WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System

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1. PREFACE

1.1 Scope

This User Guide describes the WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System. This guide is written for the scientist or engineer (student or professional) and assumes a basic knowledge of scientific measurement and data presentation. Portions of this manual devoted to electrochemical concepts assume some familiarity with the subject of electrochemistry.

A small portion of this guide is dedicated to using the HDCV software package to control the WaveNeuro instrument, primarily in the context of installing the instrument and verifying that it is working correctly. More extensive descriptions of how to use the HDCV software are provided in the additional documents listed below:

- *HDCV User Guide* (describes data acquisition software functions)
- *HDCV Analysis User Guide* (describes data analysis with HDCV data)

At the time of this user guide revision, both of the additional documents listed above are available for download from The University of North Carolina at Chapel Hill Electronics Shop Software download page:

http://chem.unc.edu/facilities/electronics_software.html

1.2 Copyright

1.3 Trademarks

1.4 Use Limitation

The WaveNeuro instrument is not designed for use in experiments involving human subjects and/or the use of electrodes inside or on the surface of the human body.

Any use of this instrument other than its intended purpose is prohibited.

1.5 Service and Warranty Information

For questions about proper operation of the WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System or other technical issues, please use the contact information below to contact Pine directly.

TECHNICAL SERVICE CONTACT
Pine Research Instrumentation, Inc. **http://www.pineresearch.com** **Phone: +1 (919) 782-8320** **Fax: +1 (919) 782-8323**

If the WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System or one of its components or accessories must be returned to the factory for service, please contact Technical Service (see above) to obtain a Return Material Authorization (RMA) form. Include a copy of this RMA form in each carton and ship to the Factory Return Service Address (below).

FACTORY RETURN SERVICE ADDRESS
Pine Instrument Company **ATTN: RMA # <RMA number>** **104 Industrial Drive** **Grove City, PA 16127** **USA**

Return Material Authorization Required!

Do not ship equipment to the factory without first obtaining a Return Material Authorization (RMA) form from Pine Research Instrumentation, Inc.

LIMITED WARRANTY

The Pine WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System (Pine part number AF01FSCV1, hereafter referred to as “WARRANTED INSTRUMENT”) offered by Pine Research Instrumentation, Inc. (hereafter referred to as “SUPPLIER”) is warranted to be free from defects in material and workmanship for a one (1) year period from the date of shipment to the original purchaser (hereafter referred to as the "CUSTOMER") if used under normal laboratory conditions. SUPPLIER’s obligation under this warranty is limited to replacing or repairing parts which shall upon examination by SUPPLIER personnel disclose to SUPPLIER’s satisfaction to have been defective. The CUSTOMER may be obligated to assist SUPPLIER personnel in servicing the WARRANTED INSTRUMENT. SUPPLIER will provide remote support (via telephone or internet) to guide the CUSTOMER to diagnose and effect any needed repairs. In the event that remote support is unsuccessful in resolving the defect, SUPPLIER may recommend that the WARRANTED INSTRUMENT be returned to SUPPLIER for repair. This warranty is expressly in lieu of all other warranties, expressed or implied and all other liabilities. All specifications are subject to change without notice. The CUSTOMER is responsible for charges associated with non-warranted repairs, such charges including but not limited to travel expenses, tariffs, labor, parts and freight charges.

This warranty does not apply to headstage amplifiers, cables, or other accessories that are used in conjunction with the WARRANTED INSTRUMENT.

1.6 Icons (*Icônes*)

Special icons (see: Table 1-1) are used to call attention to safety warnings and other useful information found in this document.

Des icônes spéciales (voir: tableau 1-1) sont utilisées pour attirer l'attention sur des avertissements de sécurité et autres renseignements utiles disponibles dans ce document.

	**CAUTION:** **Indicates information needed to prevent injury or death to a person or to prevent damage to equipment.** ***ATTENTION:*** ***Indique les informations nécessaires à la prévention de blessures corporelles ou de mort d'un individu ou à la prévention des dommages aux équipements.***
	**STOP:** **For a procedure involving user action or activity, this icon indicates a point in the procedure where the user must stop the procedure.** ***ARRÊT:*** ***Dans une opération impliquant l'action ou l'activité d'un utilisateur, cette icône indique la partie de l'opération où l'utilisateur doit arrêter l'opération.***
	**NOTE:** **Important or supplemental information.** ***REMARQUE:*** ***Renseignements importants ou complémentaires.***
	**TIP:** **Useful hint or advice.** ***CONSEIL:*** ***Astuce ou conseil utile.***

Table 1-1. Special Icons used in this Document

(Tableau 1-1. Icônes spéciales utilisées dans ce document)

1.7 Back Panel Markings

The side/end panel of the WaveNeuro bears information to identify the instrument (see: Figure 1-1).

Figure 1-1: WaveNeuro Side/End Panel Markings

1.7.1 Serial Number

For purposes of uniquely identifying a particular instrument, there is a label on the side/end panel of each WaveNeuro which indicates the serial number. The serial number is also encoded with a machine readable barcode on the same label (see: Figure 1-1).

1.7.2 Model Numbers

Pine Research part numbers for the WaveNeuro instrument, power cords, and accessories are described in more detail later (see Section 2.5). WaveNeuro model numbers have the format *AF01FSCV#*, where the # indicates the WaveNeuro series model.

1.8 Safety Warnings (Avertissements de sécurité)

CAUTION:

Connect the Power Supply to the AC mains using the Power Cord supplied with the WaveNeuro and certified for the country of use. (see: Section 0 of this User Guide for more details).

AVERTISSEMENT:

Connectez le bloc d'alimentation au secteur à l'aide du cordon d'alimentation fourni avec l'appareil WaveNeuro et conforme aux réglementations du pays d'utilisation (pour plus de détails, consultez la partie 0 du présent mode d'emploi).

CAUTION:

Do not block access to the Power Supply or the Power Cord. The user must have access to disconnect the Power Supply or the Power Cord from the AC mains at all times.

AVERTISSEMENT:

Ne bloquez pas l'accès au bloc d'alimentation ou au cordon d'alimentation. L'utilisateur doit être en mesure de déconnecter le bloc d'alimentation ou le cordon d'alimentation du secteur à tout moment.

CAUTION:

Connect the Power Supply to the AC mains using the Power Cord and appropriate plug style adapter supplied with the WaveNeuro.

AVERTISSEMENT:

Branchez l'alimentation au secteur à l'aide du cordon d'alimentation et de l'adaptateur de type de prise fourni avec WaveNeuro.

CAUTION:

The switch on the back of the WaveNeuro turns the power to the potentiostat on and off. Do not block access to the switch. The user must have access to the switch at all times.

AVERTISSEMENT:

L'interrupteur à l'arrière du WaveNeuro permet l'allumage et l'arrêt du potentiostat. Ne pas bloquer l'accès à l'interrupteur. L'utilisateur doit avoir accès à l'interrupteur en tout temps.

CAUTION:

Provide proper ventilation for the WaveNeuro. Maintain at least two inches ($50\ mm$) of clearance around the sides (left, right, and back) and above (top) the instrument.

AVERTISSEMENT:

Assurez-vous que l'appareil WaveNeuro. Soit correctement ventilé. Laissez au moins $50\ mm$ (2 po) autour de l'appareil (à gauche, à droite et derrière), ainsi qu'au-dessus.

CAUTION:

Do not operate the WaveNeuro in an explosive atmosphere.

AVERTISSEMENT:

N'utilisez pas l'appareil WaveNeuro dans une atmosphère explosive.

CAUTION:

Do not operate the WaveNeuro in wet or damp conditions. Keep all instrument surfaces clean and dry.

AVERTISSEMENT:

N'utilisez pas l'appareil WaveNeuro dans un environnement humide. Veillez à ce que toutes les surfaces de l'appareil soient toujours propres et sèches.

CAUTION:

Do not operate the WaveNeuro if it has suffered damage or is suspected of having failed. Refer the instrument to qualified service personnel for inspection.

AVERTISSEMENT:

N'utilisez pas l'appareil WaveNeuro s'il a été endommagé ou si vous pensez qu'il est tombé en panne. Signalez l'appareil au personnel d'entretien qualifié pour qu'il soit examiné.

1.9 Electrostatic Discharge Information

Electrostatic discharge (ESD) is the rapid discharge of static electricity to ground. An ESD event occurs when two bodies of different potential approach each other closely enough such that static charge rapidly passes from one object to the next. Sensitive electronics in the path of the discharge may suffer damage. Damaging ESD events most often arise between a statically charged human body and a sensitive electronic circuit. The human body can easily accumulate static charge from simple movement from one place to another (*i.e.*, walking across a laboratory).

Potentiostat users must always be aware of the possibility of an ESD event and should employ good practices to minimize the chance of damaging the instrument. Some examples of good ESD prevention practices include the following:

- Self-ground your body before touching sensitive electronics or the electrodes. Self-grounding may be done by touching a grounded metal surface such as a water pipe.
- Wear a conductive wrist-strap connected to a good earth ground to prevent a charge from building up on your body.
- Wear a conductive foot/heel strap or conductive foot wear in conjunction with standing on a grounded conductive floor mat.
- Increase the relative humidity in the air to minimize static generation.

INFO:

The WaveNeuro instrument may be susceptible to ESD events that occur on or near the electrode cable assembly. Such an ESD event can result in data loss, corruption of data, loss of communication with PC, and instrument unresponsiveness.

1.10 Software Information

The Pine Research WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System utilizes a National Instruments Data Acquisition Device (NI PCIe-6363) to interface to a personal computer. The WaveNeuro can be operated with High Definition Cyclic Voltammetry (HDCV). HDCV is a fast-scan cyclic voltammetry data collection and analysis program written by the research lab of R. Mark Wightman. HDCV was written in NI LabVIEW™ and requires LabVIEW Run-Time 2009™ or higher, NI-DAQ™ and NI-MAX™ to operate properly. NI-MAX™ software is required for accessing the National Instruments system and is provided with the data acquisition device. Academic users can download HDCV software at the following link:

http://www.chem.unc.edu/facilities/electronics_software.html

2. INSTRUMENT INFORMATION

2.1 Background Subtracted Fast-Scan Cyclic Voltammetry Overview

Fast-scan cyclic voltammetry (FSCV) is an electrochemical technique that has been gaining popularity over the last few decades. FSCV makes analytical measurements in dynamic environments. With FSCV, a rapid potential ramp, typically $> 100\ V/s$, is continuously applied to a microelectrode. The application of this rapid potential ramp to the electrode generates large Faradaic current, due to electrochemical processes, and non-Faradaic current, mostly due to ion movement. The non-Faradaic current is stable over short periods of time (typically tens of seconds); therefore, current does not significantly change in short time periods. Faradaic current variation arising from changes in electroactive species concentration can be resolved by monitoring the difference in current over time. Typically, to visualize the changes in the concentration of electroactive species, one selects a single cyclic voltammogram (CV) and defines it as the background. During a defined collection time, the background CV is subtracted from experimental CVs. The resultant background-subtracted CV results in analytical data, which encodes changes in electroactive species as a function of time. CV peak positions provide clues as to the identity of the species measured, while the peak current magnitude corresponds a change in concentration of the species.

A series of background subtracted CVs collected during a short period of time can be visualized as an electrochemical color plot. The color plot is a graphical representation of a series of CVs, with voltage plotted on the $y-axis$, time plotted on the $x-axis$, and faux-color representing the current magnitude. The color plot enables large numbers of background subtracted CVs to be analyzed and changes in concentration of electroactive species to be quickly identified (see: Figure 2-1).

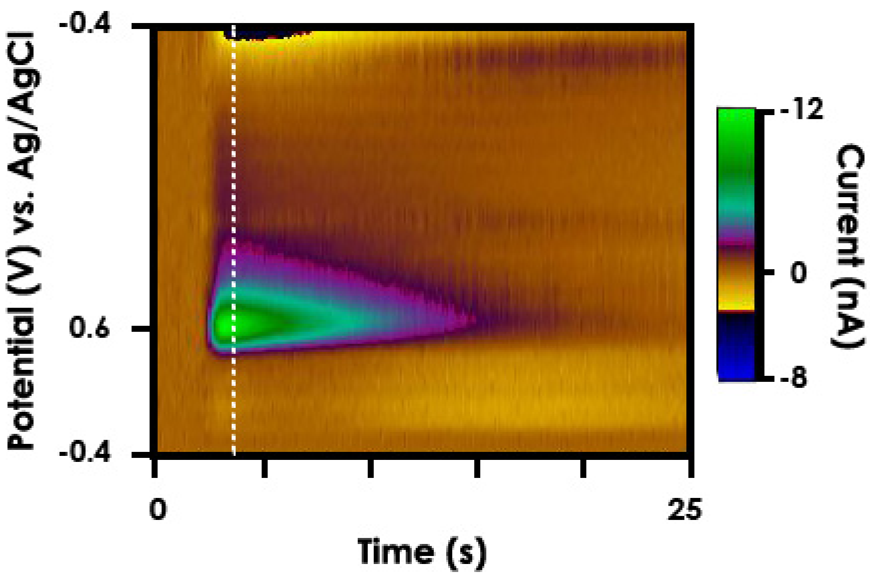


Figure 2-1. FSCV Color Plot of Evoked Dopamine Release in a Freely Moving Rat

FSCV monitors rapid fluctuations in the concentrations of electroactive species, such as electroactive neuromodulators, in small volumes of solution with very good limits of detection. As the technique has evolved, there are now protocols to maximize responsivity and selectivity to monitor changes in the extracellular concentration of electroactive neurotransmitters, such as catecholamines, in functioning tissue and even behaving animals. The typical data representation for FSCV is a colorplot, which is a stack of many CVs in time (see: Figure 2-1). Viewing CV data as a colorplot highlights differential electrochemical behavior, such as for the release of dopamine in the dorsal striatum of a rat (see: Figure

2-1). Studies utilizing FSCV have led to new insights in neuroscience. FSCV is quickly becoming an established technique in neuroscience for monitoring extracellular changes in concentration of electroactive biomolecules, such as catecholamines.

2.2 Instrument Description

The WaveNeuro Fast-Scan Cyclic Voltammetry System is a benchtop potentiostat specially designed for FSCV measurements. The WaveNeuro connects to a personal computer via a National Instruments data acquisition card and is controlled by a third party software, such as High Definition Cyclic Voltammetry (HDCV) software. The WaveNeuro controls a two-electrode electrochemical system. The electrochemical accessories that connect to the WaveNeuro are one working electrode, typically a carbon fiber microelectrode, and a reference electrode often Ag/AgCl (see: Section 2.7.7 for more details about electrode connections).

The WaveNeuro applies a potential waveform that is controlled by the software. Note that the WaveNeuro is designed to apply the electrochemical waveform to the working electrodes and maintain the reference electrode at ground (see: Section 3).

INFO:

The WaveNeuro applied the waveform to the working electrode and maintains the reference electrode at ground. As a result, when no electrodes are connected (connector just in air) to the headstage amplifier, HDCV will show a current response that follows the applied waveform. This is the expected observation. Further details are provided in Section 3.

The measured current range is defined by the gain of the electrochemical headstage. The standard Pine Research headstage provides a current range of $\pm 2{,}000\ nA$ $(5\ M\Omega)$. Additional headstage amplifier gains and configurations may be available from Pine Research. Please contact us with any special requests/custom gain levels.

An optional WaveNeuro Dual Headstage Adapter is available as an add-on item. The Dual Headstage Adapter converts the single channel WaveNeuro to a dual channel WaveNeuro, enabling the use of two head stages with a single WaveNeuro and data acquisition card (see: Section 7).

2.3 Software Description

The WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat system has been designed for use with third party data collection and analysis software. Specifically, our engineers have targeted the use of High Definition Cyclic Voltammetry (HDCV).

The HDCV software was written the R. Mark Wightman Research Group at the University of North Carolina at Chapel Hill. The HDCV scientific software is a LabVIEW based application which interfaces the WaveNeuro to a National Instruments interface board. HDCV performs complex electrochemical experiments and HDCV Analysis generates high quality graphs from experimental results.

HDCV Software can be downloaded free of charge to qualified academic users, as described on the website here: http://www.chem.unc.edu/facilities/electronics_software.html.

2.4 Instrument Specifications

The WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System offers a complete solution to perform fast-scan cyclic voltammetry.

INFO:

All specifications provided in this section are subject to change without notice.

EXTERNAL ELECTRODE CONNECTIONS	
Headstage Connector	Female DB-25 provides electrode signal lines and power to the electrochemical headstage and supports electrical stimulation (top panel)
Behavioral Connector	Female DB-25 provides digital input lines for external sources (top panel)
Stimulus Connector	Male, two-prong connector receives stimulation current from external source
Reference Electrode	White lead wire from the electrochemical headstage
Working Electrode	Yellow lead wire from the electrochemical headstage

GROUNDING	
Signal Ground (DC Common)	The signal ground is isolated from the power plug and floats with respect to the instrument chassis and earth ground.
Chassis Terminal	The chassis terminal is a banana binding post (back panel) which may optionally be used to connect the chassis to earth ground to improve noise screening (shielding).

MEASURED CURRENT	
Current Range	Defined by electrochemical headstage, $\pm 200\ nA/V\ (5\ M\Omega)$ headstage amplifier included
Resolution	16-bit resolution (provided by the interface board)
Filters	Selectable: low-pass up to 14 kHz, low-pass up to 40 kHz, bandpass (100 Hz to 14 kHz) or bypass (no filtering)
Aux Input Resolution	16-bit resolution (provided by the interface board)

APPLIED POTENTIAL (POTENTIAL RAMP WAVEFORM)

Ranges	$\pm 3.3\ V$ (maximum range)
Resolution	$100\ \mu V$ (minimum resolution defined by the interface board)
DAC Output	16-bit resolution (provided by the interface board)
Filters	Selectable; low-pass up to $2\ kHz$, $5\ kHz$ or $10\ kHz$, and bypass (no filtering)
CV Scan Rate (max)	$5{,}000\ V/s$

MEASURED POTENTIAL (FRONT PANEL LABELED AS NON-CV IN)

Ranges	$\pm 10.0\ V$ (maximum range defined by the interface board)
Resolution (at each range)	$313\ \mu V$ (minimum resolution defined by the interface board)
ADC Input	16-bit resolution (provided by the interface board)
Filter	Up to 20 kHz low-pass filter

APPLIED POTENTIAL OUT (FRONT PANEL LABELED AS ABS OUT)

Ranges	$\pm 10.0\ V$ (maximum range defined by the interface board)
Resolution (at each range)	$313\ \mu V$ (minimum resolution defined by the interface board)
ADC Input	16-bit resolution (provided by the interface board)
Filter	Up to 5 kHz low-pass filter

DATA ACQUISITION

Point Interval	$500\ ns$ (minimum)
Synchronization	simultaneous sampling of all analog input signals
Raw Point Total	>10 million per experiment

ACCESSORIES

Dummy Cell	external dummy cell (included)
Headstage Cable	DB-25 to Pine Research electrochemical headstage
Headstage Amplifier	$200\ nA/V$ $(5\ M\Omega)$, working electrode driven (other gains available by special order)
Electrode Connections	white = reference; yellow = working
Power Supply	C14-type socket (includes plug-specific $10\ A$ cord)

OUTPUT CONNECTIONS (TOP PANEL)

All output connections to the WaveNeuro are via female BNC-type connectors.

STIM +	Digital, TTL compatible
STIM -	Digital, TTL compatible
CVF	CV frequency; Digital, TTL compatible
SPARE	Digital, TTL compatible
FLOW	Flow cell trigger; Digital, TTL compatible
E-PHYS	Timing Trigger; Digital, TTL compatible
STIM OUT	Stimulus waveform; Analog, max $\pm 10.0\ V$

INPUT CONNECTIONS (TOP PANEL)

PASSTHRU	External stimulus to headstage input; Analog, BNC female
BEHAVIORAL	Female DB-25 connector for recording up to 15 TTL signals

INTERFACE BOARD CONNECTIONS	
Connector 0	68 –pin VHDCI male connector
Connector 1	68 –pin VHDCI male connector
Compatible DAQ Interface	National Instruments PCIe-6363 (sold separately)
DAQ Interface Cable	National Instruments SHC8-68 EPM x 2
Control Software	Industry standard LabVIEW based software available to researchers (not provided)

GENERAL SPECIFICATIONS	
Power Required	$12.0\ VDC\ (\pm 5\%)$
Power Supply	input: $100\ to\ 240\ VAC, 2.3\ A, 50\ to\ 60\ Hz$ output: 12.0 VDC, 2.5 A
Power Cord	various international cables available ($10\ A$, C13 Connector)
LED Indicators	power, input and output filter selection, trigger activity
Instrument Dimensions	$58 \times 242 \times 242\ mm\ (2.25 \times 9.5 \times 9.5\ in)$
Instrument Weight	$2.0\ kg\ (1.8\ lb)$
Temperature Range	$10°C$ to $40°C$
Humidity Range	$80\%\ RH$ maximum, non-condensing

2.5 Major System Components

A WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System, as shipped from the production facility, includes the parts listed in the following table (see: Table 2-2). The individual part numbers associated with these parts is also provided. An image representation of the included components is provided (see: Figure 2-2).

Part Number	Description
AF01FSCV	WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System
RRCH0124-T or RRCH0136-T	Headstage amplifier cable, DB-25 connector, 24" or 36"
AC01HS1	FSCV headstage amplifier, $200\ nA/V$ ($5\ M\Omega$) or $1{,}000\ nA/V$ ($1\ M\Omega$) working driven
AC01HC0315-5	1.5 " or 2.5" Microelectrode-headstage couplers (package of 5)
RRPA12V	AC Adapter/Power Supply; $12\ V, 2.5\ A$ input
AFDUM4	WaveNeuro dummy cell

Table 2-2: WaveNeuro System Components

In addition, the WaveNeuro will ship with a power cord for use with the AC Adapter. The cord will have the plug-style suitable for the country of use. Power cords are rated for $10\ A$ and are designed to mate with the standard C13 type connector on the power supply.

INFO:

See Section 8 of this User Guide for a selection of international power cords which may be used with the WaveNeuro instrument.

A National Instruments interface board and interface cables will be required to control the WaveNeuro under software control. These may be obtained from Pine Research or users may obtain them separately, directly from National Instruments (see: Table 2-3). A PC with an open PCIe slot on the motherboard is not supplied by Pine Research and must be present for operation as well.

Pine Research Part Number	Description
EANPCIE6363	National Instruments PCIe-6363 Interface Board
EWCP6102	National Instruments SHC68-68 Interface Cables x2

Table 2-3: Additional WaveNeuro System Components (Sold Separately)

1	**WaveNeuro System**	WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System
2	**Power Supply & Power Cord**	$12\ VDC$ ($2.5\ A$) power supply; $100\ to\ 240\ VAC$ ($50/60\ Hz$); C13 Connector
3	**Headstage Amplifier Kit**	Cable with DB-25 connector, headstage amplifier, microelectrode connectors.
4	**WaveNeuro Dummy Cell**	Network of known resistors and capacitors used to test WaveNeuro and ensure system in proper operation/configuration.

Figure 2-2: Major WaveNeuro System Components (not to scale)

2.6 WaveNeuro Side/End Panel

The side/end panel of the WaveNeuro features several input and output connections whose location and function is described below (see: Figure 2-3).

1	**Power Switch**	Switch to turn the main power on/off
2	**Power Input**	A low voltage direct current ($12.0\ VDC$ ($\pm 5\%$), $1.0\ A$) power input connector. Please use power supply included with original shipment for best performance
.3	**Connector 0**	National Instruments type-connector to connect to the National Instruments data acquisition card in the computer (68-Pin Connector)
4	**Connector 1**	National Instruments type-connector to connect to the National Instruments data acquisition device in the computer (68-Pin Connector)
5	**Chassis Terminal**	The chassis terminal is a banana binding post (back panel) which may optionally be used to connect the chassis to earth ground to improve noise screening (shielding)

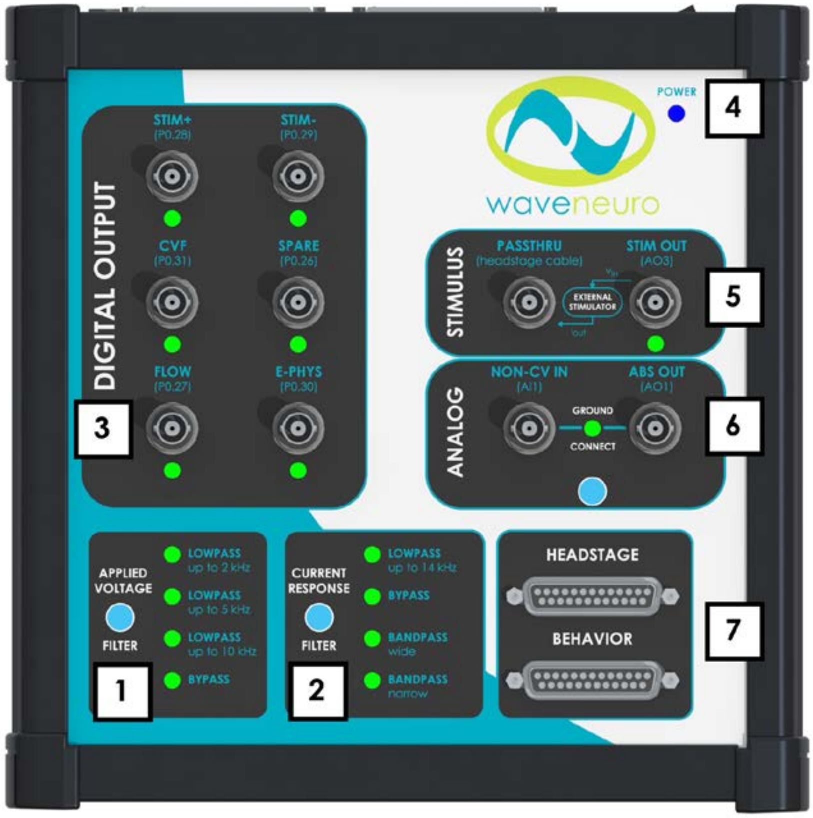
Figure 2-3: Description of the WaveNeuro Side/End Panel

2.7 WaveNeuro Top Panel

The top panel of the WaveNeuro (Figure 2-4) has BNC connectors, filter selection hardware, electrochemical headstage cable connector, behavioral input connector (digital inputs), and LED indicators.

2.7.1 WaveNeuro Connectors

The top panel of the WaveNeuro is organized by the function of the connectors and buttons. The BNC connections are categorized as DIGITAL OUTPUT, STIMULUS, and ANALOG connectors. A fuller description of the connectors in each category is described below. Other categories of connector and buttons on the front panel include HEADSTAGE and BEHAVIOR, which are male DB-25 connectors that accept headstage and behavioral inputs. Lastly are the APPLIED VOLTAGE and CURRENT RESPONSE filter selections. Filter selections for current or voltage are made by pressing a button on the top panel (see: Figure 2-4).



1	**Voltage Filter Selection**	Push button selection to filter applied voltage signal. Green LED light indicates which setting is engaged (see: Section 2.7.3)
2	**Current Filter Selection**	Push button selection to filter measured current signal. Green LED light indicates which setting is engaged (see: Section 2.7.3)
3	**Digital Output Connectors**	Female BNC Connectors, NI Interface Board address location listed in parentheses (e.g. P0.27), below name for connector, which corresponds to HDCV software naming conventions (see: Section 2.7.4)
4	**Power Indicator**	Blue LED power indicator
5	**Stimulus Connectors**	Female BNC connectors for stimulus functions (see: Section 2.7.5)
6	**Analog Input/Output Connectors**	Two female BNC connectors providing analog input and output signals to computer via the interface board. Green LED light indicates ground configuration, selectable with push button See: Section 2.7.6)
7	**Headstage and Behavioral Input Connectors**	Female DB-25 Connectors for Headstage input and/or behavioral signals (see: Section 2.7.7)

Figure 2-4: Top Panel of the WaveNeuro.

2.7.2 WaveNeuro LED Indicators

The LED lights indicate power, digital trigger activation, and filter selection. The primary purpose of the LEDs is to indicate the state of the instrument and whether or not the instrument has power. The various LED indicators are described in more detail below (see: Table 2-4).

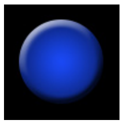
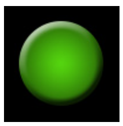
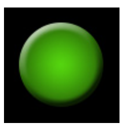
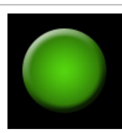
LED	LED Color/State	Indication
Power	 not illuminated	When the power LED is off, the WaveNeuro power switch is in the *off* position (or the power supply is not providing power).
	 solid blue	When the power LED is solid blue, the power supply is providing power to the WaveNeuro and the power switch is in the *on* position.
Filter Select	 solid green	The selected filter LED will be illuminated a solid green. Push button next to the LED bank changes selected filters.
Triggers	 blinking green	The application of a waveform will cause the E-Phys and the CVF LED to blinking. Blinking is indicative of the number of waveforms being applied per second.
	 solid green	While a trigger line is activate (high) this LED will be illuminated.

Table 2-4: Overview of the WaveNeuro LED Light Operation

2.7.3 WaveNeuro Filter Settings

The WaveNeuro has selectable filters on the waveform output and on the measured current. The waveform filter can be set by pressing the button below the filter options and LEDs (see: Label 1 in Figure 2-4). The current response filter can be set by pressing the button below the filter options and LEDs (see: Label 2 in Figure 2-4). In both cases, the LED light next to the filter options is illuminated when selected. The default settings for applied waveform is $LOWPASS\ up\ to\ 2\ kHz$ and for the current response filter is $LOWPASS\ up\ to\ 14\ kHz$.

2.7.4 Digital Triggers

The six BNC connectors in the *DIGITAL OUTPUT* section (see: Figure 2-4) are digital (TTL) triggers that can be programed to activate external hardware. Such programming occurs in the software connected to the WaveNeuro, via the Interface board, on the PC. The most commonly used trigger application is driving a solenoid for operating the instrument with a flow cell.

2.7.5 Stimulus Connectors

The WaveNeuro is equipped with an analog output and PASSTHRU BNC connectors (see: label 5 in Figure 2-4). The STIM OUT (AO3) BNC is mapped to the NI Interface board and outputs an analog voltage signal for stimulation. This output signal feeds to a third-party voltage to current converter device (such as a NeuroLog®, not available from Pine Research), which then outputs a stimulating current. By making a connection between the voltage to current converter device and the PASSTHRU BNC connector on the WaveNeuro, the stimulating current feeds directly to the integrated stimulator connectors on the headstage cable. The current is independent of the WaveNeuro circuitry in this configuration.

2.7.6 Analog Input/Output Connectors

On the top panel in the section labeled ANALOG, there is one analog input connector labeled NON-CV IN and one analog output connector labeled ABS OUT (see: Figure 2-4). Each of these BNC connectors can be used to route analog signals to or from the computer. These connections are rarely used.

A feature of these BNC connectors is a push button switch that will change how the BNC shield is grounded. When the GROUND CONNECT LED is illuminated, the BNC are grounded to the WaveNeuro DC Common, so any noise on the BNC shields has the potential of coupling into the measured signals. If the LED is not illuminated then the BNC are floating with respect to the WaveNeuro chassis, providing isolation between the shield and the WaveNeuro ground. For applications with third party hardware, the BNC shields would typically be floating with respect to the WaveNeuro to provide noise isolation.

INFO:

The optional WaveNeuro Dual Channel Adapter uses the analog signal lines to provide a second working electrode channel; therefore, the BNC connectors in the ANALOG category are unavailable for use. Further, when using the Dual Channel Adapter, ensure the the GROUND CONNECT LED is illuminated by pressing the button near the LED.

2.7.7 Headstage and Behavioral Connectors

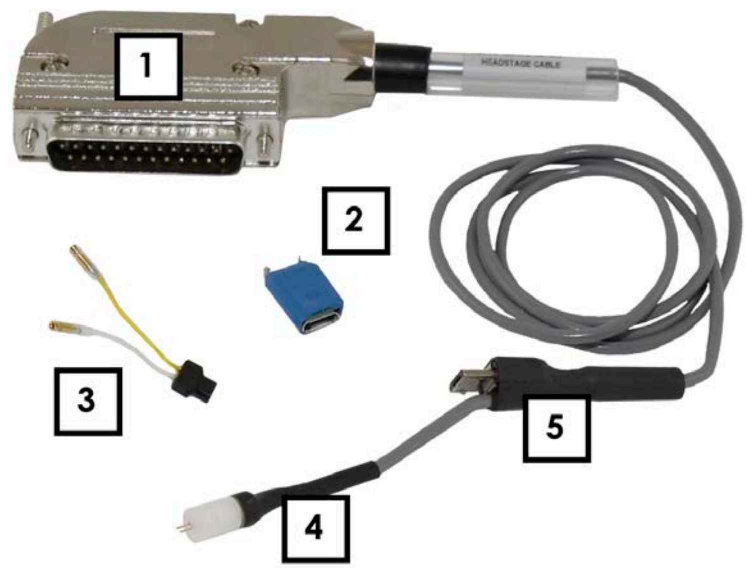
The DB-25 HEADSTAGE connector (see: label 7, Figure 2-4) is intended for use with Pine Research headstage cable kits. Such a headstage cable kit is included with the initial WaveNeuro shipment (see: Figure 2-2). Headstage amplifiers connect to directly to the cable and are interchangeable. The microelectrode-headstage couplers connect to the headstage amplifier. Replacements of the headstage cable kit components are available from Pine Research. Additional information on the headstage cable and its connections are provided in Section 3.

The DB-25 BEHAVIOR connector (see: label 7, Figure 2-4) has been configured as a series of digital (TTL) inputs lines that enable up to fifteen digital input signals. The digital input signals can be displayed by software. For example, the WaveNeuro was tested using a SuperPort™ from Med Associates, Inc. as the behavioral hardware and HDCV as the software for displaying the results. Using this combination, events can be time locked with electrochemical changes observed in the color plots.

3. INCLUDED ACCESSORIES

3.1 Headstage Amplifier Kit

The Pine Research Instrumentation electrochemical headstage amplifier is a high gain current to voltage converter for use with microelectrode systems. This electrochemical headstage is specifically designed for use in a two-electrode system using Fast-Scan Cyclic Voltammetry as the electrochemical technique (see: Figure 3-2).



1	**Headstage Cable**	Male DB-25 Connector, connects to WaveNeuro HEADSTAGE Connector. Pinout available (see: Figure 3-2). Available lengths: 24” and 36”.
2	**Headstage Amplifier**	High gain current to voltage converter for use with two microelectrode system. Working driven. Standard gains of $200\,nA/V\ (5\ M\Omega)$ or $1{,}000\,nA/V\ (1\ M\Omega)$. Other custom gain amplifiers may be available.
3	**Microelectrode-Headstage Coupler Wires**	Wires to connect microelectrodes to headstage. Yellow = working electrode, White = reference electrode. Available in packages of 5 with lengths of 1.5” or 2.5”. Custom length wires may be available.
4	**Integrated Stimulation Connector**	Two-prong 305-CP type plug for delivering stimulating current.
5	**Headstage Amplifier Connector**	Male connector onto which the Pine Research headstage amplifiers are mounted.

Figure 3-1: Components of Pine Research Headstage Amplifier Cable Kit

3.1.1 Working Driven Headstage Description

Pine Research currently offers working driven headstage amplifiers. In a working driven system, the reference electrode is grounded. The FSCV potential waveform (ramp) is connected to the non-inverting input of the operational amplifier, while the working electrode is connected to the inverting input. In this arrangement, the voltage at the microelectrode will follow the ramp applied to the inverting input[1].

In this two-electrode configuration, current arising from electron-transfer reactions, such as the oxidation of dopamine, passes between reference and working electrodes. The measured current passes through the headstage amplifer, where it is converted to voltage, and sums with the ramp voltage at the inverting input. Simple mathematical rearrangement and use of Ohm's law describes a working driven headstage amplifier (see: Equation **1**),

$$V_O = -(i_{in} \times R_F) + V_R \quad \textbf{(1)}$$

where V_O is the output voltage, i_{in} is input current, R_F is feedback resistor (gain), and V_R is the CV ramp voltage. By rearrangement, the signal voltage (proportional to the current across the 5 $M\Omega$ feedback resistor in the 200 nA/V headstage) is then

$$(V_R - V_O) = i_{in} \times R_F = V_{signal} \quad \textbf{(2)}$$

HDCV software, which supports the WaveNeuro FSCV Potentiostat system, performs software subtraction of the ramp according to this relationship, resulting in only the true differential current measurement[1].

REFERENCE

Takmakov, P.; McKinney, C. J.; Carelli, R. M.; Wightman, R. M. Instrumentation for Fast-Scan Cyclic Voltammetry Combined with Electrophysiology for Behavioral Experiments in Freely Moving Animals. Rev. Sci. Instrum. 2011, 82, 74302.

INFO:

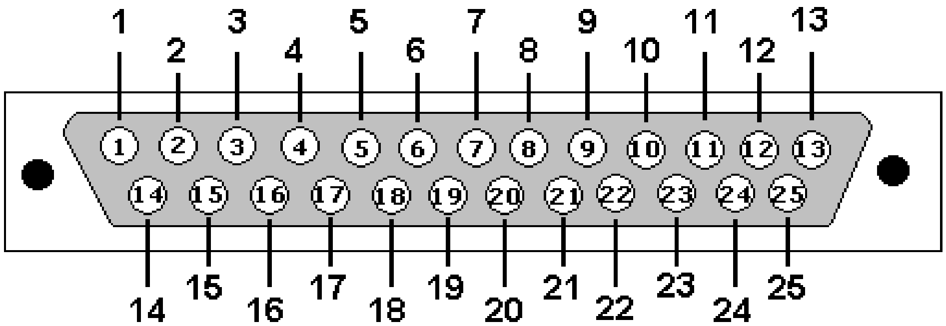
Even when no electrode are connected (connected just in air) to the headstage amplifier cable, which is connected to the WaveNeuro, HDCV will show a current response that follows the applied waveform. Such behavior is by design and expected.

3.1.2 Headstage Cable Kit Detailed Description

The headstage is comprised of three components 1) headstage cable with male DB-25 connector; 2) headstage amplifier; and 3) microelectrode-headstage couplers (see: Figure 3-1).

The connector pinout for the headstage DB-25 connector provides the user with detailed information about the pin configuration (see: Figure 3-2).

The cable has an integrated stimulator line that terminates in a 305-CP plug. The headstage amplifier module provides the amplification of the measured electrochemical signal. The module has two connectors, one for the headstage cable and one for the electrode connection wires. The connection wires are used to connect the amplifier to the working (yellow) and reference (white) electrodes.



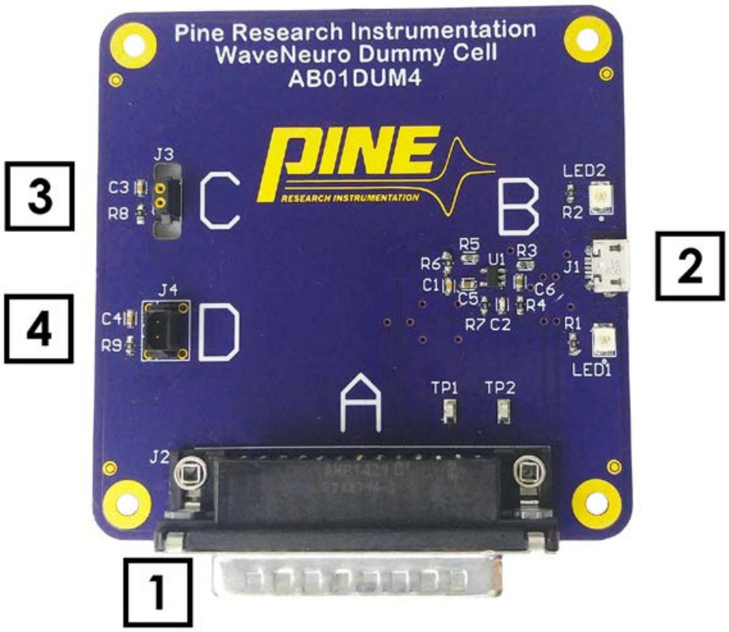
Pin #	Signal
5	Power In $+15\ V$
6	I/E Output
7	Ground
9	Power In $-15\ V$
12	Stim Connection
12	Stim Connection
14	Ramp In

Figure 3-2: Headstage Cable DB-25 Connector Pinout Diagram

3.2 WaveNeuro Dummy Cell Description

A dummy cell is a network of known resistors and capacitors that can be used to test a potentiostat to ensure that the instrument is working properly. The dummy cell included with the WaveNeuro also provides for an easy way to diagnose headstage and cable concerns.

The WaveNeuro Dummy Cell (part number: AFDUM4) is included with the initial purchase of a system. The WaveNeuro Dummy Cell has four separate connections to enable testing of the potentiostat and cabling (see: Figure 3-3). Description of how to utilize this dummy cell to verify the performance of the WaveNeuro and to check environmental noise is provided in Section 4. Upon initial setup of a system the dummy cell should be used to evaluate if any environmental noise is entering into the measurement system. The dummy cell is also used for troubleshooting and isolating issues with the potentiostat and headstage cable components. Please store the WaveNeuro Dummy Cell in a safe place for future use.



1	**Male DB-25 Connector**	*A* connects to HEADSTAGE input on WaveNeuro. This configuration can be used for direct testing of the WaveNeuro
2	**Headstage Connector Test Site**	*B* connects to the headstage connector on the cable, for evaluating the cable up to the headstage connector
3	**Headstage Amplifier Test Site**	*C* connects directly to a headstage amplifier, to independently test the function of the amplifier circuit
4	**Microelectrode-Headstage Coupler Wire Test Site**	*D* connect to each coupler wire to test the function of the coupler independently.

Figure 3-3: WaveNeuro Dummy Cell Connections

4. SYSTEM INSTALLATION

Setting up the WaveNeuro system in a laboratory consists of three basic steps: 1) software and data acquisition system installation; 2) physical installation of the WaveNeuro; and 3) system testing. The installation process typically requires 60 minutes.

For illustrative purposes, this User Guide will describe the installation of WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System under control of High Definition Cyclic Voltammetry (HDCV) software (see: Section 1.10). There are other software programs, such as WCCV®, Demon®, and TarHeel CV®, which may be compatible; however, Pine Research cannot provide software support for these programs and are limited in our ability to assist with software-hardware-interface board communication.

INFO:

The Installation instructions are based on the HDCV software platform. Please consult with your software vendor for issues arising from the use of their software with the WaveNeuro.

INFO:

Experiments should be conducted within a Faraday cage to enable low-current (picoamp) measurements.

4.1 Software and Data Acquisition System Installation

After obtaining a software application (see: Section 1.10) proceed with the following installation. The data acquisition software for the interface board are included with the purchase of a National Instruments interface board. For both software, ensure the computer used in conjunction with this system meets minimum specifications (see: Table 4-5).

Processor Class	Intel Pentium IV or equivalent/compatible
Processor Speed	1 GHz minimum recommended
Physical Memory	1 GB (32-bit OS); 2 GB (64-bit OS) minimum
Screen Resolution	1024 x 768 pixels or greater required
Operating System	Windows XP (32 bit), 32 or 64 bit Windows 7, 8, 10
Data Acquisition	NI PCIe-6363
Prerequisite Software	NI Max, LabVIEW Run-Time 2009 or higher

Table 4-5: Recommended Minimum PC Specifications

Note that the prerequisite software (NI MAX and Labview Run-Time 2009 or higher) are provided with the purchase of the National Instruments PCIe-6363 interface board. In the event that they are missing from the computer, these components are available for free download from the National Instruments website (see: http://www.ni.com/downloads/ni-drivers/).

TIP:

If any problem is encountered during software installation, please contact Pine Research directly (see: Section 10).

Pine Research offers a complete system purchase option. This option will include an adequate PC pre-installed with all software and interface board hardware. If you have purchased this option, you can skip the remainder of Section 4.1. If you have obtained the PC, interface board, and HDCV separately, the sections that follow will describe each software installation step.

4.1.1 Installation of NI-DAQ™mx

NI-DAQ™mx software is supplied with the National Instruments (NI) PCIe-6363 data acquisition card. A standard installation of the NI-DAQ™mx software is demonstrated through a series of screenshots shown below (see: Figure 4-1 through Figure 4-5). The screenshots were obtained for NI-DAQ™mx 14.0 installed from the DVD supplied with a NI PCIe-6363.

1. Insert the DVD, supplied by NI, into the computer. A dialog window appears on the computer screen. Select and *Run autorun.exe* (see: Figure 4-1). If this window does not appear on the screen then open Windows Explorer, navigate to the DVD, and select *autorun.exe* from the file directory.

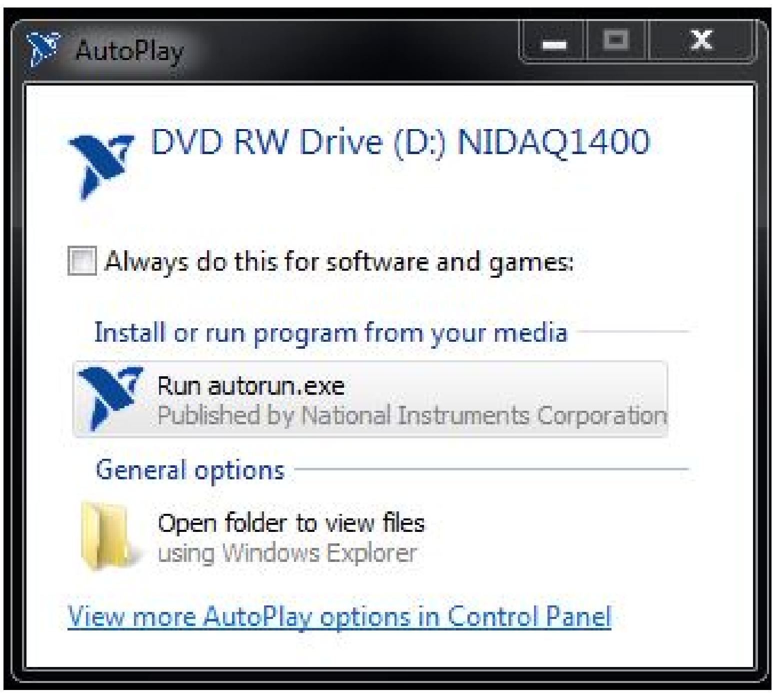


Figure 4-1: NI-DAQ™mx Installation – Step 1

2. After the installer begins to run, a dialog window with installation options will appear. Select *Install NI-DAQmx* from the list (see: Figure 4-2).

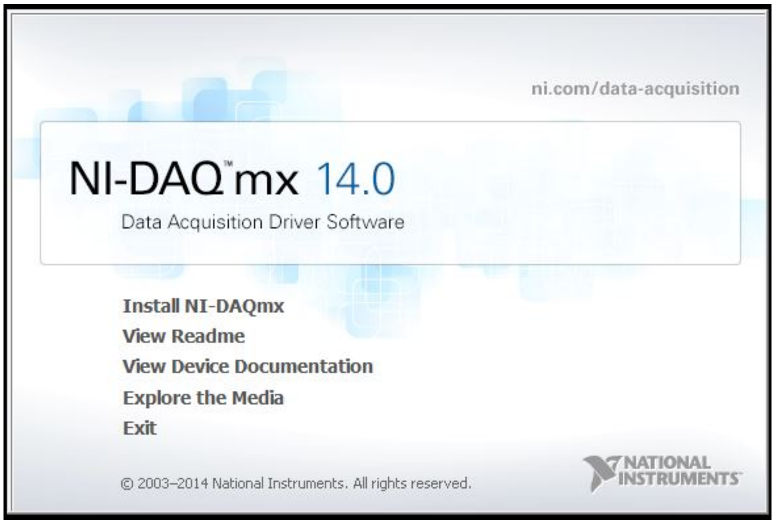


Figure 4-2: NI-DAQ™mx Installation – Step 2

3. On the next dialog window that appears, specify the destination directory and click Next. Typically, the default location is a good choice, but you can specify a location if desired (click Browse and navigate to the desired location) (see: Figure 4-3).

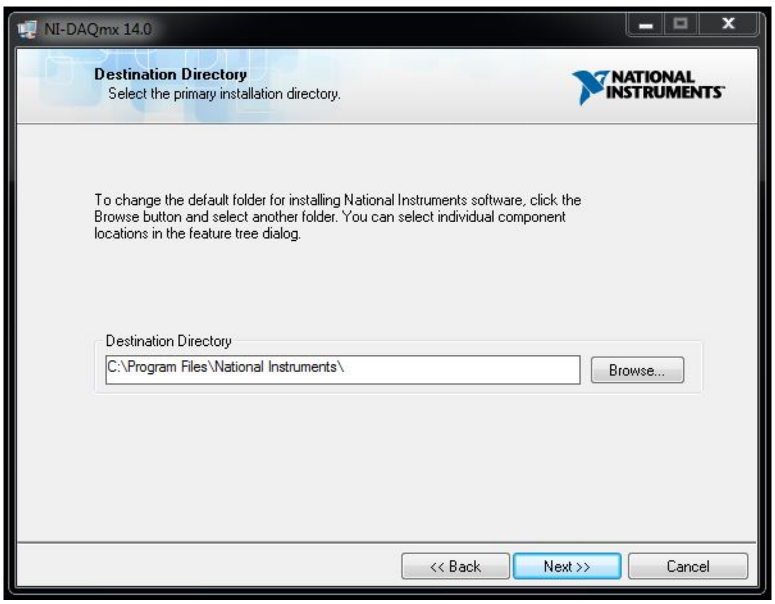


Figure 4-3: NI-DAQ™mx Installation – Step 3

4. On the next dialog window, that appears, choose *Typical Installation* (See: Figure 4-4).

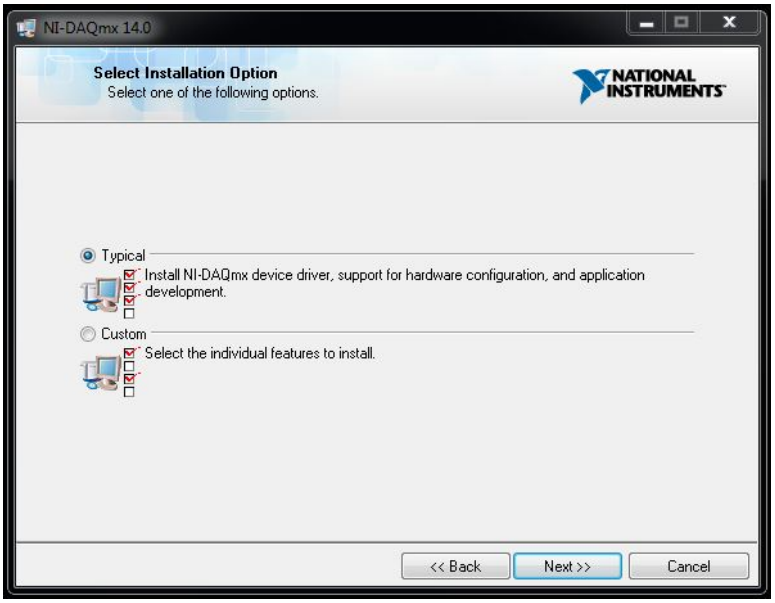


Figure 4-4: NI-DAQ™mx Installation – Step 4

5. The next dialog window will prompt for automatic updates. Users can make a choice by ticking or un-ticking the box and pressing Next. Then, on the next dialog window, review the National Instruments Software License Agreement. Select the *I accept...* radio button and press Next.
6. The next dialog window will itemize a list of changes during the installation. Press Next to continue (see: Figure 4-5).

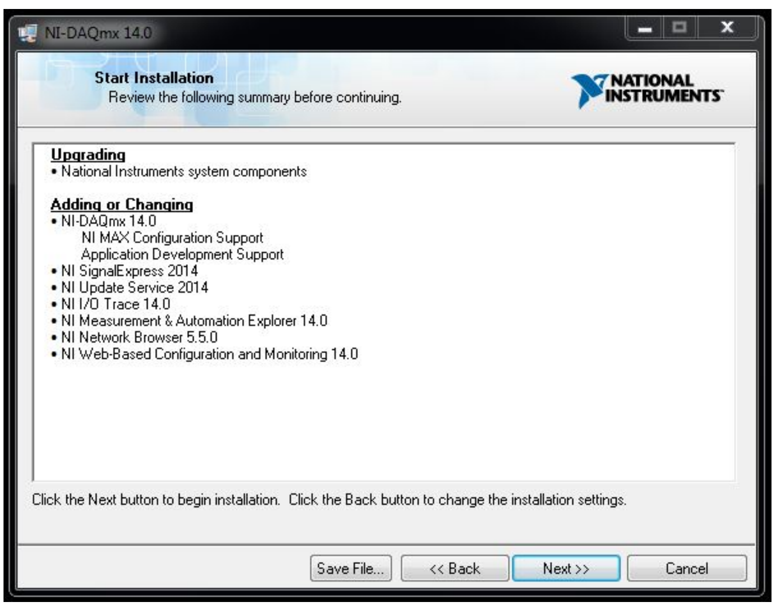


Figure 4-5: NI-DAQ™mx Installation – Step 5

7. After the progress bar dialog completes, the installer will suggest restarting the PC. After restart, the installation of NI-DAQ™mx is complete.

4.1.2 Installation of the PCIe-6363 Data Acquisition Interface Board

Ensure that the motherboard of the target PC has an open PCIe slot. Walk through the following steps to physically install the PCIe-6363.

1. Unplug the personal computer and open the computer case.
2. On the computer mother board find a PCI express expansion slot. Mother boards typically have several expansion slots. An example of an open PCIe slot has been provided (see: Figure 4-6). Refer to mother board documentation for assistance in finding the PCI express slot, if needed.
3. Ground yourself by touching the computer case and remaining in contact with the case during the installation.
4. Install the card into the empty PCI express slot and secure it, then close the computer and power it on.
5. After starting the computer run National Instruments NI MAX (Measurements and Automation Explorer) application. The application icon/link will be in the destination folder specified during installation of the software.
6. Under *My System* on the left hand side menu - select *Devices and Interfaces*.
7. NI PCIe-6363 should be listed under Devices and Interfaces with a default name *Dev0*. If the PCIe-6363 is not named *Dev0,* select the interface board on the left side of the application. On the right side, the name field appears. Change the name to 0, if not already named *Dev0.*
8. Use the NI MAX software to interact with the PCIe-6363. You may click *self-test* or *calibrate* buttons at the top. If physical installation of the board is successful, you are finished. If not, repeat steps 1 - 8, ensuring that the board seats securely and completely into the PCI express slot. There are often screws/connectors that keep expansion boards in place.

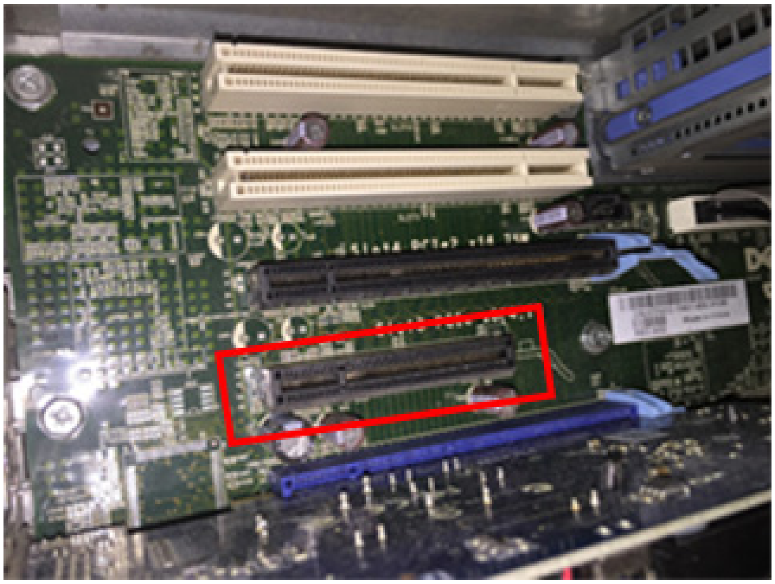


Figure 4-6: PCIe Slot (Red Rectangle) on a PC Motherboard

4.1.3 Installation of Data Acquisition Software (HDCV Shown Here)

The WaveNeuro is designed to run on third party data acquisition software applications, such as HDCV. Review previous information about obtaining software (see: Section 1.10). To install HDCV follow these steps:

8. Select and run the setup application file provided. After starting the file a series of dialog boxes will appear. First, select a directory for HDCV (see: Figure 4-7).

> **INFO:**
>
> **If installing HDCV, DO NOT install HDCV inside *Program Files*. HDCV must be installed outside of Program Files. The root drive (C:/) or *My Documents* are ideal locations to install the software. It is permissible for National Instruments product to install within Program Files.**

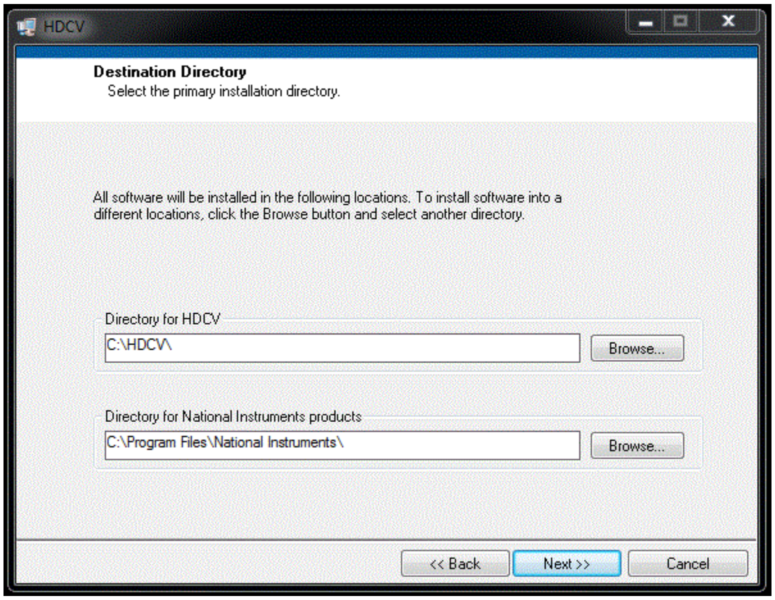


Figure 4-7: HDCV Installation – Step 1

9. As with NI-DAQ™mx, review and accept the license, the installation summary, watch the installation progress, and after complete, restart the computer.
10. After installation of the National Instruments software, the PCIe-6363 data acquisition device, and HDCV software, the HDCV software should be operational. Open the installation file folder for HDCV and select the HDCV application. HDCV can be started by double clicking on the application. After a few seconds, HDCV should appear on the monitor (see: Figure 4-8). If HDCV does not start please refer to troubleshooting or contact Pine Research Instrumentation.

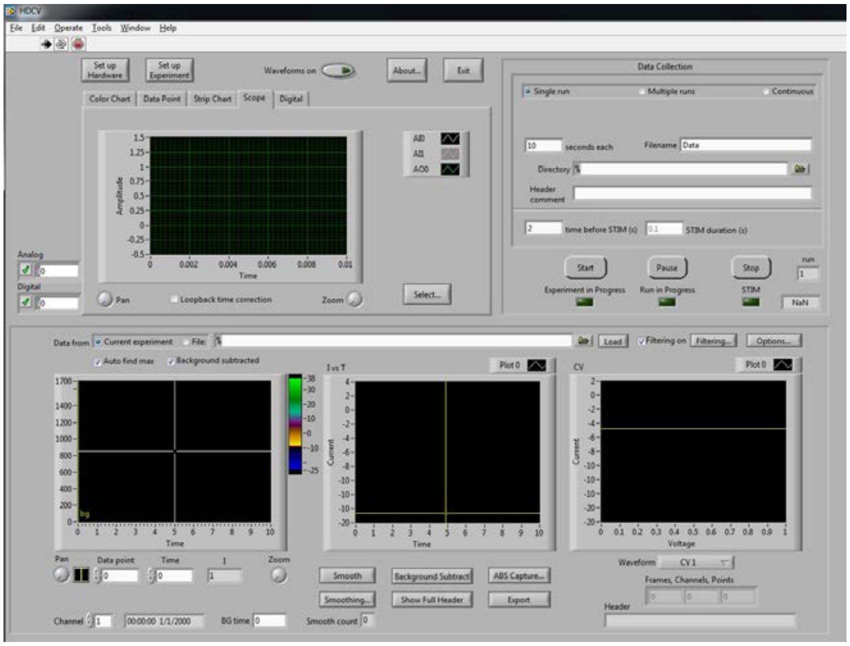


Figure 4-8: HDCV Application upon First Startup

4.2 Physical Installation

The WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System is a benchtop instrument designed for use in a typical laboratory environment. Physical installation involves positioning the instrument (and the computer which controls the instrument) in a suitable location and connecting the instrument to a source of electrical power (*i.e.*, the AC Mains) and to the computer (via National Instruments Acquisition Device). Care should be taken to position the WaveNeuro in a stable, clean and dry location that has limited power cords in close proximity to the instrument.

4.2.1 Location

The WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System should be placed on a sturdy lab bench or table in such a way that there is unobstructed access to the top of the instrument and at least ten inches ($150\ mm$) of clearance in the back of the instrument, so that cables can be connected to the potentiostat. There should be at least two inches ($50\ mm$) of clearance around the sides (left and right) of the instrument. Particular care should be given to selecting a stable, clean and dry location. During normal use, the instrument is connected to an electrochemical headstage via a cable plugged into the top panel of the instrument.

4.2.2 Electrical Power

The Power Supply provides the DC power required by the WaveNeuro instrument ($12\ VDC, 2.5\ Amps$) via a low voltage cable that is permanently connected to line power. The end of this low voltage cable is connected to the back panel of the WaveNeuro at the location marked *POWER INPUT* (see: Figure 4-9).

1	**Power Switch**	Switch to turn the main power on/off
2	**Power Input**	A low voltage direct current (12.0 VDC ($\pm 5\%$), 1.0 A) power input connector. Please use power supply included with original shipment for best performance
3	**Connector 0**	National Instruments type-connector to connect to the National Instruments data acquisition card, connector 0, in the computer (68-Pin Connector)
4	**Connector 1**	National Instruments type-connector to connect to the National Instruments data acquisition card, connector 1, in the computer (68-Pin Connector)
5	**Chassis Terminal**	The chassis terminal is a banana binding post (back panel) which may optionally be used to connect the chassis to earth ground to improve noise screening (shielding)

Figure 4-9: Installation Connections to the WaveNeuro

Caution:

Connect the Power Supply to the AC mains using the Power Cord supplied with the WaveNeuro and certified for the country of use. (See Section 7 of this User Guide for more details.)

Attention:

Connectez le bloc d'alimentation au secteur à l'aide du cordon d'alimentation fourni avec l'appareil WaveNeuro et conforme aux réglementations du pays d'utilisation (pour plus de détails, consultez la partie 7 du présent mode d'emploi)

Caution:

Do not block access to the Power Supply or the Power Cord. The user must have access to disconnect the Power Supply or the Power Cord from the AC mains at all times.

Attention:

Ne bloquez pas l'accès au bloc d'alimentation ou au cordon d'alimentation. L'utilisateur doit être en mesure de déconnecter le bloc d'alimentation ou le cordon d'alimentation du secteur à tout moment.

Caution:

The Power Switch located on the back panel of the instrument disconnects the instrument from the power source. Do not block access to the Power Switch. The user must have access to the Power Switch at all times.

Attention:

L'interrupteur situé sur le devant de l'appareil permet de couper l'alimentation. Ne bloquez pas l'accès à l'interrupteur. L'utilisateur doit avoir accès à l'interrupteur à tout moment.

The WaveNeuro power supply connects to the AC Mains via a Power Cord. The Power Cord must be rated to carry at least $4\,A$. One end of the Power Cord is connected to the power supply module (included), and the other end is connected to the AC Mains (wall outlet). Pine Research offers power cords with plug adapters suitable for use in a variety of different countries and regions (see: Section 7).

The local source of electrical power (the AC Mains) must be a branch circuit protected by a circuit breaker rated at least $5\,A$. The AC voltage supplied by the AC Mains must be $100 - 240\,VAC$, and the AC frequency must be $50 - 60\,Hz$. The Power Supply and Power Cord must be positioned in such a way that the instrument user has free and unobstructed access to these items. The user must be able to disconnect the instrument from the Power Supply and disconnect the Power Cord from the AC mains (wall outlet) without any obstructions.

4.2.3 National Instruments Interface Cables

Connect the WaveNeuro to the computer using two National Instruments SHC68-68-EPM cables (NI Part No: 192061-02) (see: Figure 4-9). The cables connect the NI PCIe-6363 acquisition device in the computer to the WaveNeuro.

TIP:

The external facing metal plate of the NI PCIe-6363 is stamped with 0 and 1, corresponding to *CONNECTOR 0* and *CONNECTOR 1* on the side/end panel of the WaveNeuro. Be sure to connect the cables to the correct NI board.

4.3 Installation Checklist

Before System Testing, ensure the following installation steps have been completed:

- ✓ National Instruments NI-DAQ™mx software installed on the computer
- ✓ Data acquisition software installed on the computer (e.g. HDCV)
- ✓ National Instruments PCIe-6363 data acquisition interface board installed in the computer
- ✓ WaveNeuro connected to the computer with two National Instruments cables connected correcting between CONNECTOR 0 and CONNECTOR 1
- ✓ Electrical power is connected to the WaveNeuro

5. SYSTEM TESTING

This section describes how to test your WaveNeuro potentiostat system using the WaveNeuro Dummy Cell that shipped with the potentiostat. By connecting the potentiostat to this well-behaved network of resistors and capacitors, the potentiostat circuitry can be tested to assure that it is working properly. Using the potentiostat with the Dummy Cell will also assist in troubleshooting environmental noise.

TIP:

Anytime that it is necessary to verify that the WaveNeuro is operating correctly, perform the system tests described here. These tests are the first actions that will be suggested by technical support personnel in the event that a user contacts Pine Research technical service.

5.1 System Verification and Tests

5.1.1 Verify Installation of Data Acquisition Interface Board

Launch the NI MAX software installed on the computer (see: Section 4.1.1). NI MAX enables the user to check the install National Instruments software and hardware. To check the installation of the PCIe-6363, select *Devices and Interfaces*, complete the following steps:

1. From Devices and Interfaces section, select the NI PCIe-6363.
2. A series of settings will appear on the right side of the application. Verify that the PCIe-6363 is named *Dev0*, highlighted (see: Figure 5-1).
3. Run a self-test of the PCIe-6363 to ensure it is installed properly by clicking the *self-test* button at the top of the application window. A dialog window will alert you of the outcome of the self-test.
4. Verify the software version by clicking on the software section on the left side of the application window.

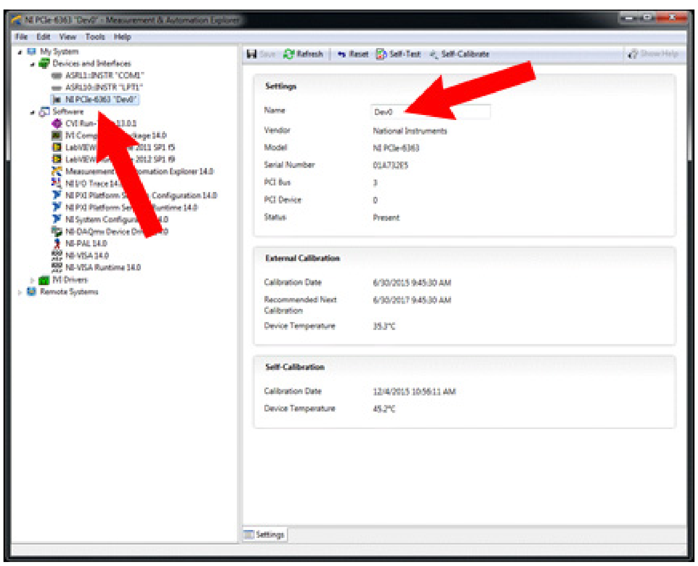


Figure 5-1: Device Name in NI MAX Software

5.1.2 Verify Instrument Status

Verify the status of the WaveNeuro by completing the following steps:

1. Turn the WaveNeuro on using the back panel power switch. The Power LED should illuminate blue (see: Table 2-4 and Figure 2-4).
2. Start data acquisition software (e.g. HDCV). The remainder of this section uses HDCV for screen captures and descriptions.
3. After a few seconds, the HDCV application should open.
4. Review some of the most common control features in HDCV, such as the *Set Up Hardware* (see: Section 5.1.3 for details) and *Set Up Experiment* buttons (see: Section 5.1.4 for details), Waveform toggle button, analysis controls section, and the data collection settings panel (see: Figure 5-2).
5. When HDCV is set-up as desired, continue to Section 5.1.5 to verify connectivity between computer and WaveNeuro.

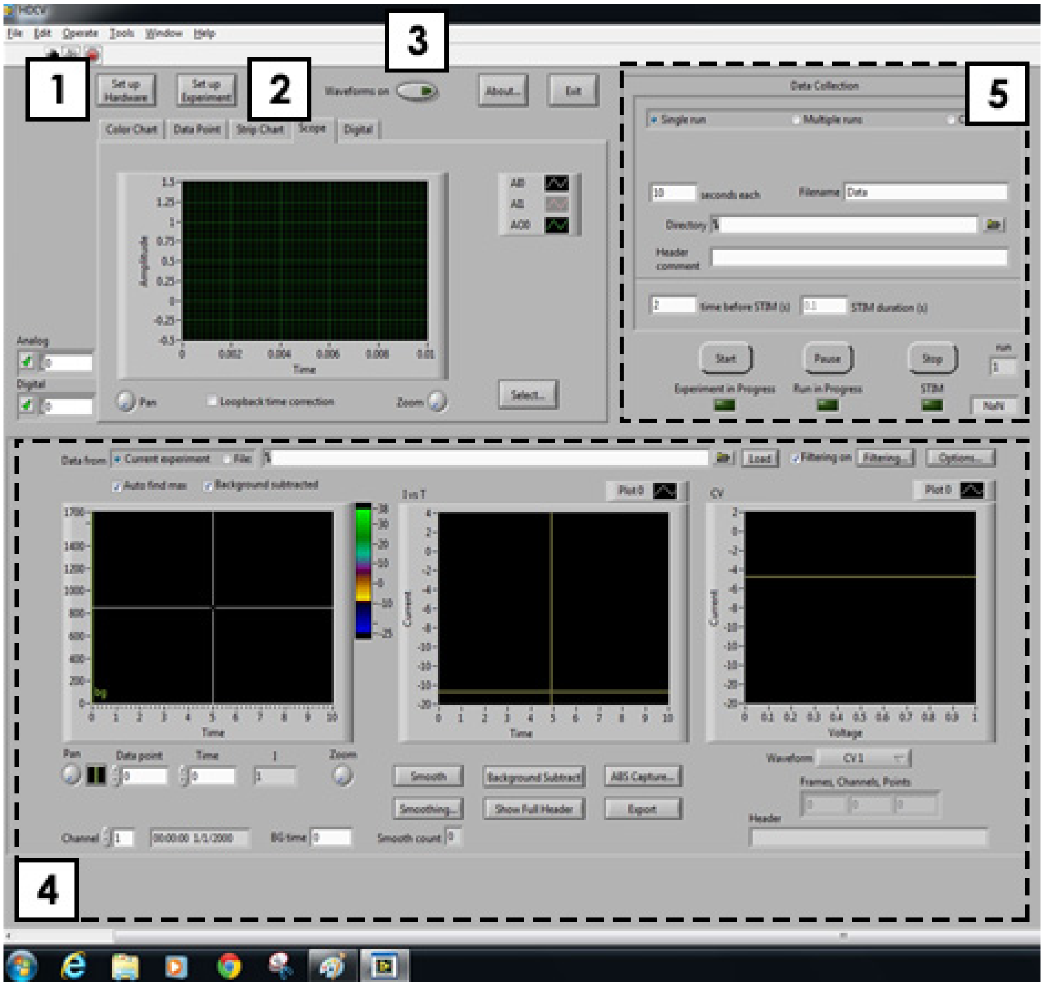
5.1.3 Review Hardware Settings

After starting HDCV click the *Set Up Hardware* button (see: Figure 5-2). A pop-up menu should appear with the hardware setting for the experiment (see: Figure 5-3). The hardware menu enables the user to select and map BNC connectors in the hardware to the appropriate pin on the NI interface board. Typically, users will select the *Simplified Setup* radio button, but to enable access to other input/output on the WaveNeuro, *Manual Setup* radio button should be selected.

For example, if the WaveNeuro is being used with a flow cell and the *Simplified Setup* radio button is selected to trigger the flow cell will require one (1) *Other Digital Outputs*. When one (1) *Other Digital Outputs* is added in the *Set Up Hardware* window then *Dev0/port0/line27* will automatically be assigned as the trigger line. So, the flow cell trigger will be available at the *FLOW/P0.27* BNC on the WaveNeuro front panel (see: Figure 2-4).

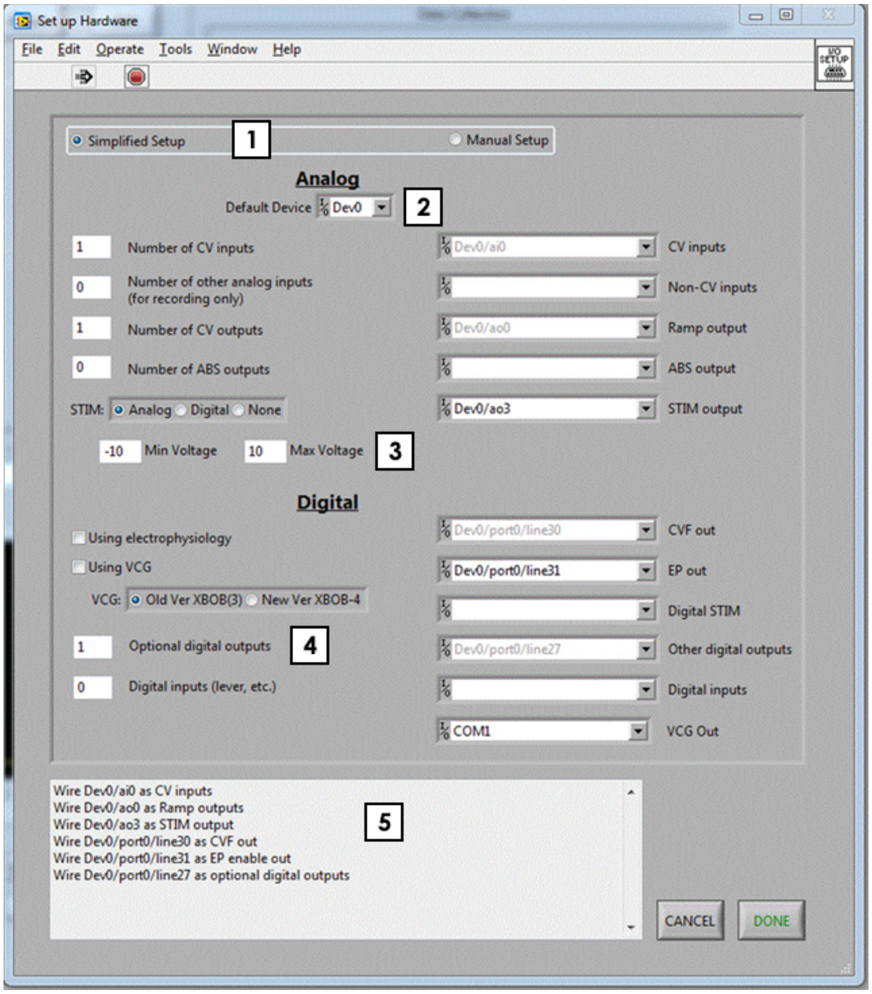
If the *Manual Setup* is selected the flow cell trigger could be routed to a different BNC on the front panel of the WaveNeuro by assigning its port (P0) line number at *Other Digital Outputs.* While this option is possible, Pine Research does not recommend re-routing or significantly altering the default settings within HDCV.

Another example would be to assign the trigger to the appropriate BNC *FLOW/P0.27* BNC, the *Other Digital Outputs* line that should be selected is *Dev0/port0/line27* (see: Figure 5-3). Always select *DONE* button when finished modifying options on this hardware setup screen.



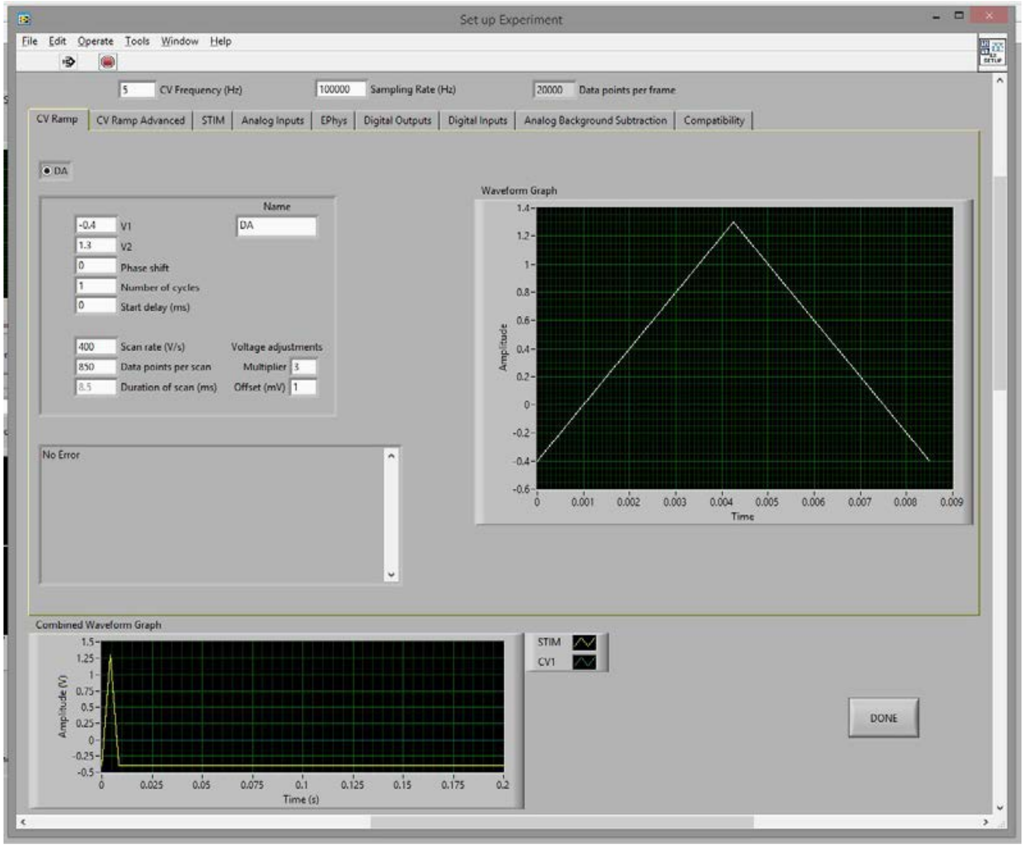
1	**Set Up Hardware Button**	Opens a panel to setup hardware features, inputs, outputs, and setup BNC connectors on the WaveNeuro
2	**Set Up Experiment Button**	Opens a panel to define waveform, frequency, and other experimental parameters.
3	**Waveform Toggle Button**	Click to turn on (apply) the established voltage vs. time waveform and to turn off (disconnect) the waveform.
4	**Analysis Controls Panel**	Panel contains live color-plot, background subtracted measured current plot, and a single-background subtracted cyclic voltammogram. Pertinent settings for each of these displays are included in this panel.
5	**Data Collection Setting Panel**	Panel to define file name and saving location, experiment duration, stim duration, and start/stop/pause experiment options.

Figure 5-2: HDCV Application Primary Panels and Buttons



1	**Setup Type Button**	In general, select researchers use *Simplified Setup* although the *Manual Setup* option allows for enabling/disabling and mapping of the various input and output BNC connectors through the software.
2	**NI Board Device Name**	Dropdown selection reads from the NI-DAQ™mx setting to define the appropriate data acquisition device. Pine Research recommends setting the device to *Dev0* as described in Section 5.1.1.
3	**Potential Range for Data Acquisition Interface Board**	Full range for the WaveNeuro and PCIe-6363 is $\pm 10\ V$.
4	**Digital Output Entry**	Total number of software-defined digital outputs, which, for example, are used for flow cell trigger.
5	**HDCV Hardware Setup Dialog**	Summarizes hardware setup and reports errors, if any.

Figure 5-3: Set Up Hardware Window in HDCV



Setting	Value (for Dopamine)
V1	-0.4
V2	1.3
Phase Shift	0
Number of Cycles	1
Start Delay (ms)	0
Scan Rate (V/s)	400
Data point per Scan	850
Multiplier	3
Offset (mV)	0

Figure 5-4: CV Ramp on Experiment Setup Tab in HDCV

5.1.4 Review Experimental Setup

Review the experimental settings by clicking the *Set up Experiment* button (see: Figure 5-2). The *Set up Experiment* panel defines the digital triggers, such as the flow cell trigger and the analog inputs and outputs to be controlled, including defining the applied electrochemical waveform. The first two tabs of the *Set up Experiment* panel, CV Ramp and CV Ramp Advanced, enables options to define the potential waveform. Advanced users may wish to adjust parameters on the CV Ramp Advanced tab, however it is rarely required for most conventional analyses.

11. On the CV Ramp tab, setup the waveform parameters. Here, the parameters for typical dopamine measurements are shown (see: Figure 5-4). **This guide will assume this common dopamine waveform for the remainder of this HDCV walkthrough guide.**
12. The standard dopamine waveform is a sweep from $-0.4\,V$ to $+1.3\,V$ and back to $-0.4\,V$ at a scan rate of $400\,V/s$. This ramp should be $8.5\,ms$ in duration and contain 850 data points at a collection rate of $100{,}000\ data\ point/second$. All other typical settings to setup this dopamine waveform are provided in the screen capture (see: Figure 5-4). **Importantly, for the WaveNeuro the Multiplier should be set as 3.**
13. On the Analog Inputs tab, enter the appropriate gain for the headstage amplifier used. The standard Pine Research headstage amplifier gain is $200\,nA/V$ (see: Figure 5-5). Entering a Name is optional.

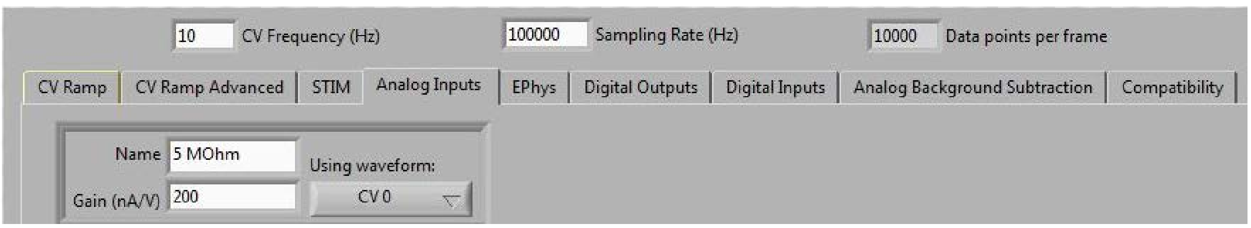


Figure 5-5: Analog Inputs Tab on the Experiment Setup in HDCV

14. On the Digital Outputs tab, you can define the parameters used for optionally enable triggers. This guide will provide an example case for when a flow cell trigger will be used.
15. Enter a Name for the trigger (*e.g.*, *Flow Cell*), select the *Once per run* radio button, enter a rise time (initiation time of the trigger pulse), a fall time (end time of trigger pulse), and select *On during run* with the toggle switch (see: Figure 5-6). Note that the times for the rise and fall should be in units of milliseconds, so for the trigger to start 2 seconds after the start of a run with a duration of 3 seconds, enter a Rise time of $2{,}000$ (ms after start) and a Fall time of $5{,}000\ ms$. Notice that as parameters are entered, the panel to the lower right will display the pulse as entered, in this case a digital pulse from 2 seconds to 5 seconds during the experimental run (see: Figure 5-6).

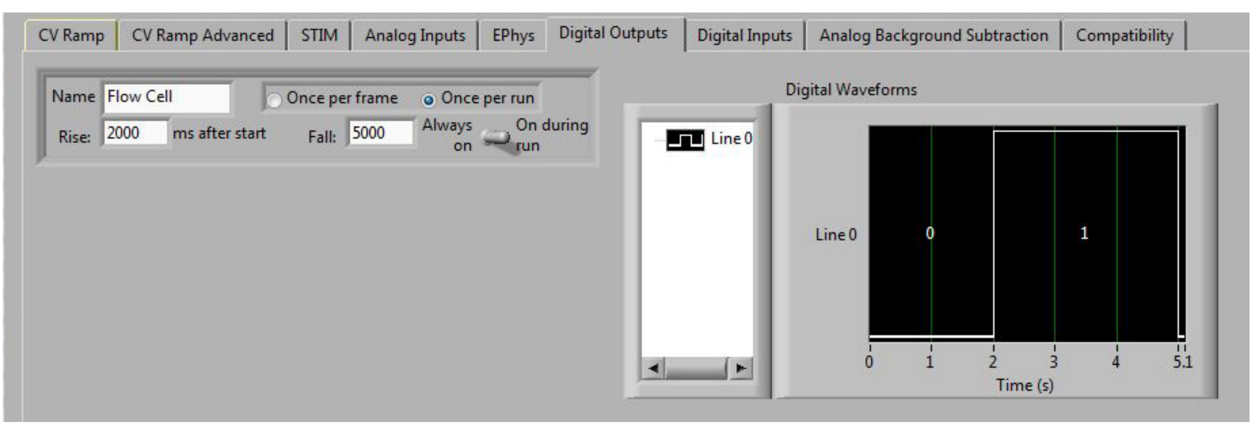


Figure 5-6: Analog Inputs Tab on the Experiment Setup in HDCV

After verifying the setup parameters discussed here, push the *Done* button to close the *Set up experiment* panel.

5.1.5 Verifying the Connection between the Computer and WaveNeuro

To test the connection between the WaveNeuro and the computer apply the waveform by pressing the *Waveform on* button on the front panel of HDCV (see: Figure 5-2).When the waveform is turned on, the CVF indicator LED on the front panel of the WaveNeuro should continuously flicker (see: Table 2-4). This flickering indicates that the waveform is being applied to the electrochemical system through the HEADSTAGE connector. At this point, verify the performance of the WaveNow with a dummy cell test (see: Section 5.2).

5.2 WaveNeuro Dummy Cell Performance Test

As described previously, the dummy cell is a network of known resistors and capacitors that mimic an electrochemical interface (see: Section 3.2). This network is a stable electrical system to use in evaluating instrument performance and function. Complications which arise from electrodes, electrode placement, and chemistry are avoided when a dummy cell is used. The output from a dummy cell test can be predicted, allowing for the observation of system anomalies, which aids in resolving any setup or hardware issues. After installation of the WaveNeuro, a dummy cell test will verify the connections between the potentiostat and the computer. The subsequent tests will evaluate and help eliminate sources of environmental noise. Follow these steps to perform a dummy cell test.

5.2.1 Testing the WaveNeuro Performance

16. Connect the DB-25 connector of the WaveNeuro Dummy Cell A (see: label 1 in Figure 3-3) to the HEADSTAGE connector on the front panel of the WaveNeuro (see: Figure 2-4 and Figure 5-7).

Figure 5-7: Connection of Dummy Cell to WaveNeuro HEADSTAGE Connector

17. Apply the electrochemical waveform to the dummy cell by pressing the *Waveform on* button in HDCV (see: Figure 5-2). The live view window has five tabs, with Scope being the default view. Select Scope (see: Figure 5-8).

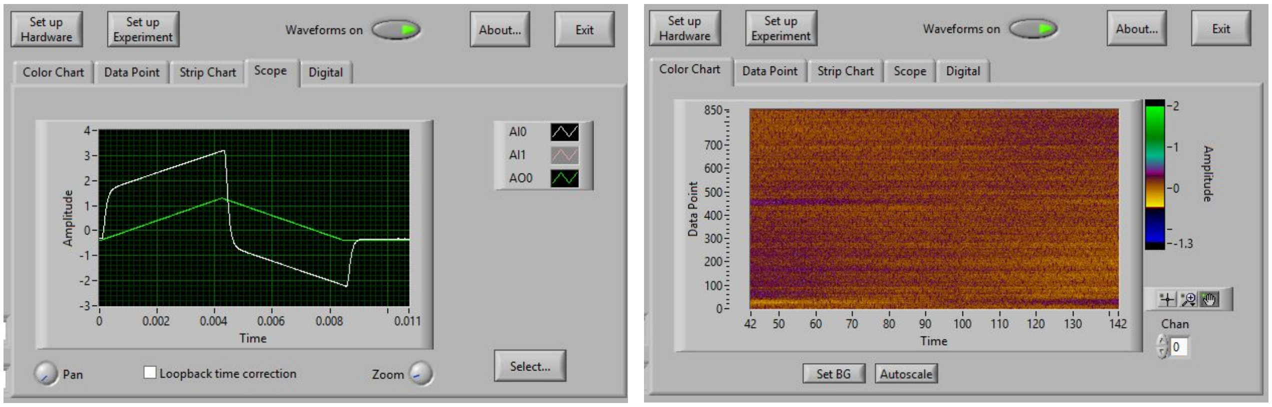


Figure 5-8: Scope (left) and Color Chart (right) Live Display Panels in HDCV

18. With the dummy cell connected to the WaveNeuro and the standard dopamine waveform applied to the dummy cell (green trace on Scope), the current response of that applied waveform across the dummy cell circuit is displayed on the Scope in white (see: Figure 5-8). If the observed current response does not follow waveform as expected, double check all cable connections between the WaveNeuro and the computer and that the WaveNeuro is powered on.
19. Select Color Plot tab in the live display area. In this mode, the electrochemical response will be continually recorded and displayed as an electrochemical color plot. The time of the CV for background subtraction can be define by pressing the *Set BG* buttons on the bottom of the display. Pressing the *Autoscale* button will adjust the magnitude of the current response on the plot. Right click on the mouse select *Clear Chart* to reset the collection of data. Then reset the background and scale by clicking on the *Set BG* and *Autoscale* buttons. The resulting color plot should look similar to the results shown (see: Figure 5-8). Note that the scale on the color plot in Figure 5-8 is $+2\,nA$ to $-1.3\,nA$ and the scale of the colorplot can be manually adjusted by selecting (left clicking) an endpoint and entering a value. The background CV used must be periodically updated by pressing the *Set BG* button. After $\sim 5\,min.$ of applying the waveform, if the resulting color plot is not stable within $\pm 5\,nA$ over the displayed time, please take a screen shot and contact Pine Research for technical support.

5.2.2 Testing DB-25 Extension Cable Performance

The instructions in this section assume a DB-25 extension cable is being used. If a DB-25 extension cable is not being used, skip this section.

20. Turn the waveform off by clicking the *Waveform on* button (see: Figure 5-2). Remove the dummy cell from the WaveNeuro HEADSTAGE connector. Connect the DB-25 extension cable into the HEADSTAGE connector on the WaveNeuro front panel. Place the other end of the cable into a Faraday cage. Connect the DB-25 extension cable to the Dummy Cell A see: label 1 in Figure 3-3).
21. Turn the waveform on by clicking the *Waveform on* button (see: Figure 5-2). Follow the same steps 3 and 4 in Section 5.2.1 to observe the same behavior as before when the dummy cell was connected directly to the WaveNeuro. If additional noise is observed when the dummy cell is plugged into the DB-25 extension cable check the path of the cable to ensure: 1) no

other cables especially power or internet cables are in close proximity to it; 2) check the connectors are all properly seated; and 3) that the cable is not swinging or mechanically moving.

5.2.3 Testing the Headstage Cable Performance

The instructions in this section will test the various headstage cable connections. These steps will be routinely employed throughout years of research to verify the function of each headstage cable kit component.

22. Turn the waveform off by clicking the *Waveform on* button (see: Figure 5-2). Connect the headstage cable DB-25 connector into the WaveNeuro HEADSTAGE connector on the front panel. Place the other end of the cable into a Faraday cage. Connect the headstage amplifier connector on the cable (see: label 5 in Figure 3-1) to Dummy Cell B. Two green LEDs on the dummy cell will illuminate to indicate a successful connection has been made between WaveNeuro and dummy cell and that the power supplied is at the correct level (see: Figure 5-9).
23. Turn the waveform on by clicking the *Waveform on* button (see: Figure 5-2). Follow the same steps 3 and 4 to observe the same behavior as before when the dummy cell was connected directly to the WaveNeuro.

Figure 5-9. Connection of Headstage Cable to Dummy Cell B Connector

5.2.4 Testing the Headstage Amplifier Performance

Turn the waveform off by clicking the *Waveform on* button (see: Figure 5-2). Install the headstage amplifier onto the connector on the headstage cable (see: Figure 3-1). Plug the headstage (connected to the cable) into Dummy Cell C (see: Figure 5-10).

24. Turn the waveform on by clicking the *Waveform on* button (see: Figure 5-2). Follow the same steps 3 and 4 as above in Section 5.2.3 to observe the same behavior as before when the dummy cell was connected directly to the WaveNeuro.

Figure 5-10. Connection of Headstage Cable to Dummy Cell C Connector

5.2.5 Testing the Microelectrode-Headstage Coupler Wire Performance

Turn the waveform off by clicking the *Waveform on* button (see: Figure 5-2). Install the headstage amplifier onto the headstage cable and then connect the microelectrode-headstage coupler to the headstage amplifier (see: Figure 3-1). Plug the microelectrode-headstage coupler wires onto the two pins of the connector located at Dummy Cell D. Simply plug the white wire connector onto one of the pins and the yellow wire connector onto the other of the pins.

25. Turn the waveform on by clicking the *Waveform on* button (see: Figure 5-2). Follow the same steps 3 and 4 as above in Section 5.2.3 to observe the same behavior as before when the dummy cell was connected directly to the WaveNeuro.

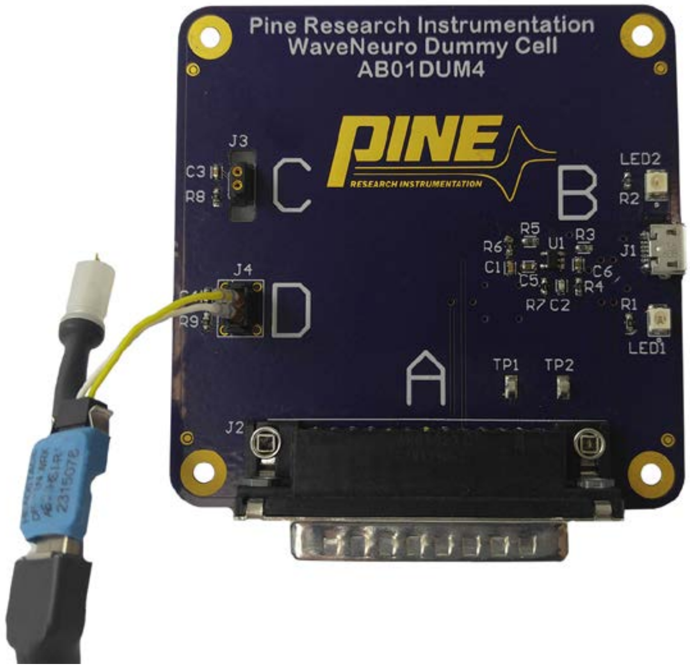


Figure 5-11: Connection of Headstage Cable to Dummy Cell D Connector.

TIP:

The dummy cell circuit is an uncomplicated system for hardware evaluation. It does not produce a typical voltammogram obtained when analyzing electroactive species. Typical dummy cell responses are provided (see: Figure 5-12).

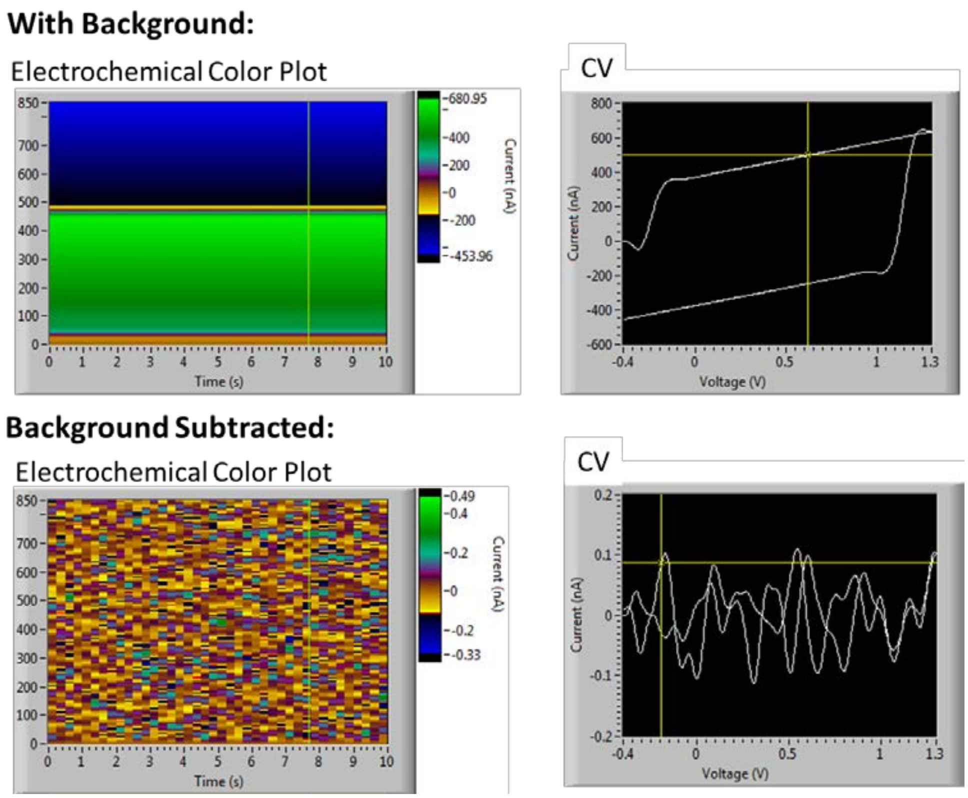


Figure 5-12: Typical Dummy Cell Responses in HDCV

If the measured noise peak-to-peak is less than a few nA with all of the performance tests, the installation of the WaveNeuro is acceptable and experiments can begin. Please save a few short background files for future reference. If any dummy cell performance measurements have signal (noise) levels that exceed 5 nA, the installation is unacceptable. Review placement of the instrument and contact Pine Research Instrumentation for technical assistance.

INFO:

For a more thorough discussion of using HDCV acquisition software, please review the HDCV User Guide, available for download from The University of North Carolina at Chapel Hill, see:

https://admin.chem.unc.edu/electronics_facility/software/ uploads/1/manual/HDCV_Data_Acquisition_v3_Manual.pdf

INFO:

For a more thorough discussion of using HDCV analysis software, please review the HDCV User Guide, available for download from The University of North Carolina at Chapel Hill, see:

https://admin.chem.unc.edu/electronics_facility/software /uploads/2/manual/HDCV_Analysis_v6_Manual.pdf

6. GROUNDING INFORMATION

To avoid issues with signal noise when making electrochemical measurements, it is important to properly ground all metal objects near an electrochemical cell to the earth ground, and this generally includes the metal chassis of the potentiostat, the clamps and supports used to physically secure the electrochemical cell, and any peripheral equipment such as an electrode rotator or Faraday cage that may be used in conjunction with the potentiostat.

6.1 Common Environmental Noise Sources

A modern laboratory is full of potential noise sources that can interfere with the measurement of small amplitude electrochemical signals. Computers, LCD displays, video cables, network routers, network cables, ovens, hotplates, stirrers, and fluorescent lighting are all examples of common laboratory equipment that may electromagnetically interfere with a delicate electrochemical measurement.

In general, the electrochemical cell, the cell cable, and the WaveNeuro should be located as far away from such noise sources as possible. It is especially important that the headstage cable be located well away from any electromagnetic noise sources (such as mouse or keyboard cables, network cables, video cables, power cords etc.). Typically, FSCV experiments are performed within a Faraday cage to limit noise from environmental sources. Ideally, the WaveNeuro should be physically located outside the Faraday cage. The headstage cable (or/and DB-25 extension cable) should extend from the WaveNeuro into the Faraday cage where the electrochemical experiment is performed. Environmental noise can be easily observed in the live color chart in HDCV software (see: Figure 6-1).

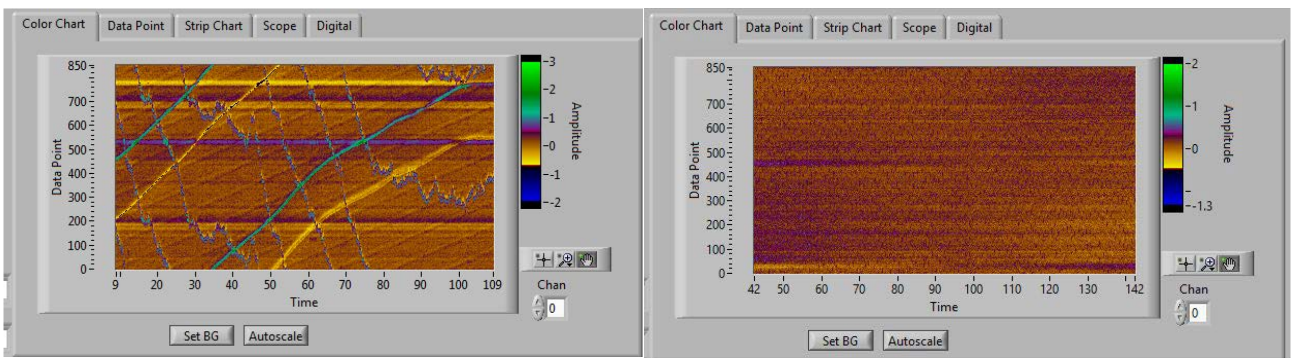


Figure 6-1: Live Color Chart Displays in HDCV with Noise (left) and without Noise (right)

A piece of laboratory equipment which intermittently draws a lot of current (such as an oven or hotplate under thermostatic control) should not be powered using the same branch circuit as the WaveNeuro. When such a piece of equipment goes through a power cycle, it may induce noise or a glitch in the electrochemical measurement. The WaveNeuro is designed to be connected to a personal computer (tower, desktop, or laptop) via a cable. The cables are long enough that the WaveNeuro can be placed at a reasonable distance (a few feet) from the computer. Do not allow the PC interfacing cables to run alongside or cross the headstage cable as this may induce noise in the electrochemical measurement.

6.2 Grounding Terminology

When using a potentiostat in conjunction with other electronic instruments, it is often necessary to make various grounding connections between the potentiostat and the other instrumentation. A proper approach to making such connections begins with a good understanding of the terminology associated with grounding. A potentiostat or other piece of electronic equipment generally has three types of grounding connections which are often confused with one another: the earth ground, the chassis terminal, and the DC Common. These are discussed in more detail below.

6.2.1 Earth Ground

EARTH GROUND:

An earth ground connection is a direct physical and electrical connection to the Earth.

An earth ground connection is available in most modern laboratories via the third prong on the power receptacle for the local power system. The power system infrastructure for a laboratory building usually has a long metal probe buried in the earth, and the third prong in the building wiring is connected to this earth connection. Many electronic devices have a three prong power cord which brings the earth ground connection to the power supply for the device. Whether or not the earth ground connection passes through the power supply and to the internal circuitry of the device depends upon the design of the power supply.

The power supply for the WaveNeuro potentiostat does not allow the earth ground connection to pass through to the instrument circuitry. This means that there is no permanent, direct connection to the earth ground when the instrument is connected to the AC Mains (*i.e.*, there is no connection to earth ground via the power cabled plugged into a receptacle). Nevertheless, an indirect, whether deliberate or inadvertent, connection to earth ground may occur when the potentiostat is connected to other electronic equipment. In fact, by using the National Instruments data acquisition interface, which makes an earth ground connection with the computer in which it is installed, the WaveNeuro chassis is tied to earth ground through the NI cable connector.

6.2.2 Chassis

CHASSIS:

A metal case which surrounds and protects the electronic circuitry is called the chassis.

The metal case that contains the WaveNeuro circuitry is the instrument chassis. The chassis helps to protect the circuitry from environmental noise sources and from ESD events.

When a NI Interface board cable is connected to the instrument, the grounds within the NI cable are connected directly to the WaveNeuro circuit board. When the other end of the NI cable is connected to the NI board installed in a computer, it is almost certain that a direct electrical connection between the instrument chassis and the computer chassis will be made. If the computer chassis happens to be connected to earth ground, then the NI cable becomes an indirect means by which the instrument chassis is connected to the earth ground.

6.2.3 DC Common

DC COMMON:

In an analog circuit, such as a potentiostat, the DC Common is the zero reference point against which voltages are measured. This point is also known as the analog ground, signal ground, or signal common.

The DC Common for the WaveNeuro potentiostat is the zero volt reference point used by the instrument circuit board.

7. DUAL CHANNEL WAVENEURO (OPTIONAL)

The previous sections of this user guide focus on single channel setup, testing, and operation. To expand the capabilities of the WaveNeuro, a Dual Channel Adapter (DCA) can be added onto the unit. With an additional headstage cable kit, the WaveNeuro can be used as a two-channel FSCV potentiostat, with HDCV software and a single NI PCIe-6363 interface board. This section will detail the installation, hardware setup, and software setup to use two working electrode channels. Previous sections apply for software installation, and Dummy Cell Performance Testing.

7.1 WaveNeuro Dual Channel Adapter (DCA)

The DCA enables two working electrode channels (*i.e.*, two headstages) to be simultaneously used with a single WaveNeuro Fast-Scan Cyclic Voltammetry Potentiostat System and a single NI PCIe-6363 interface board. The WaveNeuro can operate as either a single channel or as a dual channel potentiostat with the DCA installed. Once installed, the DCA should remain in place as the WaveNeuro can be used in single or dual channel mode with the DCA installed.

With the DCA, there are two modes of operation: 1) two working electrodes with a common reference electrode; and 2) two working and reference electrodes (i.e., two independent electrochemical cells). While both modes are possible, Pine Research recommends the first mode, that two working electrodes be connected to a single common reference electrode (ground).

To operate the WaveNeuro in two-channel mode, the DCA and an additional headstage cable kit must be purchased. The DCA bundle can be purchased with the WaveNeuro and can also be purchased separately, at a later time.

The DCA is a small circuit board with a series of DB-25 connectors on it (see: Figure 7-1). Two of the DB-25 connectors provide connections for two headstage cables, labeled as *HEADSTAGE 0* and *HEADSTAGE 1*. There is also a DB-25 connector for Behavioral connections. The *BEHAVIOR* connection behaves the same regardless of the number of Headstage cables connected (see: Section 2.7.7). There are some differences between *HEADSTAGE 0* and *HEADSTAGE 1* on the DCA. Refer to the comparison table when considering implementation of an experiment (see: Table 7-6).

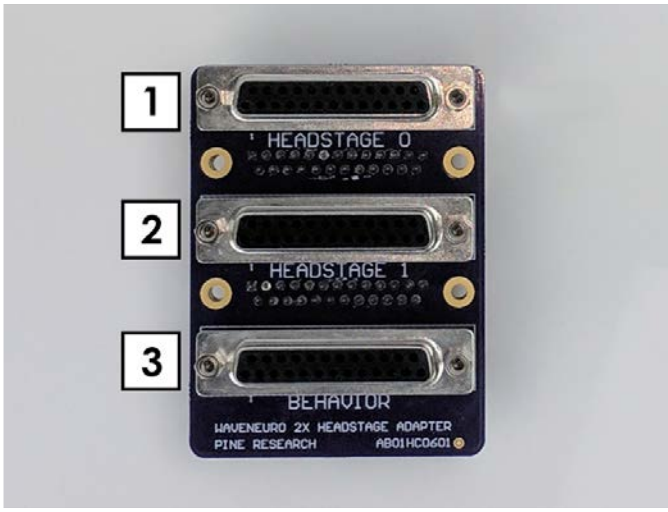
	***HEADSTAGE 0* Only**	***HEADSTAGE 1* Only**	***HEADSTAGE 0* and *1* Simultaneously**
Access Analog I/O BNCs	Yes	No	No
Filtering (see: Section 7.4.3)	Front Panel Selections	Fixed 5 kHz low-pass on applied waveform Fixed 20 kHz low-pass filter on current response	Filtering always as if independently used
HDCV Multiplier (see: Section 0)	3	1	see HDCV setup with DCA

Table 7-6. Channel 0 and Channel 1 Comparison

7.1.1 Dual Channel Adapter Installation

Installation of the adapter is fast, simple, and requires only a screwdriver (see: Figure 7-2).

To install the adapter, plug in and secure it to the existing DB-25 connectors. The adapter will only fit in one orientation (see: Figure 7-3). Ensure the adapter is seated on all the HEADSTAGE and BEHAVIOR DB-25 connectors. Secure the adapter in place by tightening four screws, included with the adapter (see: Figure 7-4).



1	***HEADSTAGE 0***	DB-25 Connector, whose connection through the WaveNeuro uses the front panel filter control.
2	***HEADSTAGE 1***	DB-25 Connector, whose connection through the WaveNeuro to the NI board uses the on board Analog input/output, has fixed current and voltage filters.
3	***BEHAVIOR***	DB-25 Connector, for use with behavior connections (*e.g.*, level press TTL inputs from external hardware)

Figure 7-1. WaveNeuro Dual Channel Adapter

Figure 7-2. WaveNeuro Potentiostat with Dual Channel Adapter Board

Figure 7-3. Connecting Dual Channel Adapter to WaveNeuro – Step 1

Figure 7-4. Tightening Dual Channel Adapter Screws – Step 2

7.2 System Verification

Before proceeding to hardware and software setup for dual channel operation, verify the following steps have been performed as previously described:

1. Verify the NI PCIe-6363 data acquisition board has been installed (see: Section 4.1.2) and its operation verified (see: Section 5.1.1).
2. Verify the WaveNeuro system status and the proper installation of HDCV (see: Section 5.1.2).
3. Verify the communication between the computer and WaveNeuro system (see: Section 5.1.5).

7.3 WaveNeuro Hardware Setup for Dual Channel Use

This section describes how to set-up HDCV and the WaveNeuro for two channel operation. Note that even with the DCA installed the WaveNeuro can function as a single channel potentiostat when a single headstage is connected to *HEADSTAGE 0*. Therefore, if the DCA has been purchased and installed, the WaveNeuro can operate with one or two headstage cables connected.

If a DCA is installed with two headstage cables connected, the WaveNeuro operates as a two channel potentiostat. The following sections will describe the set-up of dual channel operation. Dual headstage operation requires only one data acquisition board.

Ensure the following steps have been completed to setup the WaveNeuro and associated hardware to function in two channel mode:

1. Install the Dual Channel Adapter (see: Section 7.1.1).

2. In the ANALOG section on the front panel of the WaveNeuro, press the *GROUND CONNECT* button such that the LED light above the blue button is illuminated. When the LED is illuminated, the ground of the signal pathway, which is connected to both the *ABS OUT* BNC and *HEADSTAGE 1*, connects to the DC common of the WaveNeuro.

7.4 Software (HDCV) Setup for Dual Channel Use

This guide will detail how to setup software to use the WaveNeuro as a two-channel FSCV potentiostat. We will include directions specific to HDCV software. First, the user must setup the software to use two working electrode channels. Next, the user will configure each channel to apply the desired waveform and measure the resultant signal.

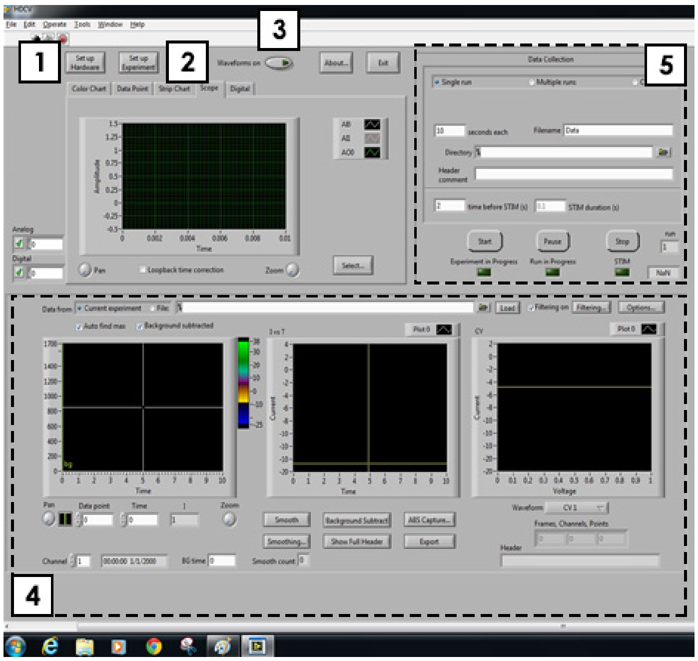
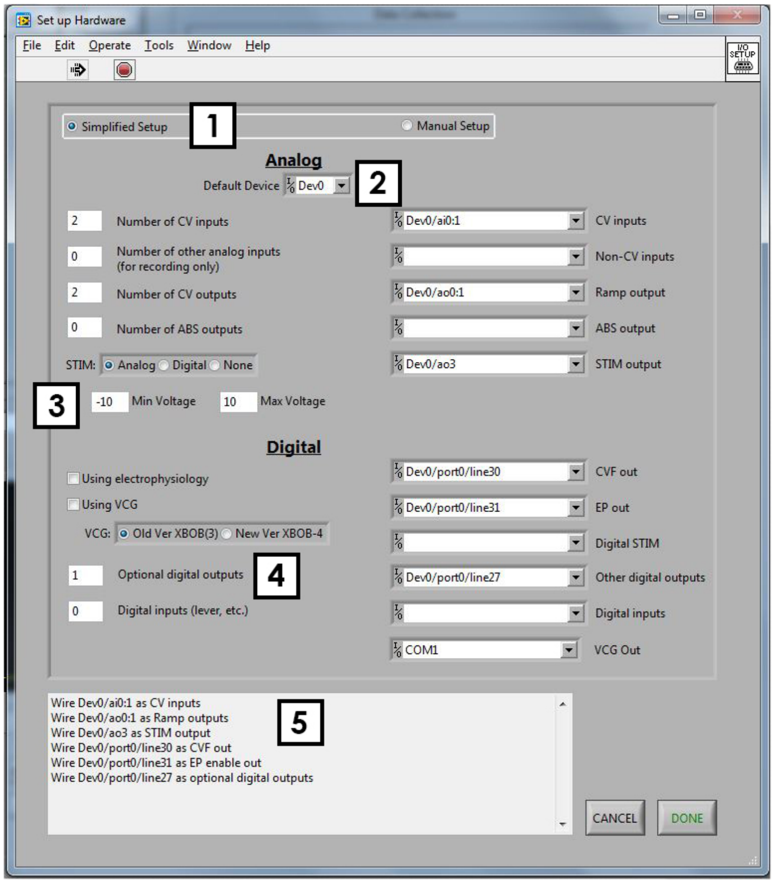


Figure 7-5. HDCV Application Primary Panels and Buttons

7.4.1 Hardware Setup in HDCV for Dual Channel Operation

1. Start HDCV software.
2. Click the *Set up Hardware* button (see: Figure 7-5). A pop-up menu appears, which enables the user to select and map the inputs and outputs of the system to the appropriate pins on the NI interface board (see: Figure 7-6). For most single working electrode experiments, users will select the *Simplified* Setup radio button In the *Set up Hardware* menu (see Figure 7-6). For dual working electrode channel operation, the following must be adjusted in the *Set up Hardware* menu:
3. Ensure the *Default Device* is set to *Dev0* (as previously explained in Section 5.1.1).
4. Under the Analog settings, Enter 2 in the Number of CV inputs box (see: Figure 7-6).
5. Under the Analog settings, Enter 2 in the Number of CV outputs box (see: Figure 7-6).
6. After committing the changes above, the dialog box on the bottom of the menu displays configuration changes. The first line will change from ***Wire Dev0/ai0 as CV inputs*** to ***Wire Dev0/ai0:1 as CV inputs***. If you do not see the message change, please revisit the first two steps to ensure the values have been appropriately updated (see: Figure 7-6).

7. The second line in the dialog box at the bottom of the screen will also change from ***Wire Dev0/ao0 as Ramp outputs*** to ***Wire Dev0/ao0:1 as Ramp outputs***. If you do not see the message change, please revisit the first two steps to ensure the values have been appropriately updated (see: Figure 7-6).
8. Press *Done* to save the changes and return to the main HDCV screen.



1	**Setup Type Radio Button**	For standard one channel use, select *Simplified Setup*. To enable and map connections for two channel use, select *Manual Setup*.
2	**Default Device**	Dropdown selection reads from the NI-DAQ™mx setting to define the appropriate data acquisition device. Pine Research recommends setting the device to *Dev0* as described in Section 5.1.1.
3	**Analog Min Voltage and Max Voltage**	Full range for the WaveNeuro and PCIe-6363 is $\pm 10\ V$.
4	**Optional digital outputs**	Total number of software-defined digital outputs. Used for flow cell trigger, most often.
5	**HDCV Hardware Setup Dialog**	Software generated messages to alert user of settings as well as any errors.

Figure 7-6. Set Up Hardware Window in HDCV

1	**CV Ramp Tab**	Select for basic waveform definitions, channel naming, and multiplier selection
2	**Channel Selection Radio Button**	HDCV allows editing of one waveform at a time. Edit the first working electrode channel by selecting leftmost radio button.
3	**Channel Name**	Name the channel as desired. This name propagates across all aspects of HDCV (*Ramp 0* suggested).
4	**Scan Rate**	Enter the desired scan rate for the waveform. For the purposes of this guide, enter 400 *V/s* for *Ramp 0*.
5	**Voltage Adjustments Multiplier**	*Ramp 0* (working electrode channel 0 / *HEADSTAGE 0*) **must be set to 3**.
6	**Channel Selection Radio Button**	HDCV allows editing of one waveform at a time. Edit the second working electrode channel by selecting the rightmost radio button.
7	**Channel Name**	Name the channel as desired. This name propagates across all aspects of HDCV (*Ramp 1* suggested).
8	**Scan Rate**	Enter the desired scan rate for the waveform. For the purposes of this guide, enter 200 *V/s* for *Ramp 1*.
9	**Voltage Adjustments Multiplier**	*Ramp 1* (working electrode channel 1 / *HEADSTAGE 1*) **must be set to 1**.
10	**Message Box**	For either channel, HDCV will report any errors with entered parameters, else *No Error*.

Figure 7-7. Dual Channel Experimental Setup Panels (*Ramp 0* and *Ramp 1* Both Shown)

7.4.2 Experiment Setup in HDCV for Dual Channel Operation

After completing the WaveNeuro hardware setup (see: Section 7.3) and HDCV hardware setup (see: Section 7.4.1) for dual channel operation, the next step is to define experimental conditions in HDCV *Set up Experiment* settings. HDCV requires experimental parameters for each channel. When the same parameters are used on both channels simultaneously, the user need copy the parameters from Channel 0 to Channel 1. Alternatively, the user can setup HDCV to apply a unique waveform to each channel.

To edit the experimental conditions, on a per channel basis, select the *Set up Experiment* button from the main HDCV page (see: Figure 7-5). A pop-up menu that contains several tabs opens (see: Figure 7-7). The Set up Experiment menu enables the user to define several different experimental parameters, including waveform (ramp) settings, flow cell trigger, and/or stimulus parameters. This section will focus on setting up the waveform parameters for each channel. The instructions are as follows:

1. Select the *CV Ramp* tab. The *CV Ramp Advanced* tab can also be selected. The advanced tab gives a greater number of options, which may be needed by the user. For the purpose of this guide, a more simplistic overview will be provided using the options on the *CV Ramp* tab. Notice, two radio buttons are present. Selecting a radio button adjusts the waveform parameters for one channel at a time (see: Figure 7-7).
2. Select the first radio button. Change the text in the *Name* field to your preferred label (HDCV labels this as CV 1 by default). Pine Research recommends the name *Ramp 0*, as it corresponds with the *HEADSTAGE 0* connector on the DCA.
3. Adjust the *Ramp 0* waveform parameters as desired. For these instructions, common dopamine waveform parameters have been used (see: Table 7-7), with a *Scan rate* of $400\ V/s$. Remember, waveform parameters are adjusted on a per channel basis. Changes you make to one channel will not carry over to the other channel automatically. For *Ramp 0* (*HEADSTAGE 0*), **set the *Voltage adjustments Multiplier* to 3**.
4. Select the second radio button. Change the text in the Name field to your preferred label (HDCV labels this as CV 2 by default). Pine Research recommends the name *Ramp 1*, as it corresponds with the *HEADSTAGE 1* connector on the DCA.
5. Adjust the *Ramp 1* waveform parameters as desired. For these instructions, common dopamine waveform parameters have been used (see: Table 7-7), with a *Scan rate* of $200\ V/s$. Remember, waveform parameters are adjusted on a per channel basis. Changes you make to one channel will not carry over to the other channel automatically. For *Ramp 1* (*HEADSTAGE 1*), **set the *Voltage adjustments Multiplier* to 1**.

INFO:

When using two channels, it is critical to set the Voltage adjustments Multiplier for each channel correctly.

The headstage cable connected to *HEADSTAGE 0* on the DCA (*Ramp 0* as suggested above) should have the Multiplier set to 3.

The headstage cable connected to *HEADSTAGE 1* on the DCA (*Ramp 1* as suggested above) should have the Multiplier set to 1.

6. If acceptable values have been entered for *Ramp 0* and *Ramp 1* waveforms, the dialog box at the bottom of the screen will report No Error (see: Figure 7-7).

Setting	*Ramp 0*	*Ramp 1*
V1	-0.4	-0.4
V2	1.3	1.3
Phase shift	0	0
Number of cycles	1	1
Start delay (ms)	0	0
Scan rate (V/s)	400	200
Data point per scan	850	1700
Multiplier	3	1
Offset (mV)	0 or 1	0 or 1

Table 7-7. Typical Dopamine Waveform Settings for Dual Channel Testing

7. Verify the headstage gain for each channel. Select the *Analog Inputs* tab. Now, two entries exist such that the headstage amplification (gain) can be set individually (see: Figure 7-8). Enter the appropriate gain ratio into the *Gain (nA/V)* field, select *Ramp 0* or *Ramp 1* from the *Using waveform* drop down list, and enter a *Name* (optional). To increase clarity on the Scope tab, when viewing all input and output signals, Pine Research recommends naming the Analog Inputs as *HS 0* (for the headstage connected to *HEADSTAGE 0*) and *HS 1* (for the headstage connected to *HEADSTAGE 1*). The WaveNeuro with DCA under HDCV control allows the use of different gains as desired. The standard headstage gain from Pine Research is 200 nA/V ($5\ M\Omega$). Alternate headstage gains may be available, contact Pine Research for more information. For more detailed information on headstage gain, review earlier sections of this guide (see: Figure 5-5).

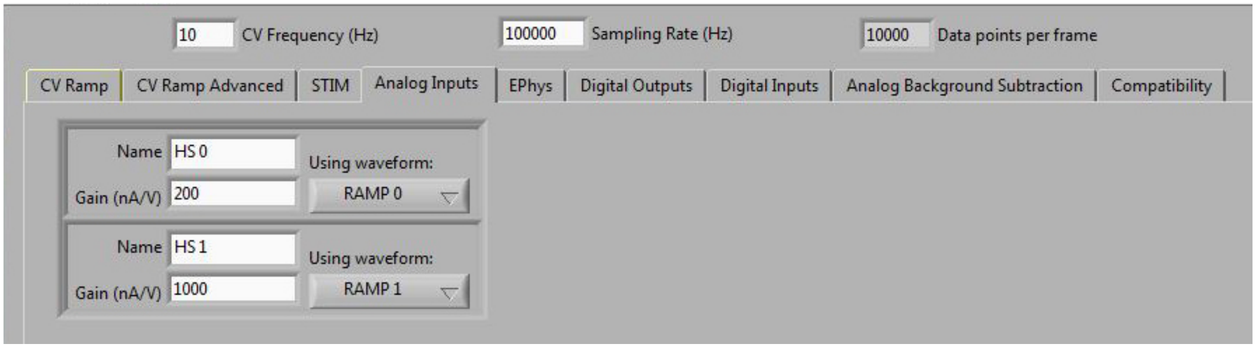


Figure 7-8. Analog Input Tab to Set Headstage Amplification Gain

8. Setup any digital inputs/outputs. For in-vitro experiments, it is common to setup a Flow Cell trigger, which is a digital output (see: Section 5.1.4 and Figure 5-6). Press *Done* to save the changes and return to the main HDCV page. Now two waveforms will be applied as defined by the researcher and two channels of data collected.

7.4.3 Filter Settings for Dual Channel Operation

Note that in addition to the different multiplier settings, the filtering is also different between the two data channels. The filtering on *HEADSTAGE 0* is controlled by the filter select buttons on the front panel of the WaveNeuro. The same as with the standard single channel WaveNeuro (see: Section 2.7.3). The

HEADSTAGE 1 working electrode channel has fixed filtering that is set in the hardware. The waveform is low-pass filtered at $5\ kHz$ and the current response is low-pass filtered at $20\ kHz$.

7.5 Testing Dual Channel Operation

Section 5.2 provides step by step instructions for testing the WaveNeuro performance, at each step along the headstage cable kit, including headstage amplifier.

> **TIP:**
>
> **Whenever it is necessary to verify that the WaveNeuro and Dual Channel Adapter are operating correctly, perform the system tests described in this guide (see: Section 5.2). These tests are the first actions that will be suggested by technical support personnel in the event that a user contacts Pine Research technical support.**

When using the DCA with two headstage cable kits connected and the *Waveforms on* button is illuminated, the different analog input and output signals can be viewed on the Scope tab of HDCV (see: Figure 7-5). The legend next to the Scope screen will show four entries, which are two analog inputs and two analog outputs. The legend will use the names entered previously (see: 7.4.2), and as recommended in this guide, as *HS 0*, *HS 1, Ramp 0*, and *Ramp 1*. The default names assigned by HDCV for these signals are *AI0*, *AI1*, *AO0*, and *AO1, respectively*. If an entry in the legend is grayed out, its trace will not be visible on the scope plot. To make the signal visible, right click on the legend entry and select *Plot Visible*.

Follow the testing procedures outlined for single channel operation (see: Section 5.2), but adjust the instructions to accommodate two working electrode channels. The Dual Channel WaveNeuro bundle includes two dummy cells, such that each working electrode channel can be connected to a dummy cell during the various testing steps (see: Figure 7-9). When performing the dummy cell tests with a dummy cell connected to each headstage cable (*HEADSTAGE 0* and *HEADSTAGE 1*), the Scope will show two current responses (*HS 0* and *HS 1*) and two waveforms (*Ramp 0* and *Ramp 1*) simultaneously (see: Figure 7-10).

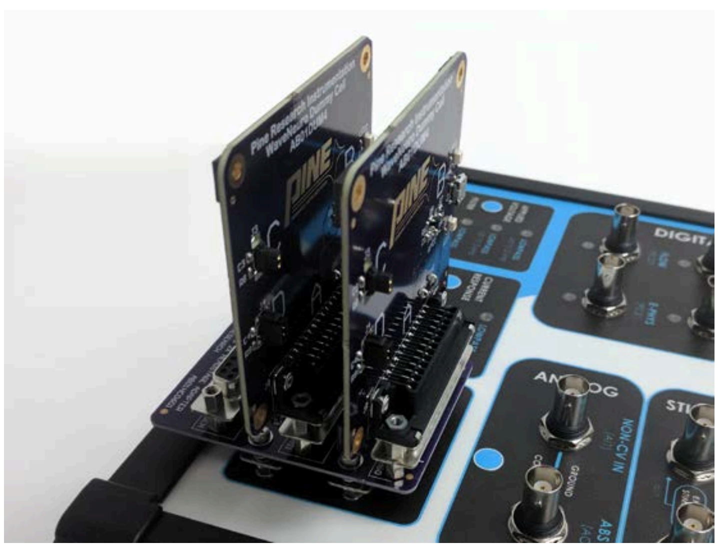


Figure 7-9. WaveNeuro with DCA and Two Dummy Cells Installed

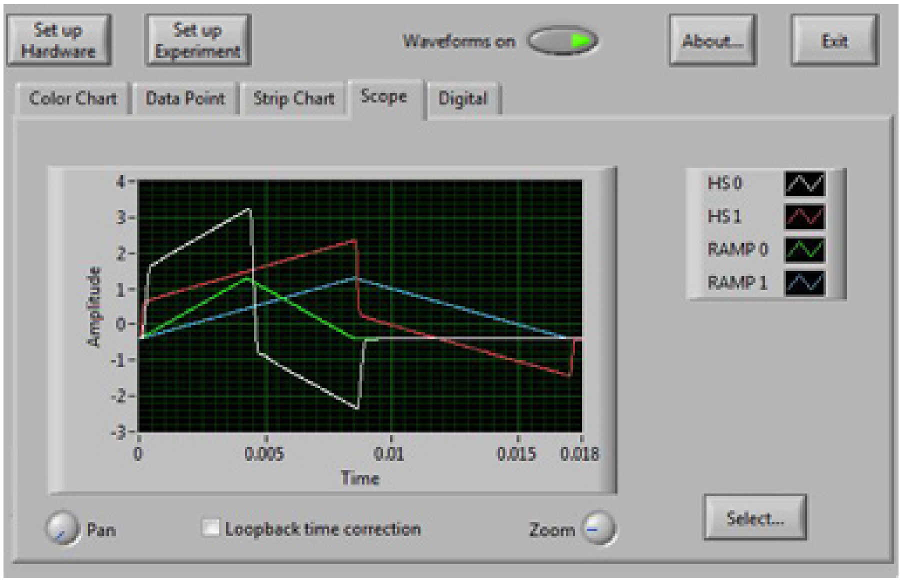


Figure 7-10. Scope Results for Simultaneous Dual Channel Dummy Cell Testing

Alternatively, test each channel separately while leaving the other channel open. In this testing mode, a single channel current signal (analog input) responds to the applied waveform (analog output), while the other channel I/O does not change (see: Figure 7-11). In the example shown, the waveforms applied to channel 0 and channel 1 remain constant, while moving the dummy cell between connections shows a change in current response (see: Figure 7-11). The current response from a channel will float when not connected to a dummy cell yet the *Waveforms on* button is illuminated. The value of this response is meaningless.

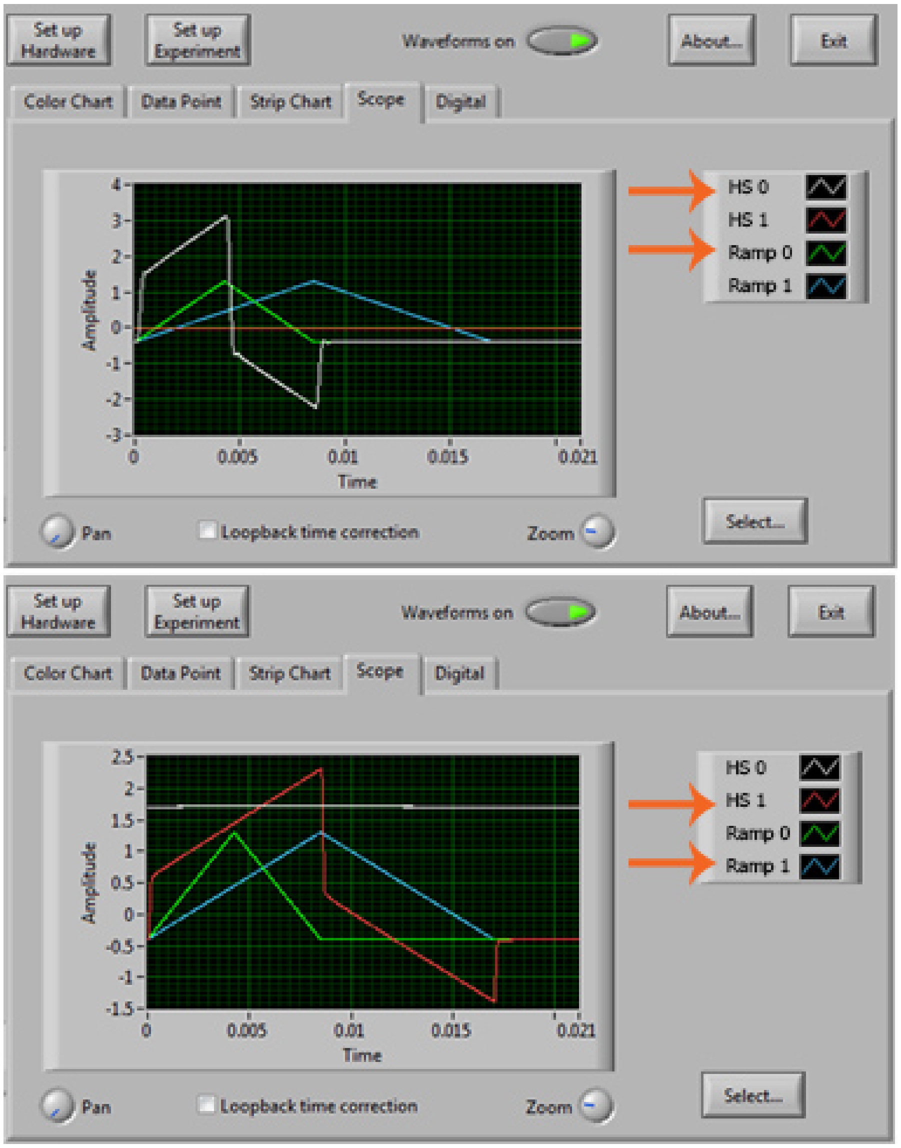


Figure 7-11. Scope Results for Dummy Cell Testing of Independent Channels

7.6 Dual Channel Data Viewing in HDCV

In the live *Color Chart* mode and in the *HDCV Analysis software*, data is only shown for one channel at a time. HDCV records data from two channels and the user must toggle views between channel 0 and channel 1. A selector switch next to each plot allows the user to choose between channel 0 and channel 1 (see: Figure 7-12). Data from either channel can be manipulated and exported independently.

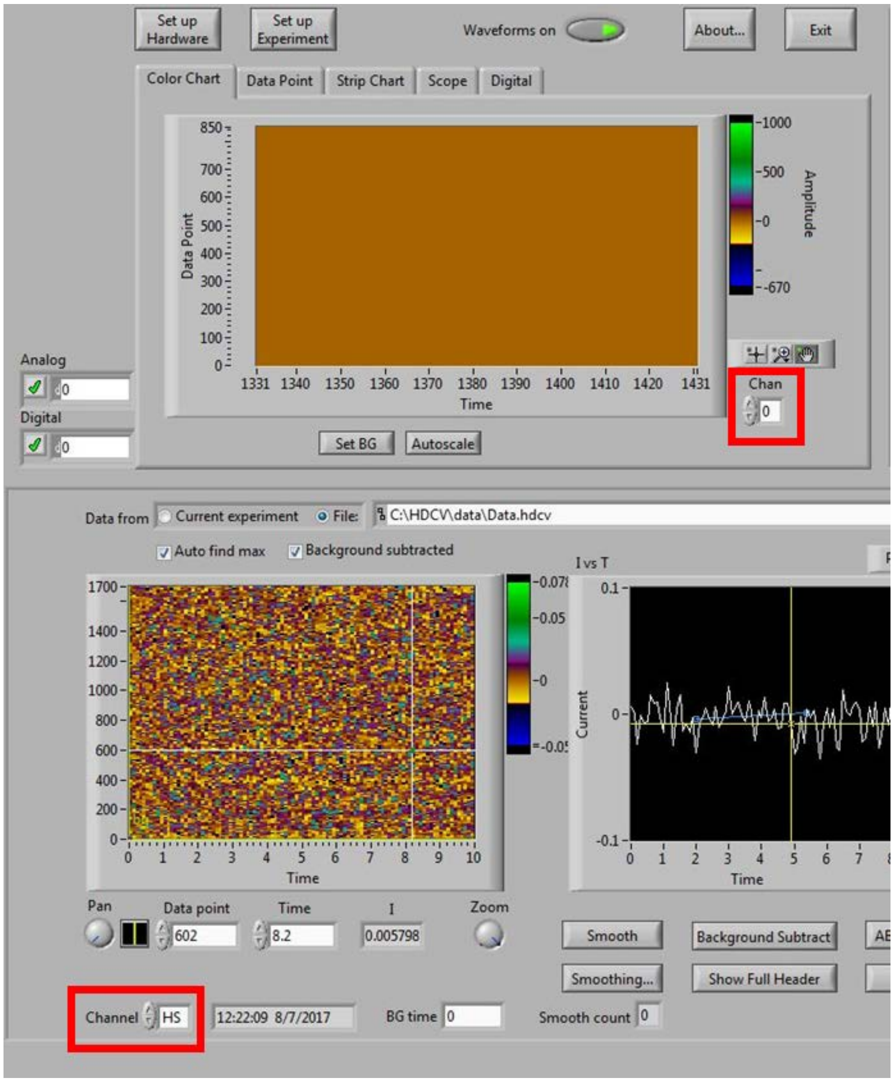


Figure 7-12. HDCV screen shot with channel selection switch highlighted.

8. POWER CORDS

The standard C13 type plug connector on the WaveNeuro power supply is compatible with a wide range of power cords available from Pine Research Instrumentation (see: Table 8-8 and Table 8-9). Each of the available power cords is rated at 10 Amps (minimum), and each cord is designed for use in a specific country or region of the world. Pine Research part numbers provided.

8.1 International Power Cords

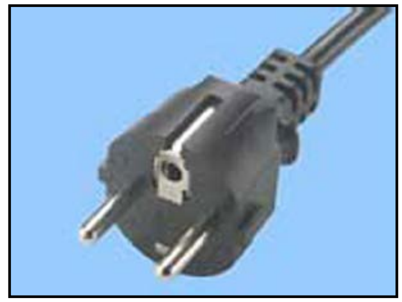
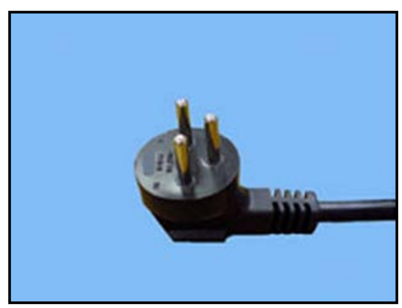
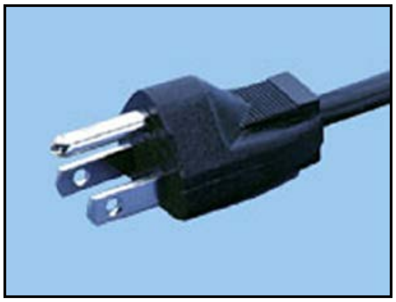
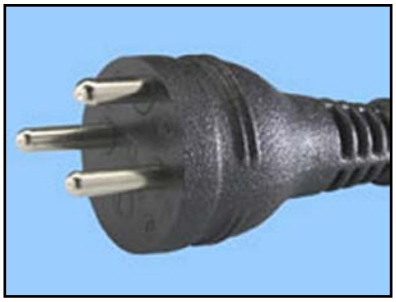
	
This cord is for use in the USA, Canada, Mexico, Brazil, Columbia, Korea, Mexico, Saudi Arabia, and Taiwan.	This cord is for use in continental Europe, Russia, and Indonesia.
Power Cord (USA) EWM18B7	**Power Cord (Europe) EWM18B8EU**
	
This cord is for use in the United Kingdom, Ireland, Oman, Hong Kong, and Singapore.	This cord is for use exclusively in China.
Power Cord (UK) EWM18B8UK	**Power Cord (China) EWM18B8CN**
	
This cord is for use in India and South Africa.	This cord is for use exclusively in Israel.
Power Cord (India) EWM18B8IN	**Power Cord (Israel) EWM18B8IL**

Table 8-8: Select Power Cords Available from Pine Research Instrumentation (Part I).



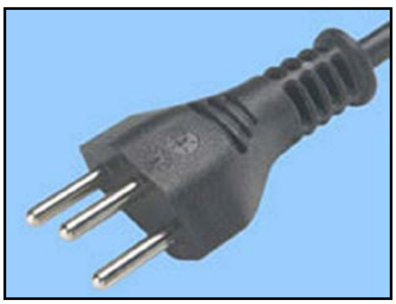
This cord is for use exclusively in Japan.
Power Cord (Japan) EWM18B8JP

This cord is for use exclusively in Argentina.
Power Cord (Argentina) EWM18B8AR

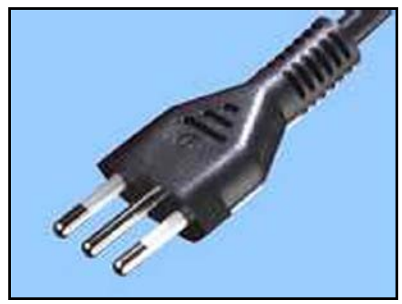


This cord is for use exclusively in Denmark.
Power Cord (Denmark) EWM18B8DK

This cord is for use in Australia & New Zealand.
Power Cord (Australia) EWM18B8NZ



This cord is for use exclusively in Switzerland.
Power Cord (Switzerland) EWM18B8CH



This cord is for use exclusively in Italy.
Power Cord (Italy) EWM18B8IT

Table 8-9: Select Power Cords Available from Pine Research Instrumentation (Part II)

9. GLOSSARY

9.1 Important Terms used in this Guide

Anodic Current	Flow of charge at an electrode as a result of an oxidation reaction occurring at the electrode surface. For a working electrode immersed in a test solution, an anodic current corresponds to flow of electrons out of the solution and into the electrode.
Banana Cable	A banana cable is a single-wire (one conductor) signal cable often to make connections between various electronic instruments. Each end of the cable has a banana plug. The plug consists of a cylindrical metal pin about 25 mm (one inch) long, with an outer diameter of about 4 mm, which can be inserted into a matching banana jack.
Banana Jack	Female banana connector
Banana Plug	Male banana connector
BNC Connector	The BNC (Bayonet Neill-Concelman) connector is a very common type of used for terminating coaxial cables (see: https://en.wikipedia.org/wiki/RF_connector).
Cathodic Current	Flow of charge at an electrode as a result of a reduction reaction occurring at the electrode surface. For a working electrode immersed in a test solution, a cathodic current corresponds to flow of electrons out of the electrode and into the solution.
Coaxial Cable	Coaxial cable, or coax, is an electrical cable with an inner conductor surrounded by a flexible, tubular insulating layer, surrounded by a tubular conducting shield. The term coaxial comes from the inner conductor and the outer shield sharing the same geometric axis. Coaxial cable is often used to carry signals from one instrument to another in situations where it is important to shield the signal from environmental noise sources.
Counter Electrode	The counter electrode, also called the auxiliary electrode, is one of three electrodes found in a typical three-electrode voltammetry experiment. The purpose of the counter electrode is to carry the current across the solution by completing the circuit back to the potentiostat.
Cyclic Voltammetry	An electroanalytical method where the working electrode potential is repeatedly swept back and forth between two extremes while the working electrode current is measured.
Dummy Cell	A known network of resistors and capacitors used to test a potentiostat. The dummy cell is used in place of an actual electrochemical cell when troubleshooting a potentiostat because the dummy cell provides a known response whereas the response from an actual cell is complicated by chemical phenomena.
Electroactive	An adjective used to describe a molecule or ion capable of being oxidized or reduced at an electrode surface.

Electrode	An electrode is an electrical conductor used to make contact with a nonmetallic part of a circuit.
Faradaic Current	The portion of the current observed in an electroanalytical experiment that can be attributed to one or more redox processes occurring at an electrode surface.
Fast Scan Cyclic Voltammetry (FSCV)	Electrochemical technique in which the working electrode potential is rapidly (>50 V/s) swept while measuring the current.
Half-Reaction	A balanced chemical equation showing how various molecules or ions are reduced (or oxidized) at an electrode surface.
	A small amplifier or pre-amplifier typically positioned near the
Linear Sweep Voltammetry	Experiment in which the working electrode potential is swept from initial value to final value at a constant rate while the current is measured.
Non-Faradaic Current	The portion of the current observed in an electroanalytical experiment that cannot be attributed to any redox processes occurring at an electrode surface.
Overpotential	The overpotential is the difference between the formal potential of a half reaction and the potential actually being applied to the working electrode.
Oxidation	Removal of electrons from an ion or molecule.
Redox	An adjective used to describe a molecule, ion, or process associated with an electrochemical reaction.
Reduction	Addition of electrons to an ion or molecule.
Reference Electrode	A reference electrode has a stable and well-known thermodynamic potential. The high stability of the electrode potential is usually achieved by employing a redox system with constant (buffered or saturated) concentrations of the ions or molecules involved in the redox half reaction.
Standard Electrode Potential	A thermodynamic quantity expressing the free energy of a redox half reaction in terms of electric potential.
Sweep Rate	The rate at which the electrode potential is changed when performing a sweep voltammetry technique such as cyclic voltammetry.
Three-Electrode Cell	A common electrochemical cell arrangement consisting of a working electrode, a reference electrode, and a counter electrode.
Two Electrode Cell	A common electrochemical arrangement when using a micro- or ultramicroelectrode consisting of a working and reference electrode
Voltammogram	A plot of current vs. potential from an electroanalytical experiment in which the potential is swept back and forth between two limits.

Working Electrode

The electrode at which the redox process of interest occurs. While there may be many electrodes in an electrochemical cell, the focus of an experiment is typically only on a particular half reaction occurring at the working electrode.

10. CONTACT SUPPORT

After reviewing the content of this user guide, please contact Pine Research Instrumentation should you have any issues or questions with regard to the WaveNeuro, WaveNeuro accessories, instrumental setup, National Instruments components, and general software questions.

Our staff is happy to help with software concerns, however, we are most familiar with HDCV and may not have the experience with different software. We will utilize our resources to secure the assistance you need based on your specific software installation.

Contact us anytime by the methods provided below:

10.1 By E-mail

Send an email to pinewire@pineresearch.com. This is the general sales email and our team will ensure your email is routed to the most appropriate technical support staff available. Our goal is to respond to emails within 24 hours of receipt.

10.2 By Phone

Our offices are located in Durham, NC in the eastern US time zone. We are available by phone Monday through Friday from 9 AM EST to 5 PM EST. You can reach a live person by calling +1 (919) 782-8320.

10.3 By Carrier Pigeon

From time to time, modern technology can be the major cause of inefficiencies. In the event you have a well-trained carrier pigeon (or dove, or hawk, we do not discriminate), you can send him/her, carrying a list of questions, to our offices at the following address:

2741 Campus Walk Avenue
Building 100
Durham, NC 27705
USA