. R., & Ashcroft, F. M., (1995). Fluorometric and amperometric study of calcium and secretion in isolated mouse pancreatic beta-cells. *PflugersArch.* **430**, 808-818.
- Souvannakitti, D., Kumar, G. K., Fox, A., & Prabhakar, N. R. (2009). Neonatal Intermittent Hypoxia Leads to Long-Lasting Facilitation of Acute Hypoxia-evoked Catecholamine Secretion from Rat Chromaffin Cells. *J.Neurophysiol.*
- Taylor, A. R., & Chow, H. (2001). A microelectrochemical technique to measure trans-plasma membrane electron transport in plant tissue and cells *in vivo*, *PlantCellEnviron*, **24**, 1-6.
- Urena, J., Fernandez-Chacon, R., Benot, A. R., Alvarez de Toledo, G., & Lopez-Barneo, J., (1994). Hypoxia induces voltage-dependent Ca²⁺ entry and quantal dopamine secretion in carotid body glomus cells. *Proc.Natl.Acad.Sci. USA* **91**, 10208-10211.
- Wightman, R.M., J.A. Jankowski, R.T. Kennedy, K.T. Kawagoe, T.J. Schroeder, D.J Leszczyszyn, J.A. Near, E.J. Dilberto, Jr., and O.H. Viveros. 1991. Temporally resolved catecholamine spikes correspond to single vesicle release from individual chromaffin cells. *Proc.Natl.Acad.Sci. USA* **88**, 10754-10758.
- Zhou, Z, Misler, S., & Chow, R. H. (1996). Rapid fluctuations in transmitter release from single vesicles in bovine adrenal chromaffin cells. *Biophys.J.* **70**, 1543-1552.
- Zhou, Z., & Misler, S., (1995). Amperometric detection of stimulus-induced quantal release of catecholamines from cultured superior cervical-ganglion neurons. *Proc.Natl.Acad.Sci. USA* **92**, 6938-6942.
- Zhou, Z., & Misler, S. (1996). Amperometric detection of quantal secretion from patch-clamped rat pancreatic beta-cells. *J.Biol.Chem.* **271**, 270-277.

8. Technical Data

Headstage:

Input voltage range:	± 1200 mV
Operating voltage:	± 12 V
Enclosure:	Size: 40 x 70 x 20 mm, driven shield (COMMAND potential) mounting plate: 50 x 70 mm, not conducting
Electrode connector:	BNC with shield (COMMAND potential)
Ground connector:	1 mm connector (black)
Command output:	1 mm connector (red)
Current range:	± 20 nA max. (depending on feedback resistor)
Feedback resistor:	500 M Ω

Noise: <1 pA

LP FILTER: Bessel Filter

Attenuation: 4-pole: -24 dB/octave; 8-pole (optional): -48 dB/octave
corner frequencies: 20, 50, 100, 200, 300, 500, 700, 1 k, 1.3k, 2k, 3k, 5k, 8k, 10k, 13k, 20k Hz

GAIN: 7-position rotary switch
(x0.5, x1, x2.5, x5, x10, x25 and x50 mV / pA)

Voltage source: COMMAND VOLTAGE set by 10-turn potentiometer, range: ± 1000 mV (standard), polarity selectable with polarity switch (+, 0, -), display XXXX mV

OUTPUTS:

Impedance:	50 Ω
Linear voltage range:	± 10 V
FILTERED:	BNC connector, sensitivity selectable with GAIN switch, FILTERED with LP Bessel FILTER
UNFILTERED:	BNC connector, sensitivity selectable with GAIN switch

INPUTS:

Resistance:	1 M Ω
COMMAND VOLTAGE:	INPUT BNC connector, also displayed

MONITORING:

Impedance:	50 Ω
GAIN:	BNC connector, 1 V per step, +1 V...+7 V
FILTER:	BNC connector, 1 V per step, -8 V...+7 V
COMMAND:	BNC connector, sensitivity: x1

Dimensions: 19" rackmount cabinet, 19" (483 mm), 10" (250 mm), 1.75" (44 mm)

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