

WaveNeuro 4 Fast-Scan Cyclic Voltammetry (FSCV) Potentiostat System



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1. PREFACE

1.1 Scope

This User Guide describes the WaveNeuro 4 Multiple Channel 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 are devoted to electrochemical concepts and 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 4 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 can be obtained from The University of North Carolina at Chapel Hill Chemical Research Instrumentation Teaching and Core Labs (CRITCL) Electronics facility, whose contact information is found at:

<https://chem.unc.edu/criticl-electronics/>

1.2 Copyright

This publication may not be reproduced or transmitted in any form, electronic or mechanical, including photocopying, recording, storing in an information retrieval system, or translating, in whole or in part, without the prior written consent of Pine Research Instrumentation.

1.3 Trademarks

All trademarks are the property of their respective owners. NI, NI-DAQ, LabVIEW, and DAQCard are trademarks of National Instruments (Austin, TX). *Windows* is a registered trademark of Microsoft Corporation (Redmond, WA). *WaveNeuro* is a registered trademark of Pine Research Instrumentation (Durham, NC).

1.4 Use Limitation

The WaveNeuro 4 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 4 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 4 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 4 Fast-Scan Cyclic Voltammetry Potentiostat System (Pine part number AF01FSCV4, 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.









	<p>CAUTION:</p> <p>Indicates information needed to prevent injury or death to a person or to prevent damage to equipment.</p>
	<p>ATTENTION:</p> <p><i>Indique les informations nécessaires à la prévention de blessures corporelles ou de mort d'un individu ou à la prévention des dommages aux équipements.</i></p>
	<p>STOP:</p> <p>For a procedure involving user action or activity, this icon indicates a point in the procedure where the user must stop the procedure.</p>
	<p>ARRÊT:</p> <p><i>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.</i></p>
	<p>NOTE:</p> <p>Important or supplemental information.</p>
	<p>REMARQUE:</p> <p><i>Renseignements importants ou complémentaires.</i></p>
	<p>TIP:</p> <p>Useful hint or advice.</p>
	<p>CONSEIL:</p> <p><i>Astuce ou conseil utile.</i></p>

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 4 bears information to identify the instrument (see: Figure 1-1).



Figure 1-1: WaveNeuro 4 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 4 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 4 instrument, power cords, and accessories are described in more detail later (see Section 0). WaveNeuro series of potentiostats have model numbers with the format AF01FSCV#, the # indicates the number of channels; therefore, the WaveNeuro 4 part number is AF01FSCV4.

1.8 Safety Warnings (Avertissements de sécurité)



CAUTION:

Connect the Power Supply to the AC mains using the Power Cord supplied with the WaveNeuro 4 and certified for the country of use. (see: Section 6 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 4 et conforme aux réglementations du pays d'utilisation (pour plus de détails, consultez la partie 6 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 4.

AVERTISSEMENT:

Branchez l'alimentation au secteur à l'aide du cordon d'alimentation et de l'adaptateur de type de prise fourni avec WaveNeuro 4.



CAUTION:

The switch on the back of the WaveNeuro 4 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 4 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 4. 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 4. 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 4 in an explosive atmosphere.

AVERTISSEMENT:

N'utilisez pas l'appareil WaveNeuro 4 dans une atmosphère explosive.

**CAUTION:**

Do not operate the WaveNeuro 4 in wet or damp conditions. Keep all instrument surfaces clean and dry.

AVERTISSEMENT:

N'utilisez pas l'appareil WaveNeuro 4 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 4 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 4 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 4 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 4 Fast-Scan Cyclic Voltammetry Potentiostat System utilizes a National Instruments Data Acquisition Device (NI PCIe-6363) to interface to a personal computer. The WaveNeuro 4 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 obtain HDCV software from The University of North Carolina at Chapel Hill Chemical Research Instrumentation Teaching and Core Labs (CRITCL) Electronics facility, whose contact information is found at:

<https://chem.unc.edu/criticl-electronics/>

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 $> 100 \text{ V/s}$, 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 to a few minutes); 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 to the change in concentration of the species.

A series of background subtracted CVs collected over a 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).

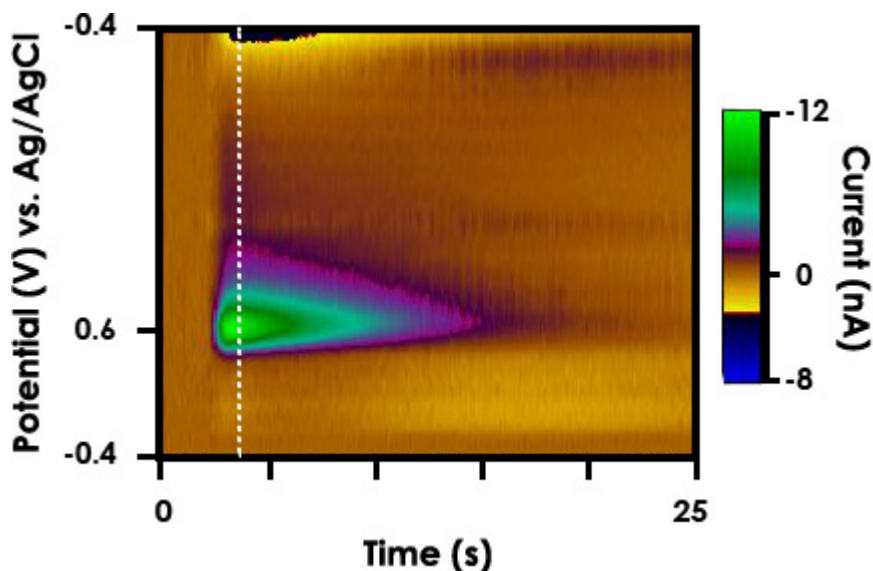


Figure 2-1. FSCV Color Plot of Evoked Dopamine Release in a Freely Moving Rat

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 color plot, which is a stack of many CVs in time (see: Figure 2-1). Viewing CV data as a color plot 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 4 Fast-Scan Cyclic Voltammetry System is a benchtop potentiostat specially designed for FSCV measurements. The WaveNeuro 4 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 4 controls up to four independent two-electrode electrochemical cells or enables up to four working electrodes to be controlled in a single system. The electrochemical accessories that connect to the WaveNeuro 4 are working electrodes, typically a carbon fiber microelectrodes, and a reference electrode often Ag/AgCl (see: Section 2.7.7 for more details about electrode connections).

The WaveNeuro 4 applies a potential waveform that is controlled by the software. Note that the WaveNeuro 4 is designed to apply the electrochemical waveform to the working electrodes and maintain the reference electrode at ground (see: Section 3). This configuration enables independent waveforms to be applied to each of the working electrodes. Some limitation exist on the software's handling of four working electrodes. See Section 6 of this user guide to review software settings on the application of multiple waveforms in HDCV.



INFO:

The WaveNeuro 4 applies the electrochemical 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.

In order to facilitate multiple working electrodes in a single electrochemical environment, the potential ramp is applied to and the change current is measured at the working electrode. 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 Ω or 200 nA/V). Additional headstage amplifier gains and configurations are available from Pine Research. Please contact us with any special requests/custom gain levels.

2.3 Software Description

The WaveNeuro 4 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) and HDCV Analysis.

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 4 to a National Instruments interface board. HDCV performs complex electrochemical

Academic users can obtain HDCV software from The University of North Carolina at Chapel Hill Chemical Research Instrumentation Teaching and Core Labs (CRITCL) Electronics facility, whose contact information is found at:

<https://chem.unc.edu/critcl-electronics/>

2.4 Instrument Specifications

The WaveNeuro 4 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 Connectors	Four female DB-25 provides electrode signal lines and power to the electrochemical headstages 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. See product instructions for details about specialty headstages, such as multiple channel headstages.
Working Electrode	Yellow lead wire from the electrochemical headstage. See product instructions for details about specialty headstages, such as multiple channel headstages.

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 the Faraday cage or to earth ground to improve noise screening (shielding). (See: 7.1 for more information on grounding for low noise operation.)

MEASURED CURRENT

Current Range	Defined by electrochemical headstage, typically $\pm 200 \text{ nA/V}$ ($5 \text{ M}\Omega$) headstage amplifiers are 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	$\pm 3.3 \text{ V}$ (maximum range)
Resolution	$100 \mu\text{V}$ (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	$\pm 10.0 \text{ V}$ (maximum range defined by the interface board)
Resolution (at each range)	$313 \mu\text{V}$ (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	$\pm 10.0 \text{ V}$ (maximum range defined by the interface board)
Resolution (at each range)	$313 \mu\text{V}$ (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 Ω), 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 4 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 ± 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 × 245 × 295 mm (2.25 × 9.7 × 11.6 in)
Instrument Weight	2.3 kg (5 lb)
Temperature Range	10°C to 40°C
Humidity Range	80% RH maximum, non-condensing

2.5 Major System Components

A WaveNeuro 4 Fast-Scan Cyclic Voltammetry Potentiostat System, as shipped from the production facility, includes the parts listed in the following table (see: Table 2-1). 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
AF01FSCV4	WaveNeuro 4 Fast-Scan Cyclic Voltammetry Potentiostat System
RRCH0124-T or RRCH0136-T	Four headstage amplifier cable, DB-25 connector, 24" or 36"
AC01HS1	Four 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 (4 packages of 5)
RRPA12V	AC Adapter/Power Supply; 12 V, 2.5 A input
AFDUM4	WaveNeuro dummy cell

Table 2-1: WaveNeuro 4 System Components

In addition, the WaveNeuro 4 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 4 instrument.

A National Instruments interface board and interface cables will be required to control the WaveNeuro 4 under software control. These may be obtained from Pine Research or users may obtain them separately, directly from National Instruments (see: Table 2-2). 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 2-2: Additional WaveNeuro 4 System Components (Sold Separately)

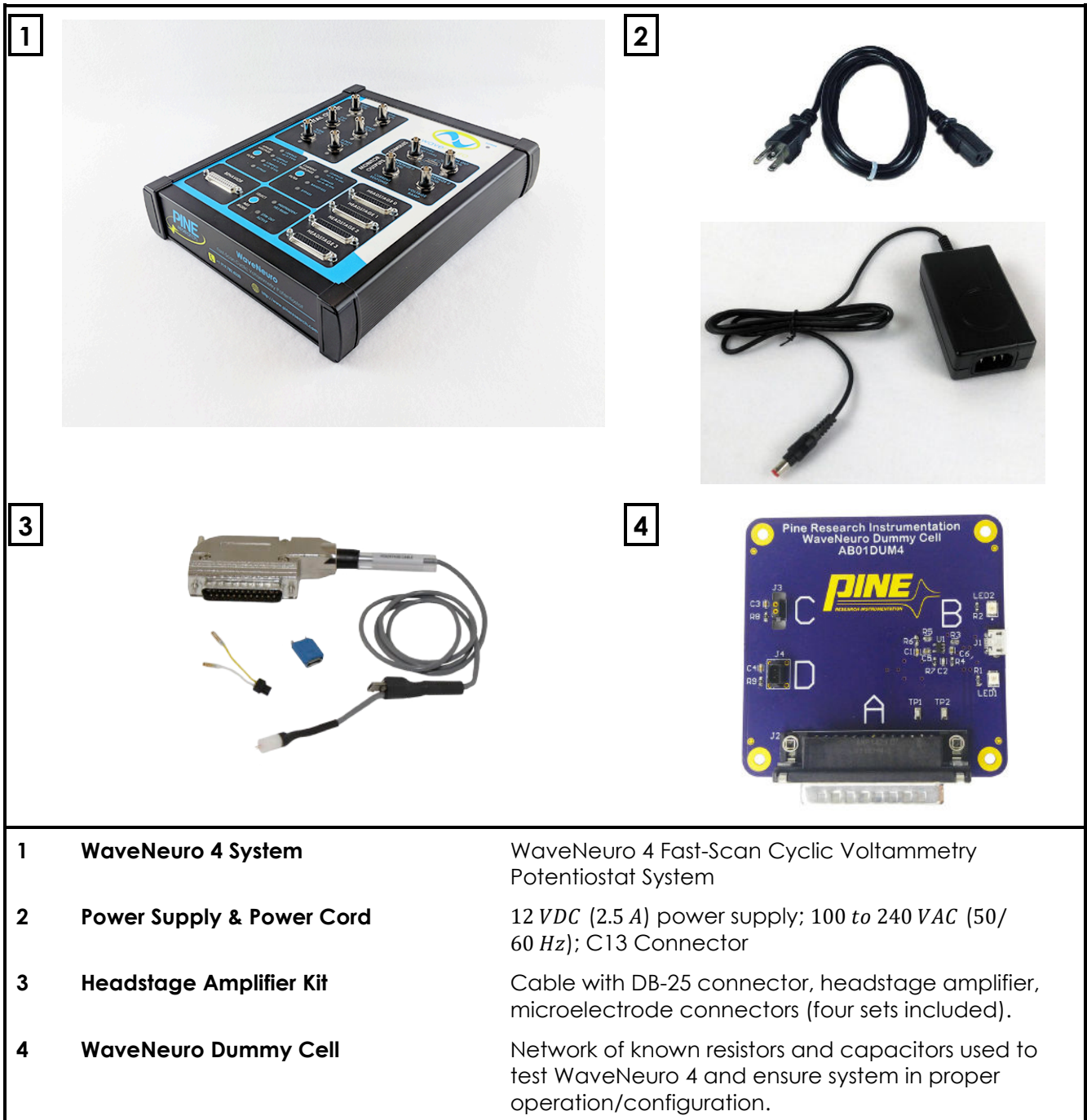


Figure 2-2: Major WaveNeuro 4 System Components (not to scale)

2.6 WaveNeuro 4 Side/End Panel

The side/end panel of the WaveNeuro 4 features several input and output connections whose location and function is described below (see: Figure 2-3).

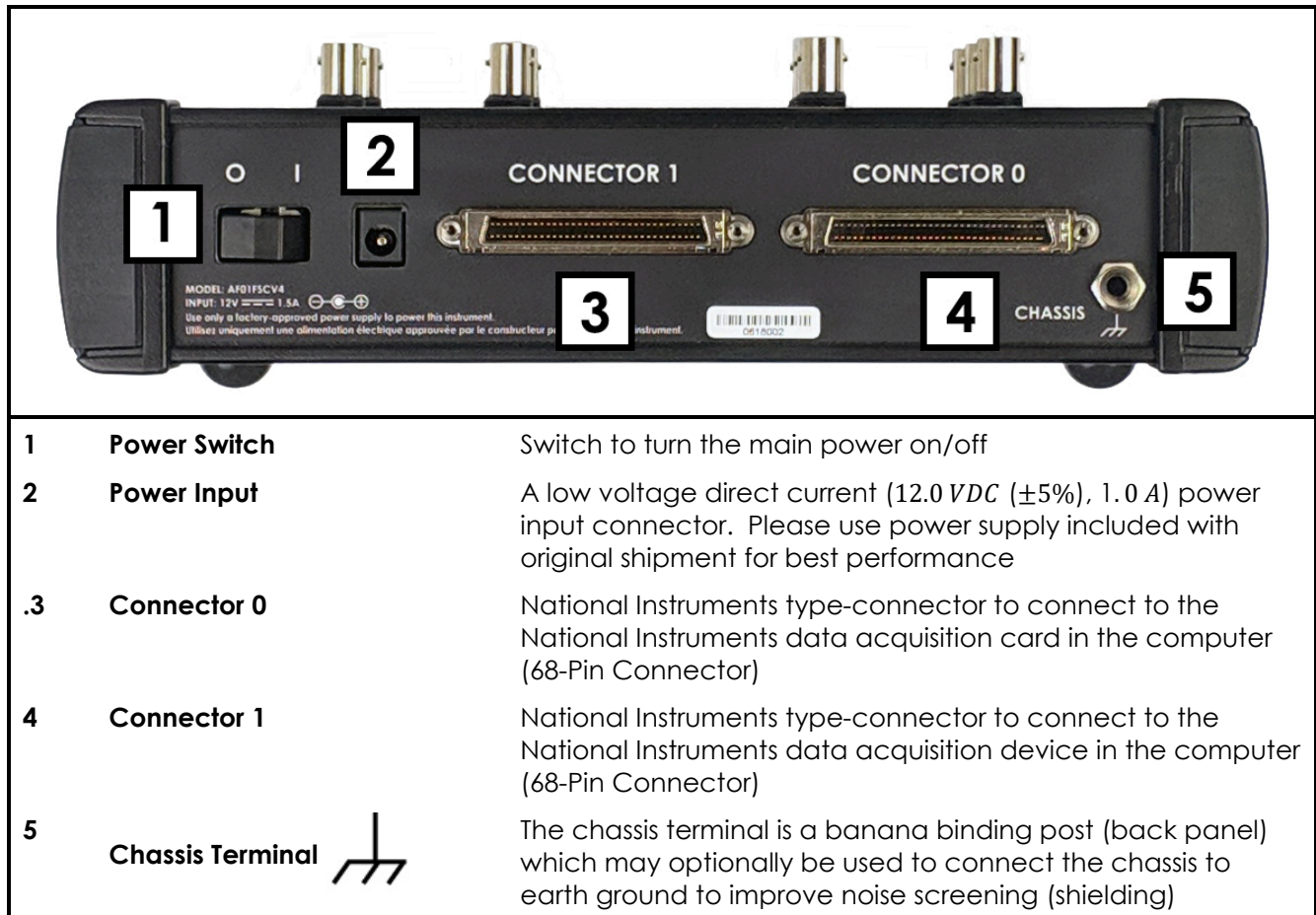


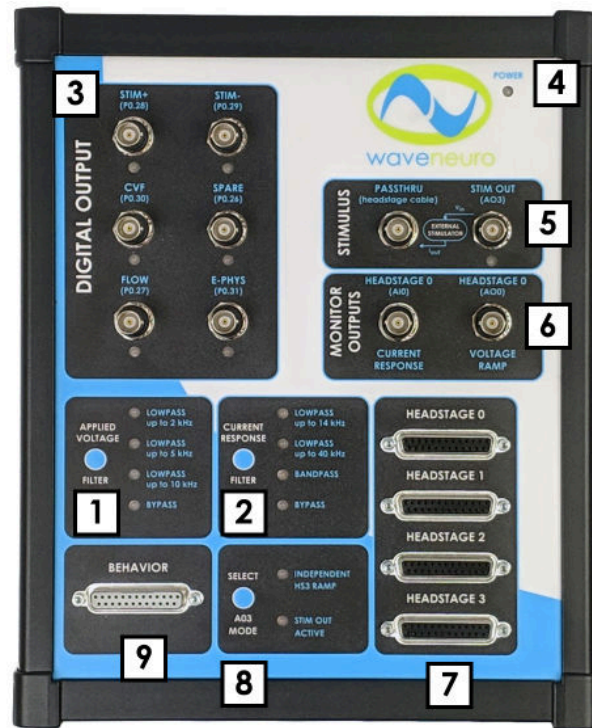
Figure 2-3: Description of the WaveNeuro 4 Side/End Panel

2.7 WaveNeuro 4 Top Panel

The top panel of the WaveNeuro 4 (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 4 Connectors

The top panel of the WaveNeuro 4 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	Monitor Outputs	Two female BNC connectors providing analog output monitoring of applied voltage (ramp) and measured current (signal) (see: Section 2.7.6)
7	Headstage Input Connectors	Female DB-25 Connectors for Headstage input signals (see: Section 2.7.7)
8	Analog Out 3 Mode Selector	Push button selection to choose whether to use line AO3 for an independent FSCV waveform on Headstage 3 (Independent HS3 Ramp) or instead to disable Headstage 3 and use Stimulus options (Stim Out Active). (see: Section 2.7.8).
9	Behavioral Input Connectors	Female DB-25 Connectors for behavioral input signals (see: Section 2.7.7)

Figure 2-4: Top Panel of the WaveNeuro 4.

2.7.2 WaveNeuro 4 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 2-3).

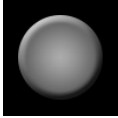
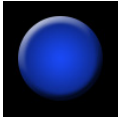
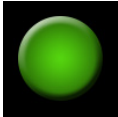
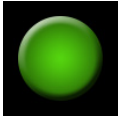
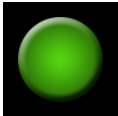
LED	LED Color/State	Indication
Power	 not illuminated	When the power LED is off, the WaveNeuro 4 power switch is in the <i>off</i> position (or the power supply is not providing power).
	 solid blue	When the power LED is solid blue, the power supply is providing power to the WaveNeuro 4 and the power switch is in the <i>on</i> 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 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 2-3: Overview of the WaveNeuro 4 LED Light Operation

2.7.3 WaveNeuro 4 Filter Settings

The WaveNeuro 4 has selectable filters on the waveform output and on the measured current. **The WaveNeuro 4 applies the same waveform filter to all four channels.** In other words, the *Applied Voltage Filter* applies the highlighted filter to all four waveform channels and the *Current Response Filter* applies the selected filter to all four current channels. The waveform filter can be set by pressing the button beside the filter options and LEDs (see: Label 1 in Figure 2-4). The current response filter can be set by pressing the button beside the *current response* 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 programmed to activate external hardware. Such programming occurs in the software connected to the WaveNeuro 4, 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 4 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 4, the stimulating current feeds directly to the integrated stimulator connectors on the headstage cable. The current is independent of the WaveNeuro 4 circuitry in this configuration. Note that to use STIM, STIM should be specified in both the hardware and the software settings (See: Section 5.1.3).

2.7.6 Monitor Outputs

On the top panel in the section labeled MONITOR OUTPUTS, there are two female BNC connectors, labeled as HEADSTAGE 0 CURRENT RESPONSE (AI0) and HEADSTAGE 0 VOLTAGE RAMP (AO0) (see: Figure 2-4). These connectors offer a method to externally monitor the voltage output (ramp, from the NI board) on Headstage 0 and/or to monitor the current input from Headstage 0. Both connections are passive monitoring connections only.

2.7.7 Headstage Connectors

Four DB-25 HEADSTAGE connectors (see: label 7, Figure 2-4) are intended for use with four Pine Research headstage cable kits. With four headstage cable kits up to four different working electrodes can be used. The headstage cable kits are included with the initial WaveNeuro 4 shipment (see: Figure 2-2). Headstage amplifiers connect 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.

2.7.8 Analog Out 3 Mode Selector

The WaveNeuro 4 offers up to four independent FSCV channels, a feature that comes with one limitation. In four-channel mode, all analog outputs on the NI PCIe-6363 interface board will be used, which means that the WaveNeuro 4 Stimulus output (STIM OUT AO3) is not available as it will be used for an FSCV waveform. In this setup, the "INDEPENDENT HS3 RAMP" LED must be illuminated.

Alternatively, The WaveNeuro 4 can be setup to perform three independent FSCV channels and a stimulus output, which means that the WaveNeuro 4 fourth channel (HEADSTAGE 3), which is also tied to AO3, is not available. In this setup, the "STIM OUT ACTIVE" LED must be illuminated. Change the mode as desired by pressing the "SELECT AO3 MODE" button (see: label 8, Figure 2-4).

2.7.9 Behavioral Connectors

The DB-25 BEHAVIOR connector (see: label 8, 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 4 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).

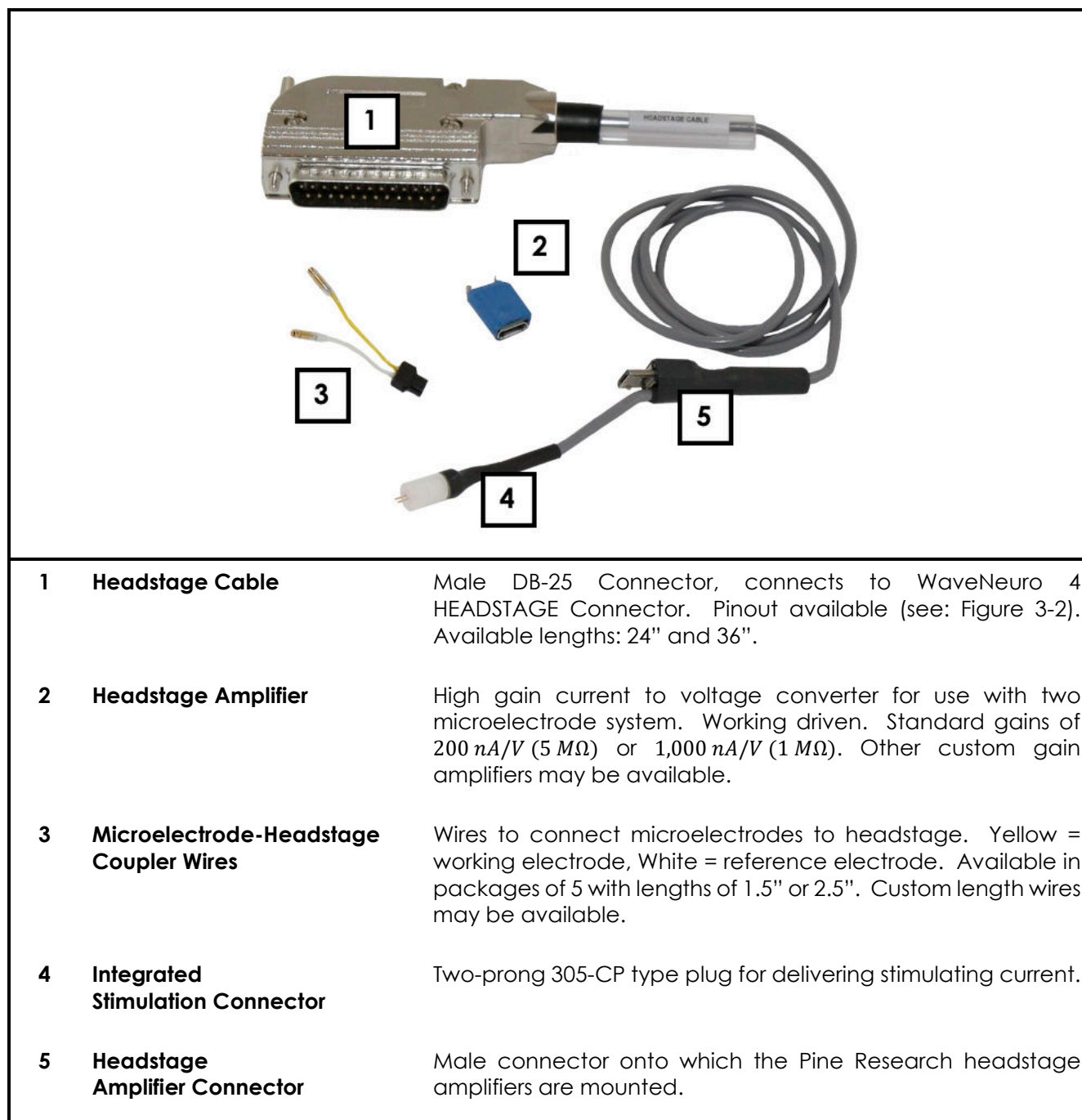


Figure 3-1: Components of Pine Research Headstage Amplifier Cable Kit

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 input¹. This configuration enables the application of independent waveforms to each working electrode.

In the working-driven 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 amplifier, 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),

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

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 $5\text{ M}\Omega$ feedback resistor in the 200 nA/V headstage) is then

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

HDCV software, which supports the WaveNeuro 4 FSCV Potentiostat system, performs software subtraction of the ramp according to this relationship, resulting in only the true differential current measurement¹.

REFERENCE

1. 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 4, 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.

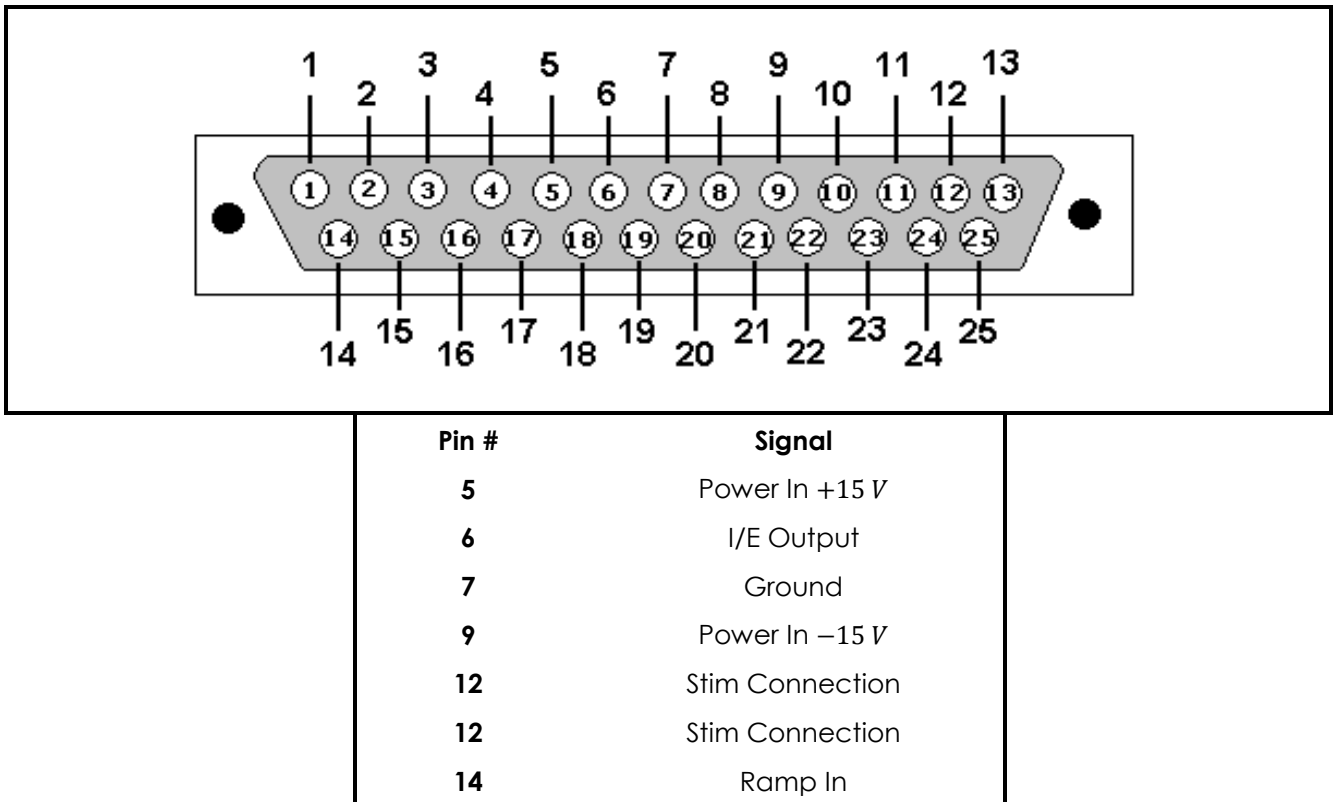


Figure 3-2: Headstage Cable DB-25 Connector Pinout Diagram

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 4 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 4 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.

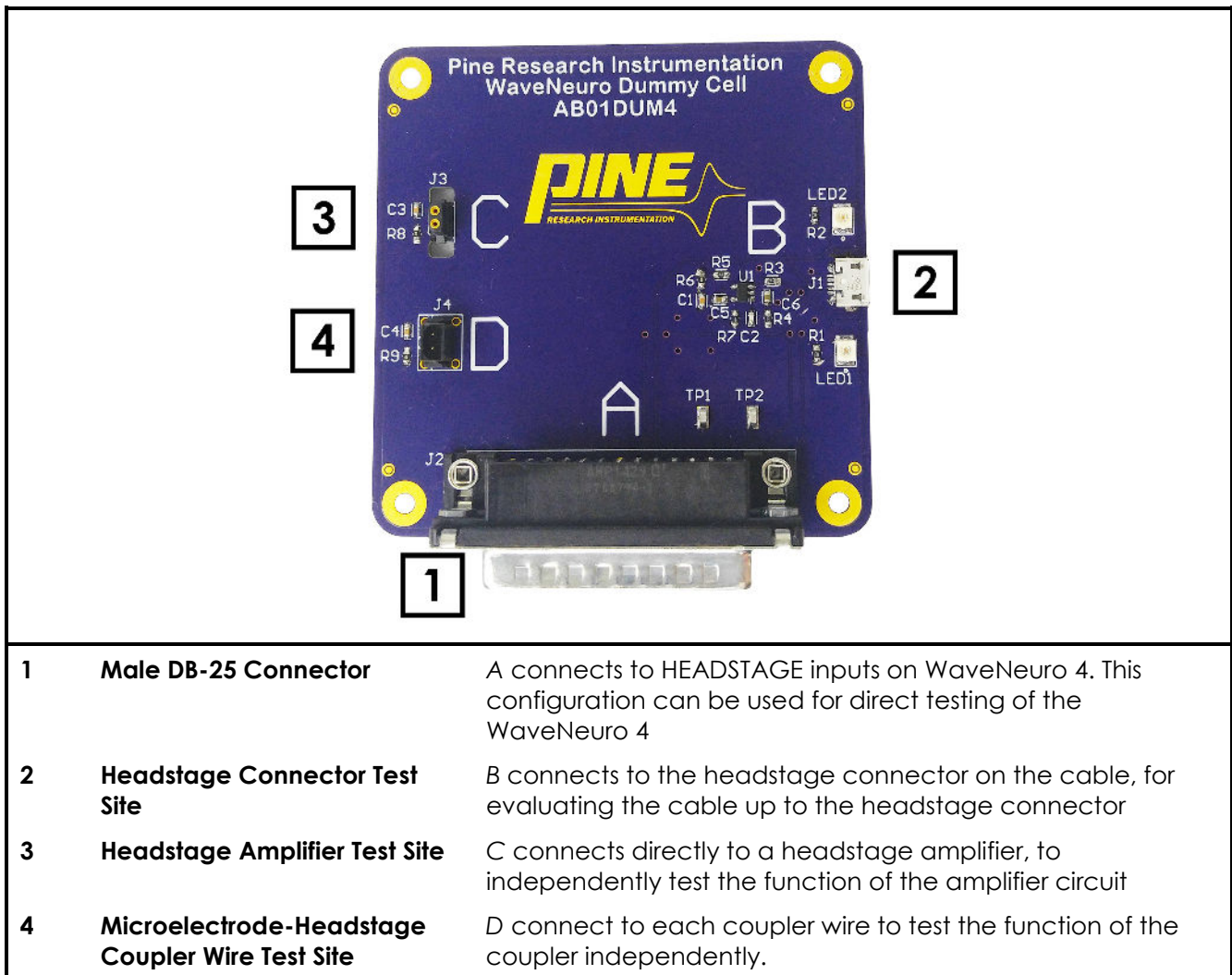


Figure 3-3: WaveNeuro Dummy Cell Connections

4. SYSTEM INSTALLATION

Setting up the WaveNeuro 4 system in a laboratory consists of three basic steps: 1) software and data acquisition system installation; 2) physical installation of the WaveNeuro 4; and 3) system testing. The installation process typically requires 60 minutes.

For illustrative purposes, this User Guide will describe the installation of WaveNeuro 4 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 4.



INFO:

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

4.1 Data Acquisition Interface 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 4-1).

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 4-1: 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).

4.1.1 Installation of National Instruments Software

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).

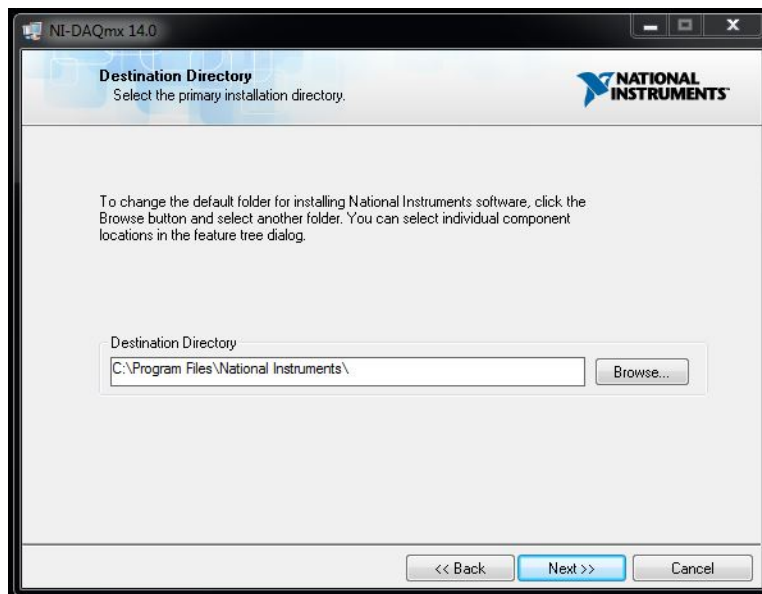


Figure 4-3: NI-DAQ™mx Installation – Step 3

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

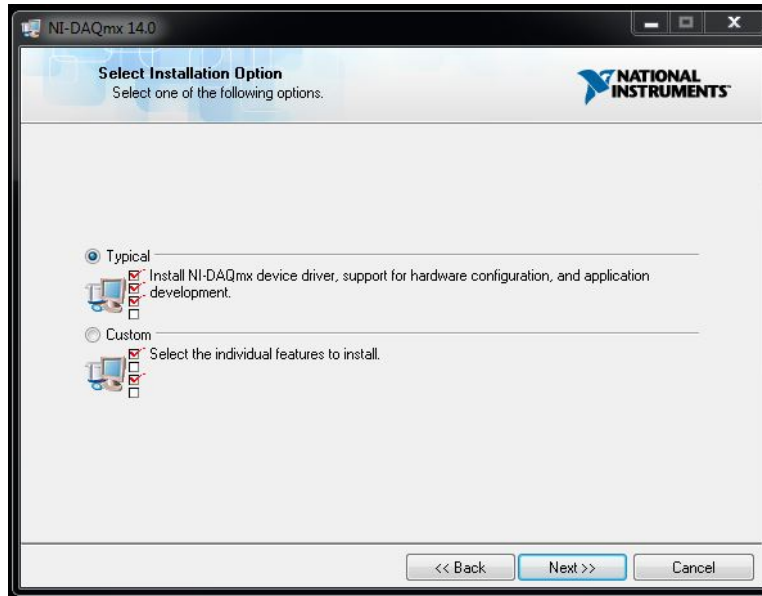


Figure 4-4: NI-DAQ™mx Installation – Step 4

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).

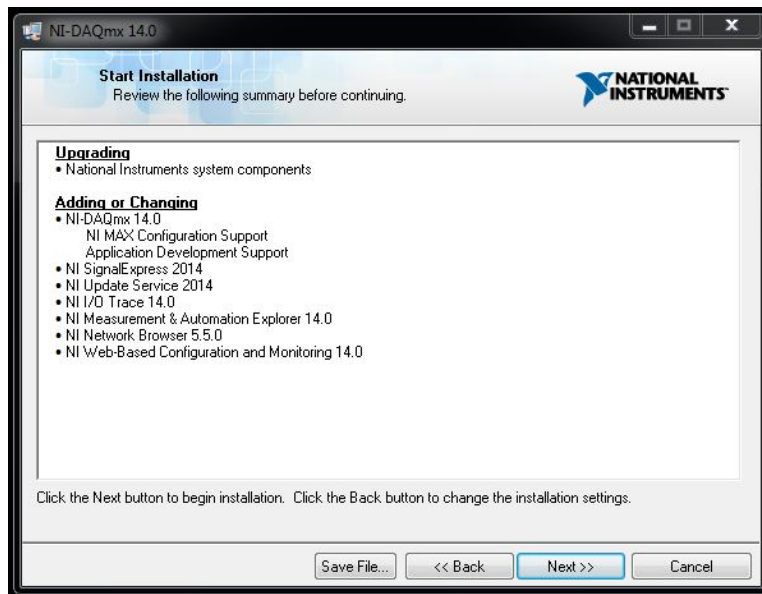


Figure 4-5: NI-DAQ™mx Installation – Step 5

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 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 4-7).
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.

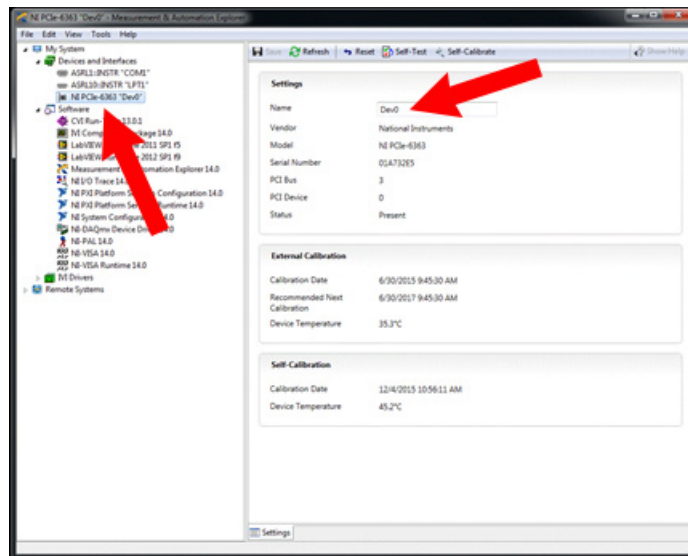



Figure 4-7: Device Name in NI MAX Software

4.2 Software (HDCV) Installation

The WaveNeuro 4 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:

1. 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 4-8).



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.

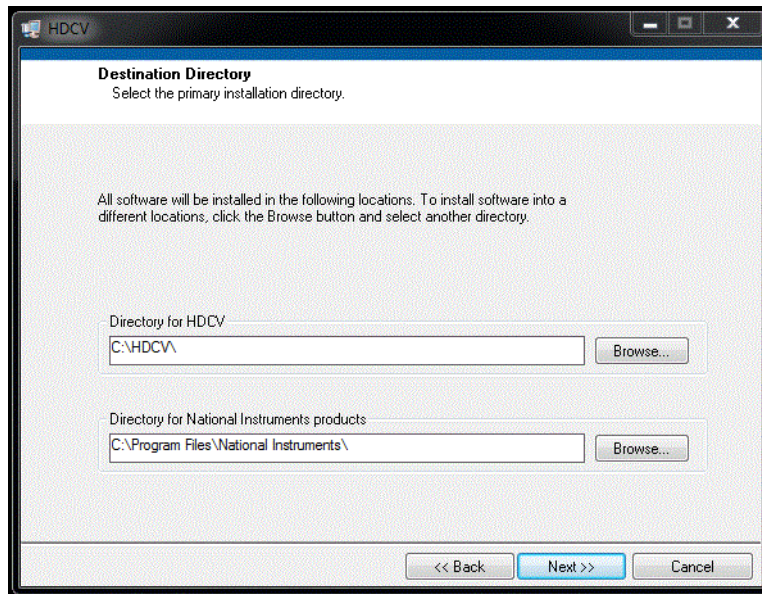


Figure 4-8: HDCV Installation – Step 1

2. As with NI-DAQ™mx, review and accept the license, the installation summary, watch the installation progress, and after complete, restart the computer.
3. 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 4-9). If HDCV does not start please refer to troubleshooting or contact Pine Research Instrumentation.

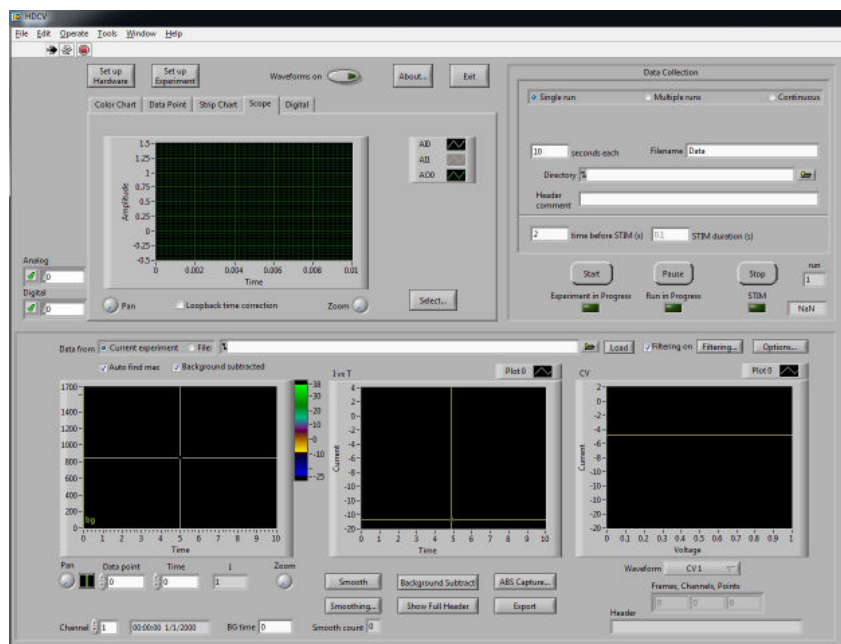


Figure 4-9: HDCV Application upon First Startup

4.3 Hardware (WaveNeuro 4) Installation

The WaveNeuro 4 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 4 in a stable, clean and dry location that has limited power cords in close proximity to the instrument.

4.3.1 Location

The WaveNeuro 4 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 one or more electrochemical headstages via a cable plugged into the top panel of the instrument.

4.3.2 Electrical Power

The Power Supply provides the DC power required by the WaveNeuro 4 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 4 at the location marked *POWER INPUT* (see: Figure 4-10).

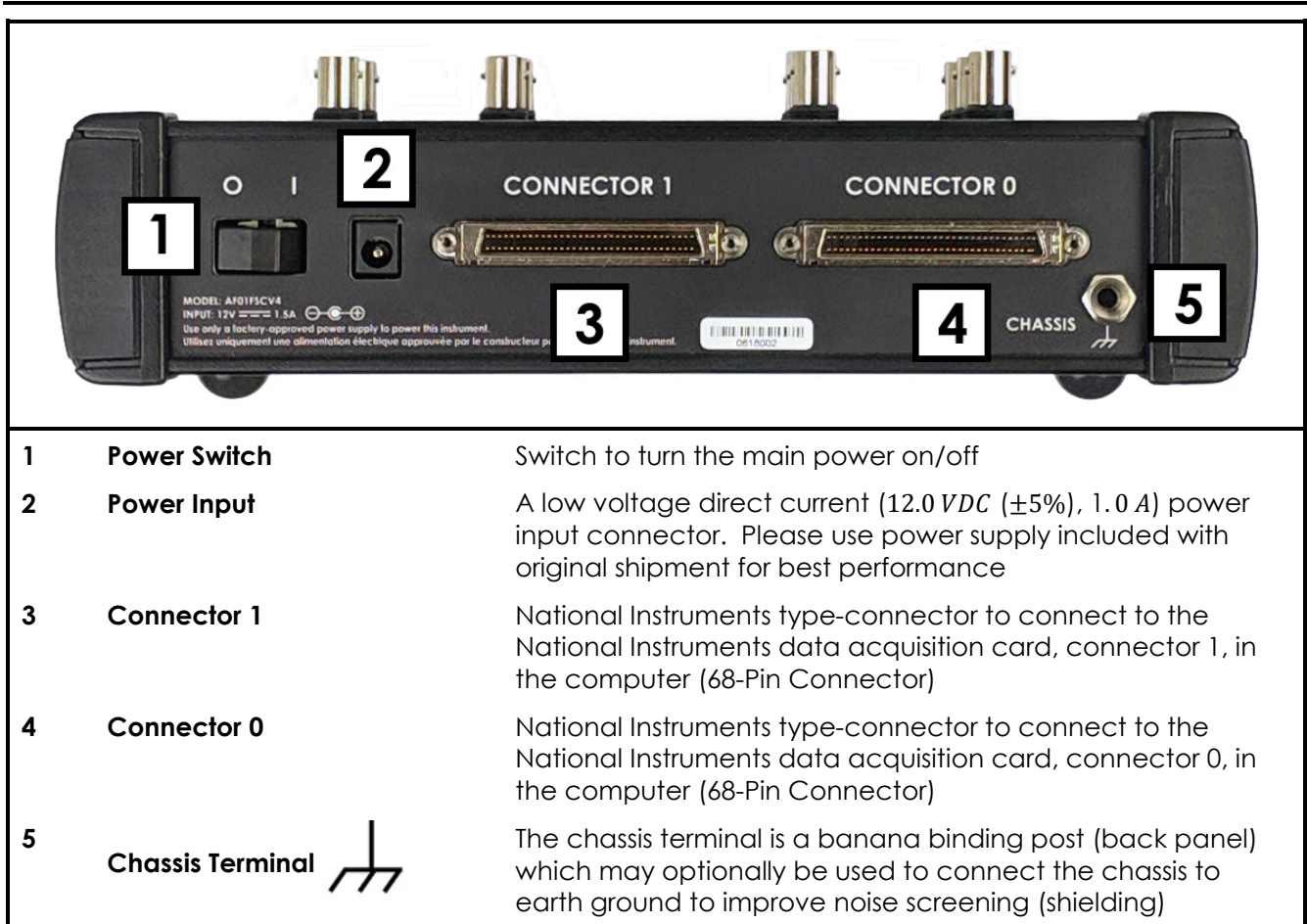


Figure 4-10: Installation Connections to the WaveNeuro 4



Caution:

Connect the Power Supply to the AC mains using the Power Cord supplied with the WaveNeuro 4 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 4 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 4 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.

4.3.3 National Instruments Interface Cables

Connect the WaveNeuro 4 to the computer using two National Instruments SHC68-68-EPM cables (NI Part No: 192061-02) (see: Figure 4-10). The cables connect the NI PCIe-6363 acquisition device in the computer to the WaveNeuro 4.

**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 4. Be sure to connect the cables to the correct NI board.

4.4 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 4 connected to the computer with two National Instruments cables connected connecting between CONNECTOR 0 and CONNECTOR 1
- ✓ Electrical power is connected to the WaveNeuro 4

5. SYSTEM SETUP AND QUICK START

After completing the installation of the interface board and related software (see: Section 4.1), acquisition software, such as HDCV (see: Section 4.2), and WaveNeuro 4 hardware installation (see: Section 4.3), users can now conduct FSCV experiments. For one's first time, Pine Research recommends use of the dummy cell (included with instrument, see: Section 3.2) to best understand the function of the software and hardware. The dummy cell test described in this section is also a very helpful diagnostic. When encountering any issues with the software and/or hardware, troubleshoot the issue(s) with this quick start guide.

**TIP:**

Anytime that it is necessary to verify that the WaveNeuro 4 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.

Before proceeding with the dummy cell performance/diagnostic test, ensure the following have been completed:

- ✓ National Instruments NI-DAQ™mx software installed on the computer
- ✓ National Instruments PCIe-6363 data acquisition interface board installed in the computer and is recognized by the NI-DAQ™mx software. Set the device name is set to "Dev0" in the software.
- ✓ WaveNeuro 4 connected to the computer via two National Instruments cables. Be sure that the cables properly connect CONNECTOR 0 and CONNECTOR 1 on the interface board to the same labeled ports on the end panel of the WaveNeuro 4.
- ✓ Electrical power is connected to the WaveNeuro 4 and the unit is turned on, as indicated by the blue POWER LED.
- ✓ Data acquisition software installed on the computer (e.g. HDCV). Start HDCV and click "Setup Hardware" and press OK. If there are any errors, they will appear in the dialog box within this setup panel. If not, and the window closes, proceed with the dummy cell performance/diagnostic test below.

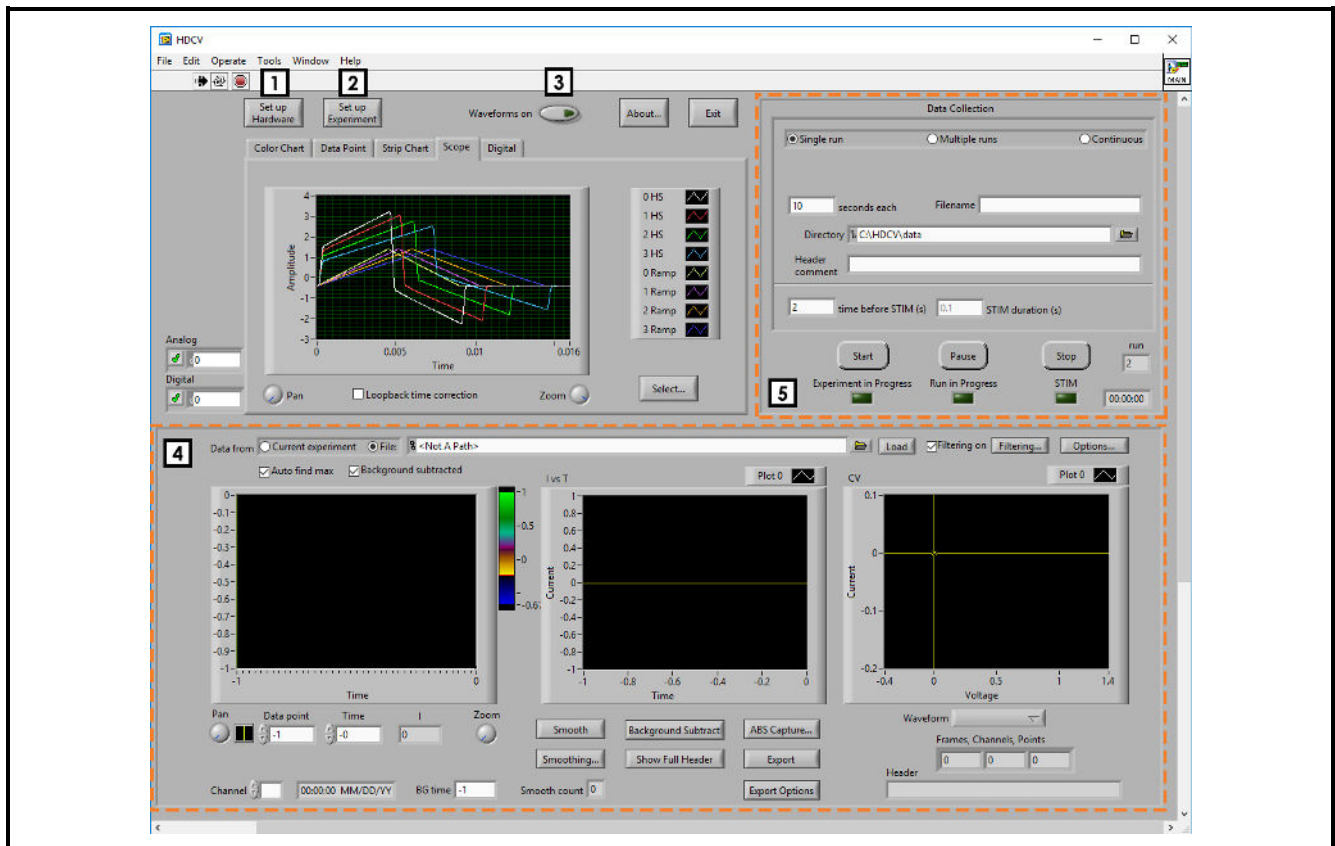
As described previously, the dummy cell is a network of known resistors and capacitors that mimic an electrochemical interface (see: Section 3.2). This network is a stable electrical system to use in evaluating instrument performance and function. Complications that arise from electrodes, electrode placement, 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 4, 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.

The dummy cell has also been designed to test and diagnose issues with the WaveNeuro 4, the headstage cables, the headstage amplifier, and/or the electrode wire connectors independently. Each of the following sections describe testing each of these parts of the FSCV experiment. The steps below apply for any number of channels (1 – 4). Special notes with regard to multichannel operation will be added as needed.

5.1 Software (HDCV) Setup

HDCV can be used with the WaveNeuro 4 for 1, 2, 3, and/or 4 channels simultaneously. HDCV consists of the hardware setup and experiment setup. Within hardware setup, map the appropriate number of channels to the ports as described. Within software setup, define the waveform parameters, headstage gain, and flow cell/triggers as desired.

When first starting HDCV, a variety of sections exist within the main window (see: Figure 5-1). Later in the guide setting up the hardware and experimenting will be discussed, which are accessed from this main window.



- | | |
|--|--|
| 1 Set Up Hardware Button | Opens a panel to setup hardware features, inputs, outputs, and setup BNC connectors on the WaveNeuro 4 |
| 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 5-1: HDCV Software Main Window Sections

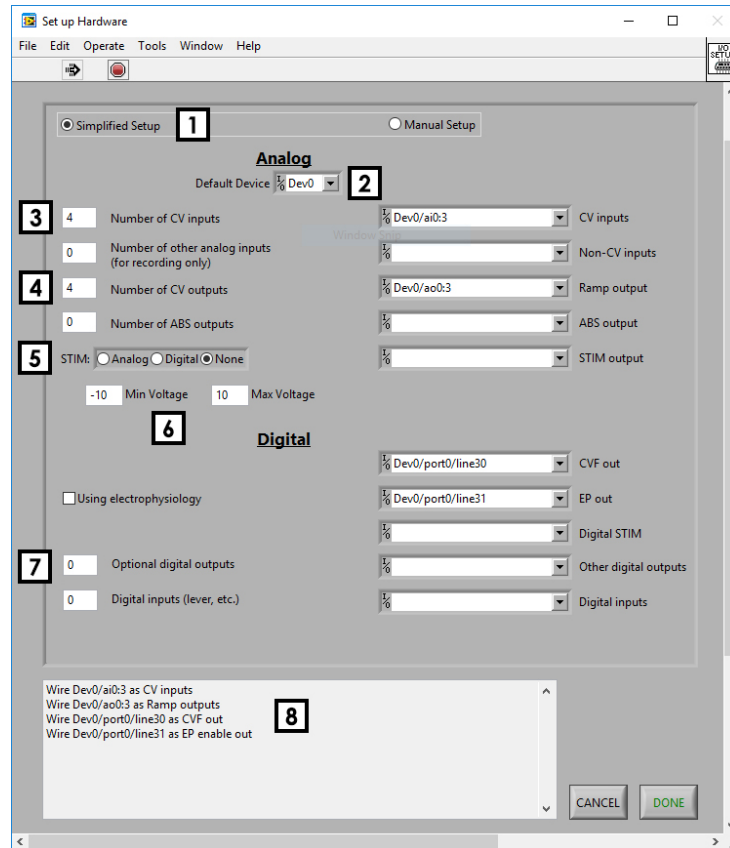
5.1.1 Hardware Setup in HDCV

After starting HDCV, select the “Set up Hardware” button in the upper left of the main screen. A separate window opens. This “Set up Hardware” window is used to define the number of FSCV channels, stimulus settings, and other trigger settings for the specific experiment. Testing the WaveNeuro 4 can be done on any number of channels (one dummy cell needed per FSCV channel).

Some settings are universal regardless of the number of FSCV channels (e.g., Min Voltage and Max Voltage, Default Device, etc.). The options for “Number of CV inputs,” “Number of CV outputs,” and “STIM:” are specific to the multichannel mode desired. Change these inputs in the Set up Hardware window to match the number of FSCV channels that are desired (see: Table 5-1). All other settings are universal and should be set as shown (see: Figure 5-2).

# FSCV Channels	# CV inputs	# of CV outputs	STIM setting
1	1	1	Analog
2	2	2	Analog
3	3	3	Analog
4	4	4	N/A

Table 5-1: HDCV Hardware Settings for Multichannel Modes



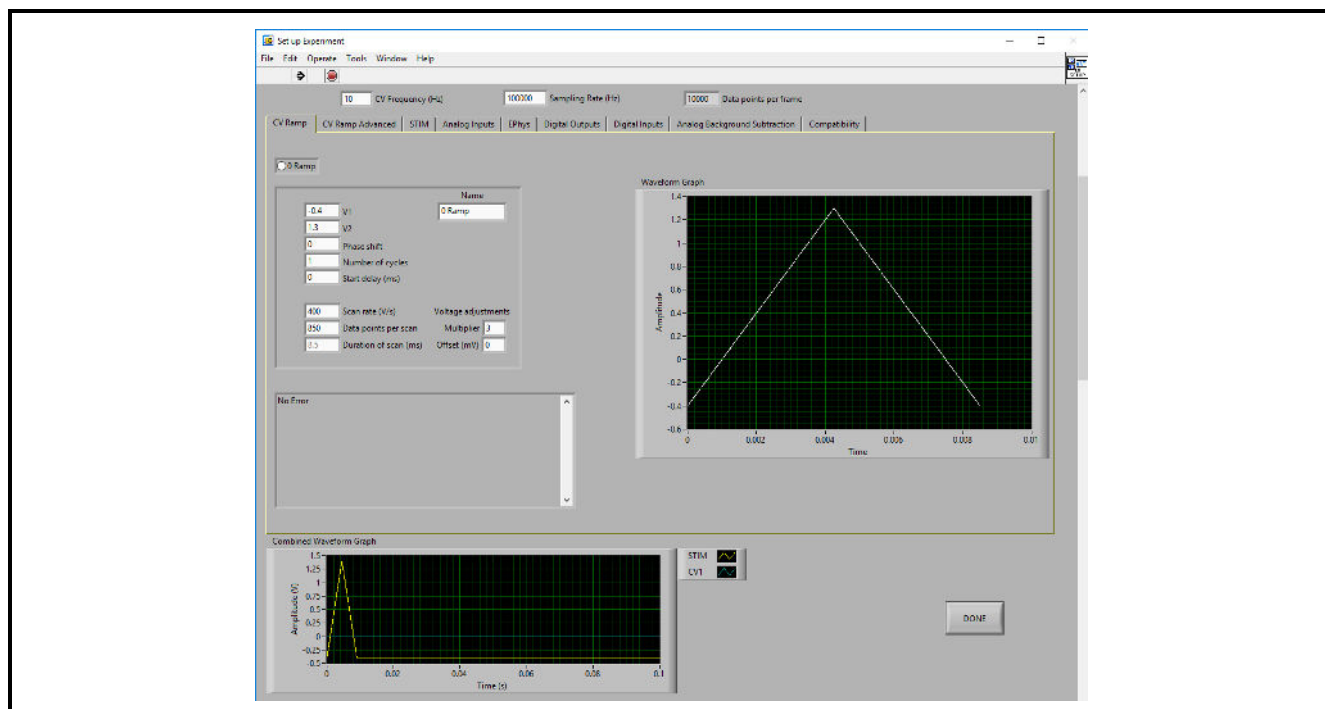
1	Setup Type Button	In general, select researchers use <i>Simplified Setup</i> although the <i>Manual Setup</i> option allows for enabling/disabling and mapping of the various input and output BNC connectors through the software.
2	NI Board Device Name	Dropdown selection reads from the NI-DAQ™mx setting to define the appropriate data acquisition device. Pine Research recommends setting the device to <i>Dev0</i> as described in Section 4.1.3.
3	Number of CV inputs	This setting chooses the number of FSCV current inputs (from the HEADSTAGE connectors). Select 1, 2, 3, or 4 as desired, but this number should match “Number of CV outputs” in this window.
4	Number of CV outputs	This setting chooses the number of FSCV waveform outputs (from the NI board to the HEADSTAGE connectors). Select 1, 2, 3, or 4 as desired, but this number should match “Number of CV inputs” in this window.
5	Stimulus Option	For 1, 2, or 3 channels, choose “Analog” if
6	Potential Range	Full range for the WaveNeuro 4 with PCIe-6363 interface board is -10 V (Min Voltage) and $+10\text{ V}$ (Max Voltage).
7	Digital Output Entry	Total number of software-defined digital outputs, which, for example, are used for flow cell trigger.
8	HDCV Hardware Setup Dialog	Summarizes hardware setup and reports errors, if any.

Figure 5-2: HDCV Set up Hardware Windows Description and Settings

5.1.2 Experiment Setup in HDCV

After setting up the hardware, the next step is to define the experiment. Defining the experiment includes establishing the waveform (ramp), scan rate, current amplifiers, any digital triggers (e.g., flow cell trigger, optional), and stimulus settings (optional). For evaluation/diagnostic purposes, we will provide typical settings used for making dopamine measurements.

First, setup the waveform (ramp) on the “CV Ramp” tab (see: Figure 5-3).



Setting	Description	Value
CV Frequency	Overall CV ramp repeat rate	10
Sampling Rate	Measured data acquisition rate (in Hz)	10000
V1	Initial Potential (in volts)	-0.4
V2	Final Potential (in volts)	1.3
Phase Shift	Shifts starting position of waveform/ramp by a fraction (in degrees)	0
Number of Cycles	Number of CV cycles (V1→V2→V1)	1
Start Delay	Delay before applying sweep/ramp (in ms)	0
Scan Rate	Potential Ramp change per time (in V/s)	400
Multiplier	The factor by which the waveform is divided when it passes through hardware	3
Offset	Potential offset (in mV)	0

Figure 5-3: HDCV Experiment Settings for Dopamine

The standard dopamine waveform is a sweep from -0.4 V to $+1.3\text{ V}$ and back to -0.4 V at a scan rate of 400 V/s . Using the parameters provided, the ramp will be 8.5 ms in duration and contain 850 data points at a collection rate of $100,000\text{ data point/second}$. All other typical settings to setup this dopamine waveform are provided in the screen capture. **Importantly, for the WaveNeuro 4 the Multiplier should be set as 3** (see: Figure 5-3).

In the case of **multichannel configuration** on the WaveNeuro 4, the experiment setup for dopamine CV Ramp is exactly the same; however, the parameters must be entered for n channels. For each pair of CV input/output used in the hardware setup (each channel), a separate CV ramp screen will be available accessed from the CV Ramp tab. Instead of only one form, a series of forms are accessible by choosing each form by radio button (see: Figure 5-4). In this case, providing a "Name" for each channel is helpful. In the example, each ramp has been named by its channel (e.g., 0 Ramp = CV ramp on Headstage 0). **In multichannel configuration on the WaveNeuro 4, it is critical to set the Multiplier = 3 for each channel.**

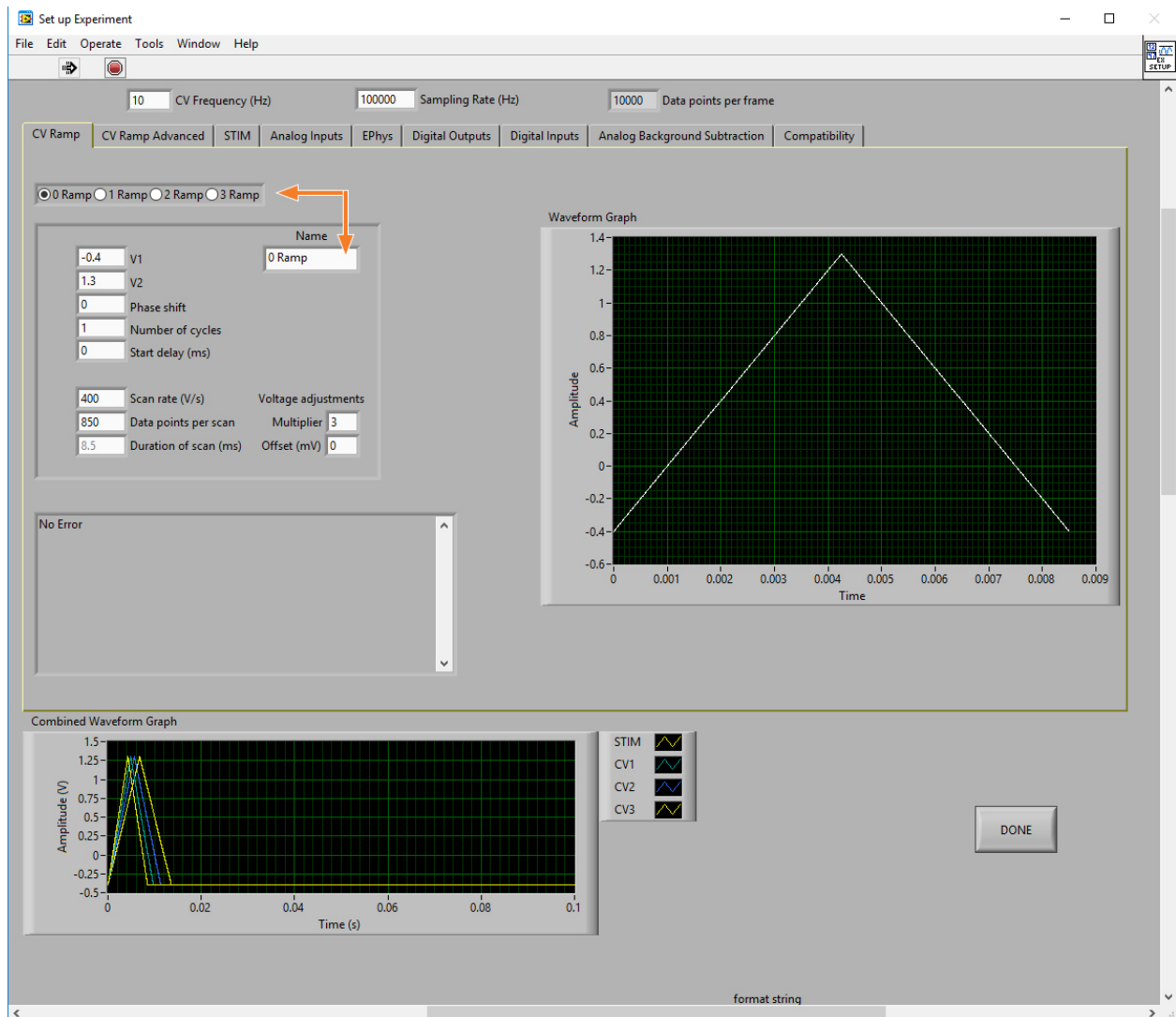


Figure 5-4: HDCV Experiment Settings for Dopamine (Multichannel Mode)

Next, define the amplifier gain on the “Analog Inputs” tab (see: Figure 5-5).

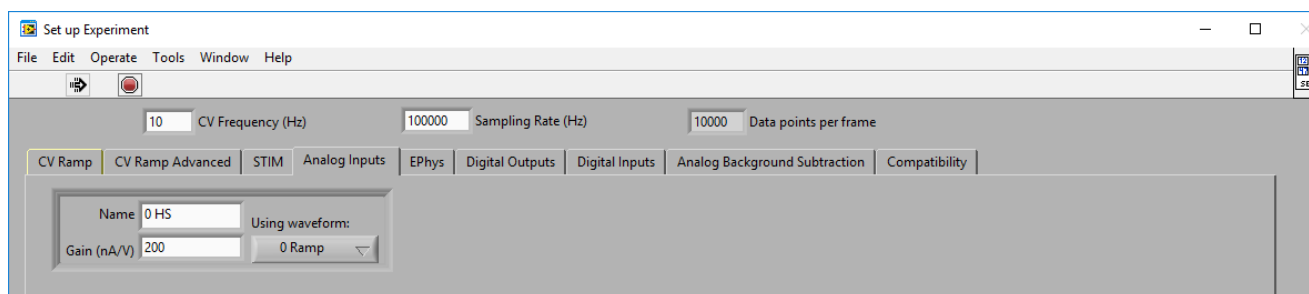


Figure 5-5: HDCV Analog Inputs Tab for Headstage Amplifier Gain

Pine Research may offer a variety of headstage amplifier gains. The most common and standard gain is 200 nA/V ($5 \text{ M}\Omega$), which is a headstage wrapped in blue shrink tubing. Enter the appropriate gain value and provide a name if you desire. In the example, “0 HS” was used to indicate HEADSTAGE 0 (channel 1).

In the case of **multichannel configuration** on the WaveNeuro 4, the experiment setup for dopamine Analog Inputs is exactly the same; however, the parameters must be entered for n channels. For each pair of CV input/output used in the hardware setup (each channel) there will be an entry for the amplification (gain) on the Analog Inputs tab (see: Figure 5-6). In this case, providing a “Name” for each channel is helpful. In the example, each headstage amplifier has been named by its channel (e.g., 0 HS = current gain on Headstage 0).

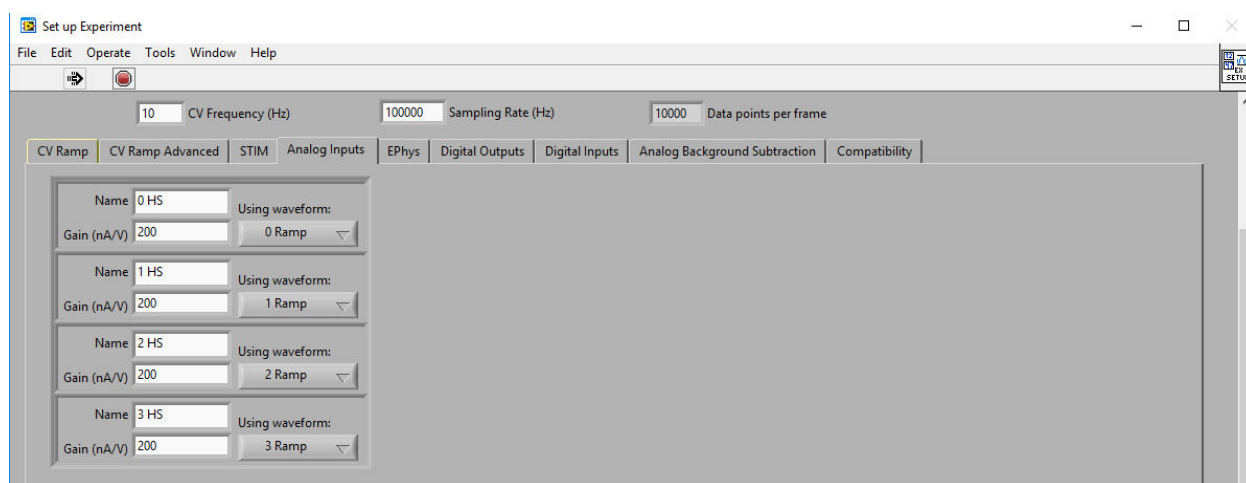


Figure 5-6: HDCV Analog Inputs Tab for Headstage Amplifier Gain (Multichannel Mode)

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

5.1.3 Optional Experiment Setup in HDCV

For the purpose of performing dummy cell tests, no additional experimental settings must be changed. For real world experiments, additional settings might want to be changed, such as digital triggering of a peripheral device (like a flow cell) or controlling stimulation. Both will be described here for

completeness, but for the remainder of the quick start, it is not necessary to adjust the following settings in the Experiment set up window in HDCV.

There are time when it is helpful to automate the start/stop of peripheral devices during an FSCV experiment. For example, for in vitro flow cell experiments, one may desire to control the injection of sample into the cell at a specific time. In this case, the WaveNeuro 4 provides additional digital output "triggers" that can be setup through HDCV.

Setup any trigger/digital outputs (e.g., flow cell) on the "Digital Outputs" tab. For this option to be available, "Other Digital Outputs" in the hardware setup would have had to be at least 1 (see: Figure 5-2). 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 4 front panel (see: Figure 2-4).

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 5-7). 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*. As parameters are entered, the panel to the lower right will display the pulse as entered.

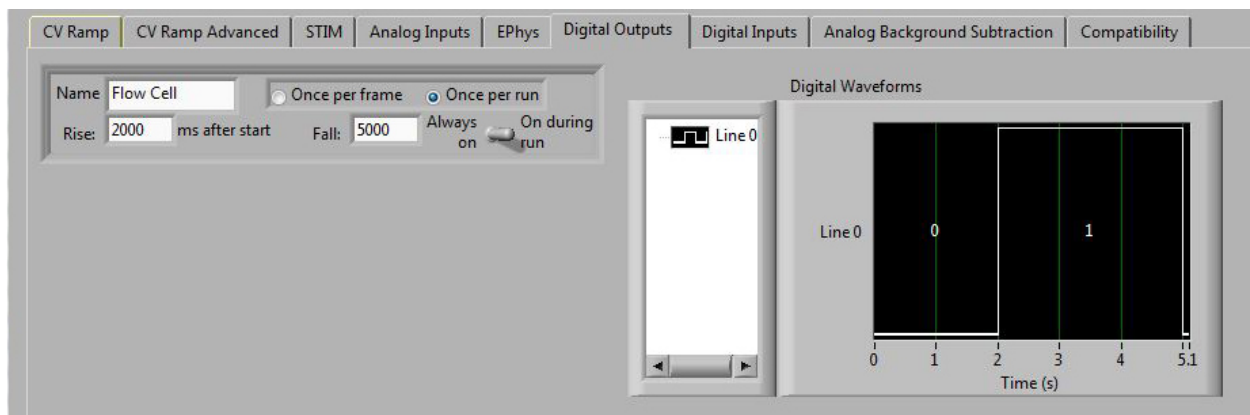


Figure 5-7: Digital Outputs Tab on the Experiment Setup in HDCV

Another common optional experimental aspect is to use HDCV to generate a stimulus pulse during the FSCV experiment. As described, the Pine Research headstage cables include a bi-polar stimulating electrode connector at the end of the cable. For those who wish to apply a stimulus during FSCV, HDCV can output an analog voltage signal, which an external stimulator box converts to a current, which then passes through the WaveNeuro 4 circuitry and maps to the stimulator connector (see: Section 2.7.5).

With the WaveNeuro 4, stimulus control through HDCV is possible for 1, 2, and 3 channel FSCV. One cannot perform four channel FSCV AND have HDCV control of stimulus output, since HEADSTAGE3 and the STIM OUT share the same aO3 line on the PCIe-6363.

Setup any stimulus settings on the "STIM" tab. For this option to be available, the "Analog" radio button on STIM output must be selected the hardware (see: Figure 5-2). When the Analog option is selected for STIM, *Dev0/ao3* will automatically be assigned as the STIM output; therefore, the stimulus output will be available at the *STIM OUT(AO3)* BNC on the WaveNeuro 4 front panel (see: Figure 2-4).

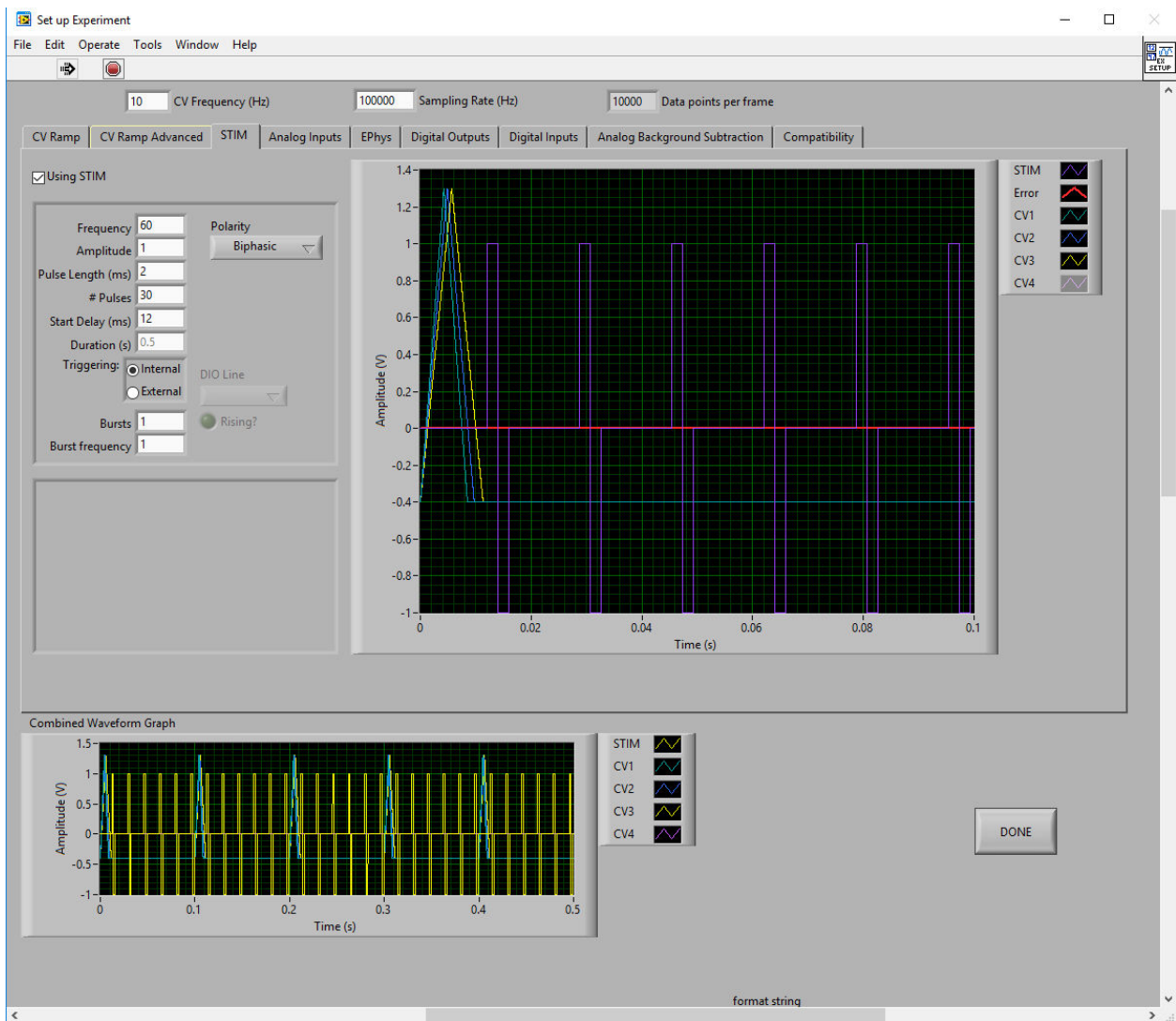


Figure 5-8: STIM Tab Settings in HDCV

Tick the “Using STIM” box on the STIM tab. Enter the desired stimulus parameters (see: Figure 5-8). HDCV will superimpose the stim waveform on top of the existing FSCV waveform/ramp. When entering parameters, watch the “Error” trace to ensure there is no collision between the FSCV waveform and the stim waveform. If the error trace encroaches the FSCV waveform, change the STIM settings to resolve the issue.

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

5.2 Hardware (WaveNeuro 4) Setup

The WaveNeuro 4 enables up to four working electrode channels to be simultaneously used with a single FSCV Potentiostat System and a single NI PCIe-6363 interface board. The WaveNeuro 4 operates as a 1, 2, 3, and/or 4 channel FSCV potentiostat.

To maximize the flexibility of the WaveNeuro 4, several operation modes are available as follows:

1. Four independent working electrodes and one common reference electrode
2. Four independent working electrodes and four independent reference electrodes (*i.e.*, four independent electrochemical cells) System Verification

5.3 WaveNeuro 4 Dummy Cell Test (Quick Start)

This test will evaluate the function and performance of the WaveNeuro 4. Typically, two dummy cells are shipped with the WaveNeuro 4; however if users have purchased additional dummy cells, it is possible to test up to four channels simultaneously.

Connect the DB-25 connector of the WaveNeuro Dummy Cell A (see: label 1 in Figure 3-3) to the HEADSTAGE 0 connector on the front panel of the WaveNeuro 4 (see: Figure 2-4 and Figure 5-9).



Figure 5-9: Connection of One Dummy Cell to WaveNeuro 4 HEADSTAGE Connector

If conducting the Dummy Cell Test in multichannel mode, insert a dummy cell into each HEADSTAGE connector on the WaveNeuro 4, as desired and as it matches the HDCV hardware and experiment set up in the previous section (see: Figure 5-10).

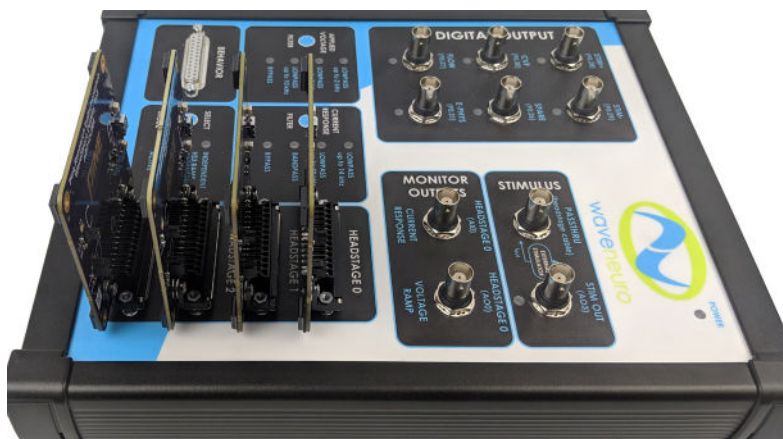


Figure 5-10: Connection of Four Dummy Cells to the WaveNeuro 4 HEADSTAGE Connectors

1. Apply the electrochemical waveform(s) to the dummy cell(s) by pressing the Waveform on button in HDCV (see: Figure 5-1). The live view window has five tabs, with Scope being the default view. Select Scope (see: Figure 5-11).
2. With the dummy cell connected to the WaveNeuro 4 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 5-11). For **multichannel mode**, each waveform and current response will appear on the Scope simultaneously (see: Figure 5-12). If the observed current response does not follow waveform as expected, double check all cable connections between the WaveNeuro 4 and the computer and that the WaveNeuro 4 is powered on.
3. Select Color Chart 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 5-11). Note that the scale on the color plot in Figure 5-11 is $+2 \text{ nA}$ 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 $\sim 5 \text{ min.}$ of applying the waveform, if the resulting color plot is not stable within $\pm 5 \text{ nA}$ over the displayed time, please take a screen shot and contact Pine Research for technical support.

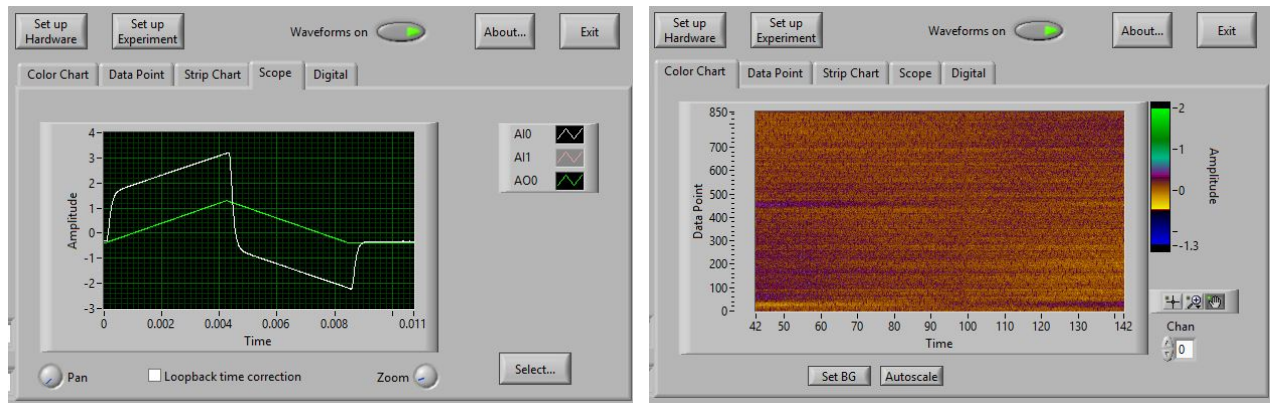


Figure 5-11: Scope (left) and Color Chart (right) Live Display Panels in HDCV

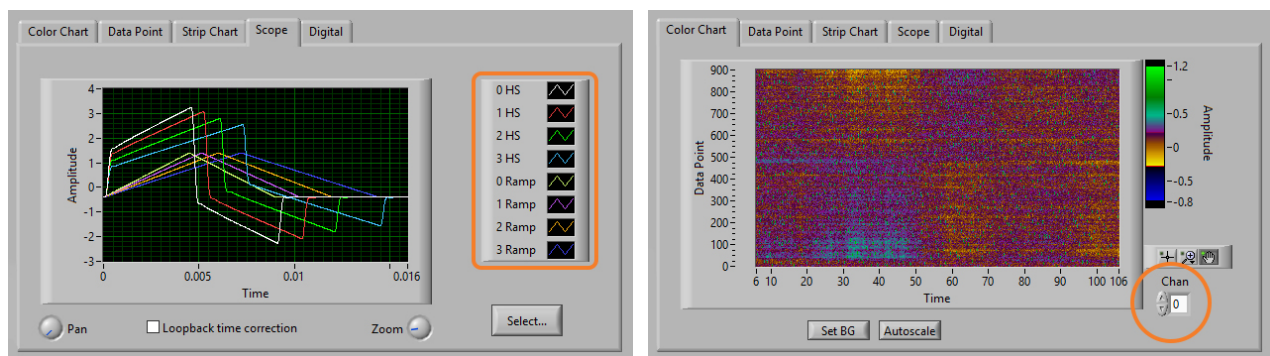


Figure 5-12: Scope (left) and Color Chart (right) Live Display Panels in HDCV (multichannel mode)

For **multichannel mode**, note that while the HDCV software is able to collect multiple channels, the software only displays a single channel at a time in most regions of the software. In both HDCV and HDCV Analysis, the data channel that is being displayed can be defined using the “Chan” or “Channel” select function (see: Color Chart orange circle in Figure 5-12). Scrolling through the channels will enable each channel to be displayed independently in HDCV.

5.4 Headstage Component Dummy Cell Tests (Quick Start)

One significant benefit of using Pine Research headstage cable kits for FSCV is that each major component of the cable are independent and replaceable, if necessary. As an expansion of the Quick Start for the WaveNeuro 4, where the actual WaveNeuro 4 was tested with the Dummy Cell, the Dummy Cell has other connections that are useful in evaluating the performance of the headstage cable components. Use the following Dummy Cell Tests as a Quick Start to learning how to use the Headstage Cable kits. You should also use the following tests as a diagnostic when you suspect there is an issue with the headstage cable kit.



TIP:

Whenever it is necessary to verify that the WaveNeuro 4 and related hardware like headstage cables are operating correctly, perform the system tests described in this guide. These tests are the first actions that will be suggested by Pine Research technical support staff.

5.4.1 Headstage Cable Test

1. Ensure the waveform is off by clicking the *Waveform on* button to its non-illuminated state (see: Figure 5-1). Connect the headstage cable DB-25 connector into the WaveNeuro 4 HEADSTAGE 0 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 4 and dummy cell and that the power supplied is at the correct level (see: Figure 5-13).



Figure 5-13. Connection of Headstage Cable to Dummy Cell B Connector

2. Turn the waveform on by clicking the *Waveform on* button (see: Figure 5-1). Repeat steps 2-3 from Section 5.3 to observe the same behavior as before when the dummy cell was connected directly to the WaveNeuro 4 (see: Figure 5-11).

For **multichannel mode**, follow the same steps outlined here, but add the appropriate number of headstage cables and dummy cells (see: Figure 6-1). The responses should match those shown for multichannel WaveNeuro 4 Dummy Cell tests (see: Figure 5-12).

5.4.2 Headstage Amplifier Test

1. Ensure the waveform is off by clicking the *Waveform on* button to its non-illuminated state (see: Figure 5-1). Connect the headstage cable DB-25 connector into the WaveNeuro 4 HEADSTAGE 0 connector on the front panel. Place the other end of the cable into a Faraday cage.
2. Install the headstage amplifier onto the headstage cable connector (see: Figure 3-1). Plug the headstage (connected to the cable) into Dummy Cell C (see: Figure 5-14). You will not observe power LEDs on the Dummy Cell in this configuration.
3. Turn the waveform on by clicking the *Waveform on* button (see: Figure 5-1). Repeat steps 2-3 from Section 5.3 to observe the same behavior as before when the dummy cell was connected directly to the WaveNeuro 4 (see: Figure 5-11).



Figure 5-14. Connection of Headstage Cable to Dummy Cell C Connector

For **multichannel mode**, follow the same steps outlined here, but add the appropriate number of headstage cables, headstage amplifiers, and dummy cells (see: Figure 6-1). The responses should match those shown for multichannel WaveNeuro 4 Dummy Cell tests (see: Figure 5-12).

5.4.3 Microelectrode-Headstage Coupler Wire Test

1. Ensure the waveform is off by clicking the *Waveform on* button to its non-illuminated state (see: Figure 5-1). Connect the headstage cable DB-25 connector into the WaveNeuro 4 HEADSTAGE 0 connector on the front panel. Place the other end of the cable into a Faraday cage.
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. The exact pin-to-wire position is irrelevant.

3. Turn the waveform on by clicking the *Waveform on* button (see: Figure 5-1). Repeat steps 2-3 from Section 5.3 to observe the same behavior as before when the dummy cell was connected directly to the WaveNeuro 4 (see: Figure 5-11).



Figure 5-15: Connection of Headstage Cable to Dummy Cell D Connector

For **multichannel mode**, follow the same steps outlined here, but add the appropriate number of headstage cables, headstage amplifiers, microelectrode-headstage couplers, and dummy cells (see: Figure 6-1). The responses should match those shown for multichannel WaveNeuro 4 Dummy Cell tests (see: Figure 5-12).

5.4.4 Headstage Extension Cable Test

It is very common that the WaveNeuro 4 will be located close to the PC, but far from the Faraday cage where experiments are conducted. While headstage cables come in 24" and 36" lengths, they are still often too short to reach between the test system in a Faraday cage and the WaveNeuro 4. In this case, Pine Research provides headstage extension cables (sold separately). As with our headstage kits, it is important to have an evaluation method available if you suspect a problem with the extension cable. These instructions in this section assume a DB-25 extension cable is being used.

1. Ensure the waveform is off by clicking the *Waveform on* button to its non-illuminated state (see: Figure 5-1). Connect the headstage cable DB-25 connector into the WaveNeuro 4 HEADSTAGE 0 connector on the front panel. Place the other end of the cable into a Faraday cage.
2. Connect the DB-25 extension cable into the HEADSTAGE 0 connector on the WaveNeuro 4 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: Figure 5-16).
3. Turn the waveform on by clicking the *Waveform on* button (see: Figure 5-1). Repeat steps 2-3 from Section 5.3 to observe the same behavior as before when the dummy cell was connected directly to the WaveNeuro 4 (see: Figure 5-11).

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.
3. Verify that the cable is not swinging or mechanically moving.

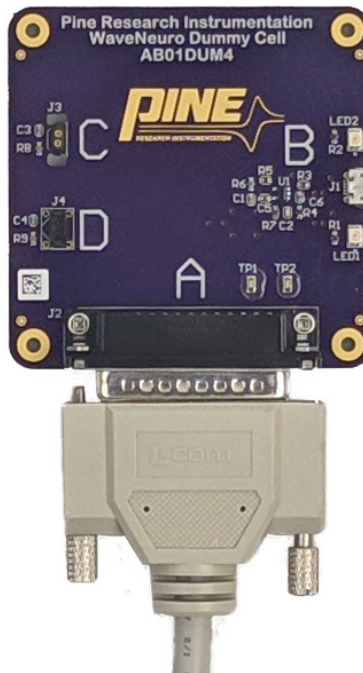


Figure 5-16: Connection of DB-25 Extension Cable to Dummy Cell A Connector

For **multichannel mode**, follow the same steps outlined here, but add the appropriate number of DB-25 extension cables and dummy cells (see: Figure 6-1). The responses should match those shown for multichannel WaveNeuro 4 Dummy Cell tests (see: Figure 5-12).

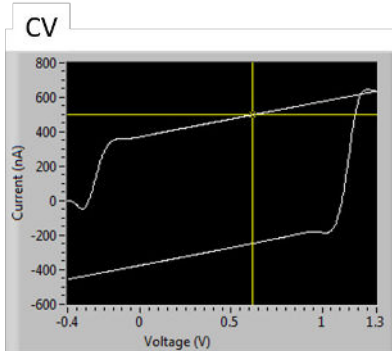
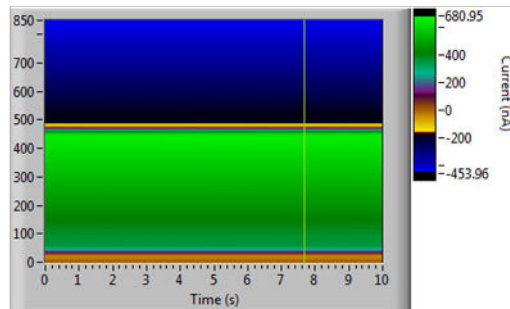


TIP:

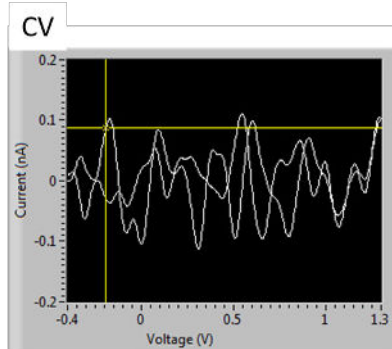
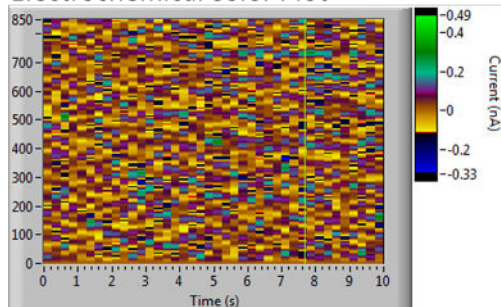
The dummy cell circuit is an uncomplicated system for hardware evaluation. It does not produce a typical voltammogram obtained when analyzing electroactive species. Typical dummy cell responses are provided (see: Figure 5-17).

With Background:

Electrochemical Color Plot

**Background Subtracted:**

Electrochemical Color Plot

**Figure 5-17: 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 4 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 and/or analysis software, please contact The University of North Carolina at Chapel Hill Chemical Research Instrumentation Teaching and Core Labs (CRITCL) Electronics facility, whose contact information is found at:

<https://chem.unc.edu/critcl-electronics/>

6. WAVENEURO 4 SETUP FOR 1, 2, 3, and 4 CHANNELS

The WaveNeuro 4 can be used to perform 1, 2, 3, and/or 4 channel FSCV. Each channel has its own headstage connector on the front panel of the WaveNeuro 4 (see: Figure 2-4). Pine Research recommends that users start with HEADSTAGE 0 (for single channel) and add additional channels (headstage cables) in ascending HEADSTAGE connector order (see: Figure 6-1).

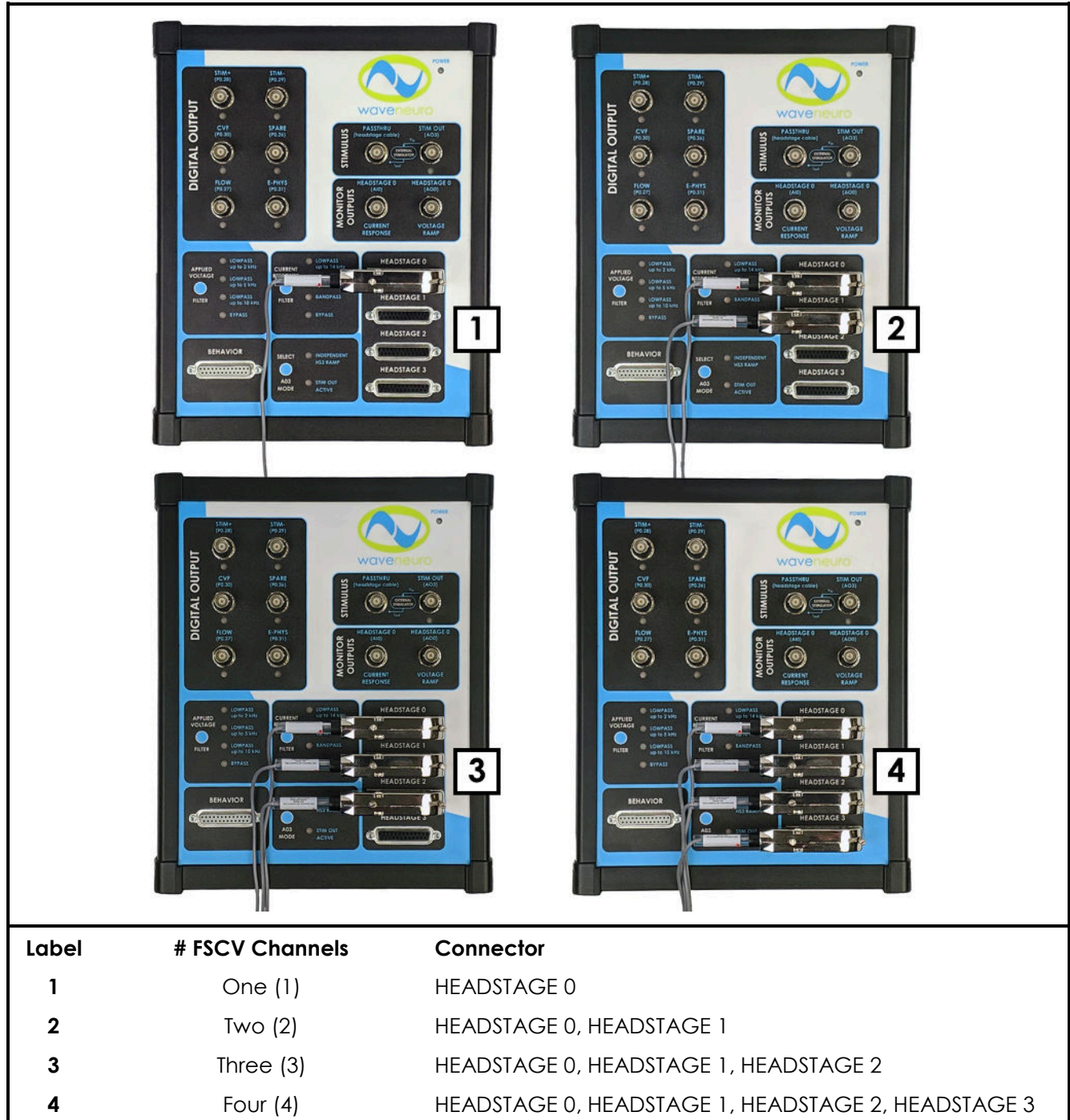


Figure 6-1: Multichannel Headstage Cable Installation for WaveNeuro 4

6.1 One Channel Operation

6.1.1 One Channel WaveNeuro 4 Hardware Setup

Connect one headstage cable to HEADSTAGE 0 connector on the front panel of the WaveNeuro 4 (see: Figure 6-1). STIM OUT may be used by pressing the “Select AO3 Mode” button until “STIM OUT ACTIVE” LED illuminates. Review Sections 2.7.5 and 5.1.3 for details on using stimulus.

6.1.2 One Channel HDCV Software Setup

Refer to the one channel hardware setup screenshot (see: Figure 6-2). In HDCV, click “Set up Hardware” button. Select the Default Device (typically, Dev0, if this guide has been followed). Enter “1” for the Number of CV inputs and the Number of CV outputs. If you wish to use stimulus, select “Analog” under STIM setting. If you wish to use any digital triggers, enter the desired number in the “Optional digital outputs” field. Press Done.

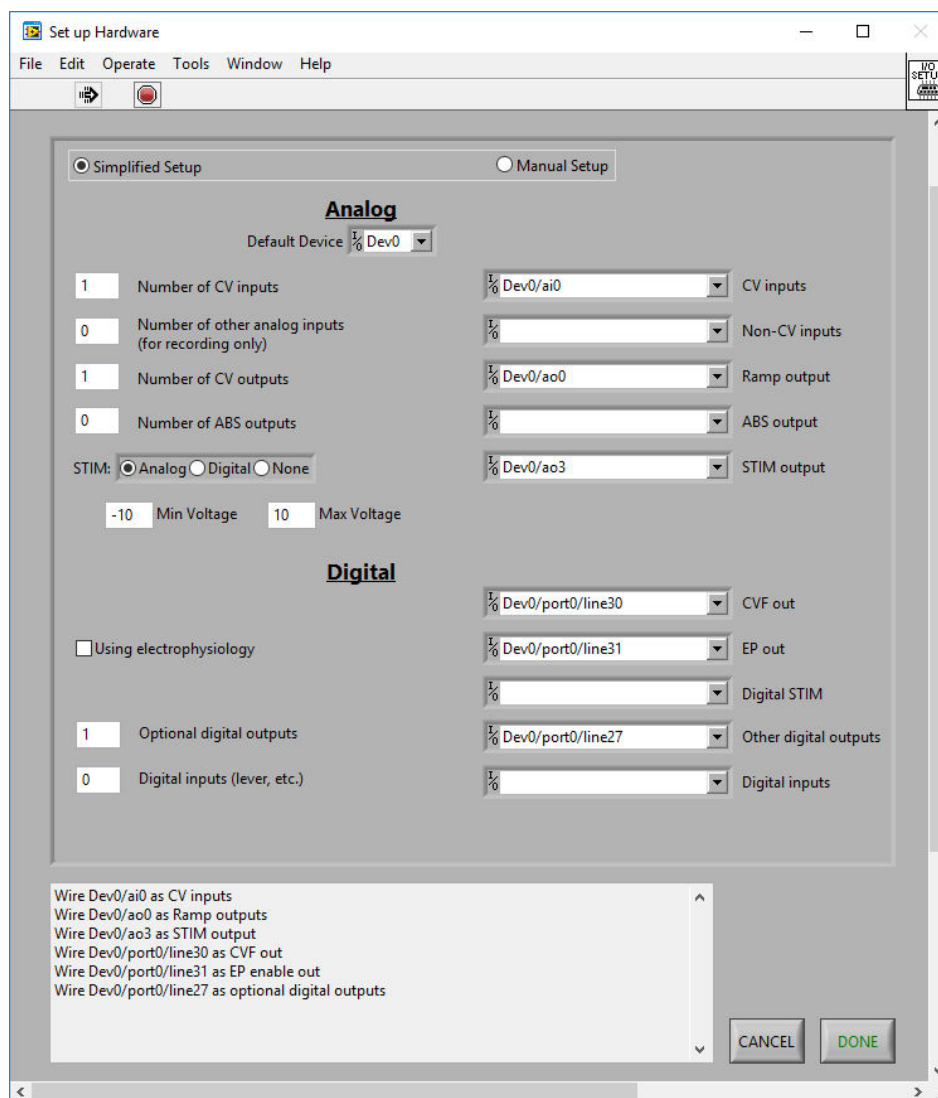


Figure 6-2: One Channel CV Ramp Setup in HDCV

Once the channel mapping (hardware setup) has been completed, click “Set up Experiment” button to enter your ramp parameters, headstage gain, and other optional controls such as flow cell digital trigger and/or stimulus settings.

Design your waveform on the “CV Ramp” tab (see: Figure 6-3). Shown is the typical dopamine waveform. Pine Research recommends to name the ramp with the channel number as the first character (e.g., “0 Ramp” as in Figure 6-3), which is more important when the WaveNeuro 4 is used in multichannel mode. Be certain the multiplier is set to 3.

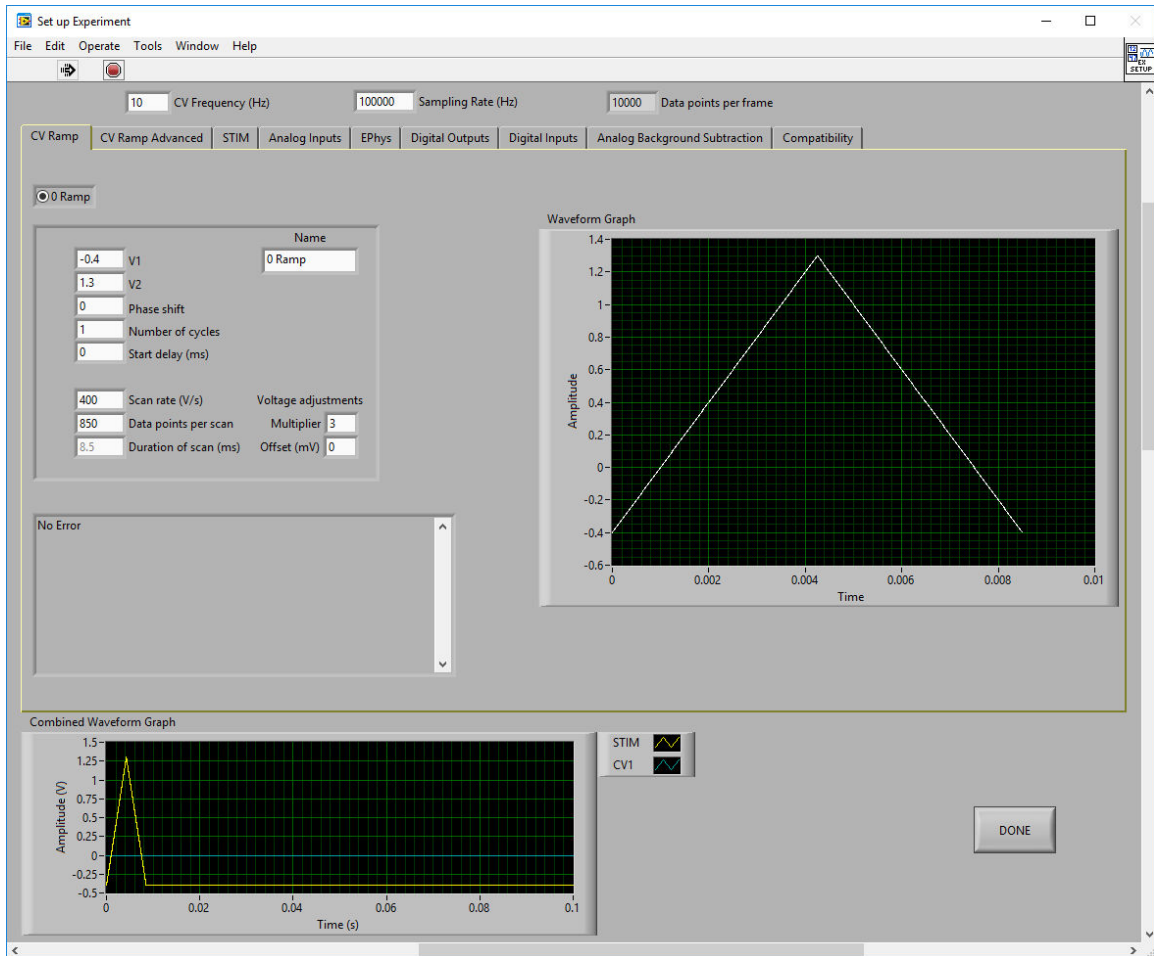


Figure 6-3: One Channel CV Ramp Setup in HDCV

Next, enter the headstage amplifier gain details on the “Analog Inputs” tab (see: Figure 6-4). Pine Research recommends that a name be provided, starting with the channel number (e.g., “0 HS” as in Figure 6-4), which is more important when the WaveNeuro 4 is used in multichannel mode.

Enter any optional digital triggers information on the “Digital Outputs” tab (see: Section 5.1.3). Enter any optional stimulus parameters on the “STIM” tab (see: Section 5.1.3). Press Done. Click the button to toggle the Waveform to the ON position.

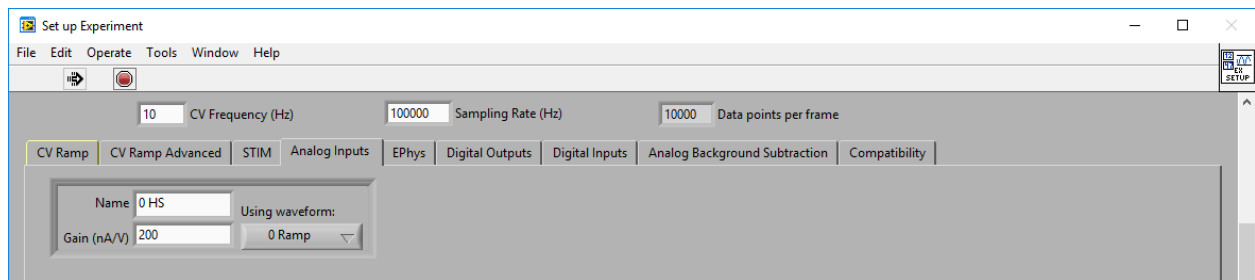


Figure 6-4: One Channel Analog Inputs in HDCV

By default, the “Scope” tab will be shown in the main window (see: Figure 6-5). When all plots are visible (right click the legend and select “Plot Visible”), the CV ramp and current response (from the HEADSTAGE 0 connector) will be shown (even when not connected to electrodes/in solution, as the system is reference grounded/working driven by default, see: Section 3.1.1 for more details).

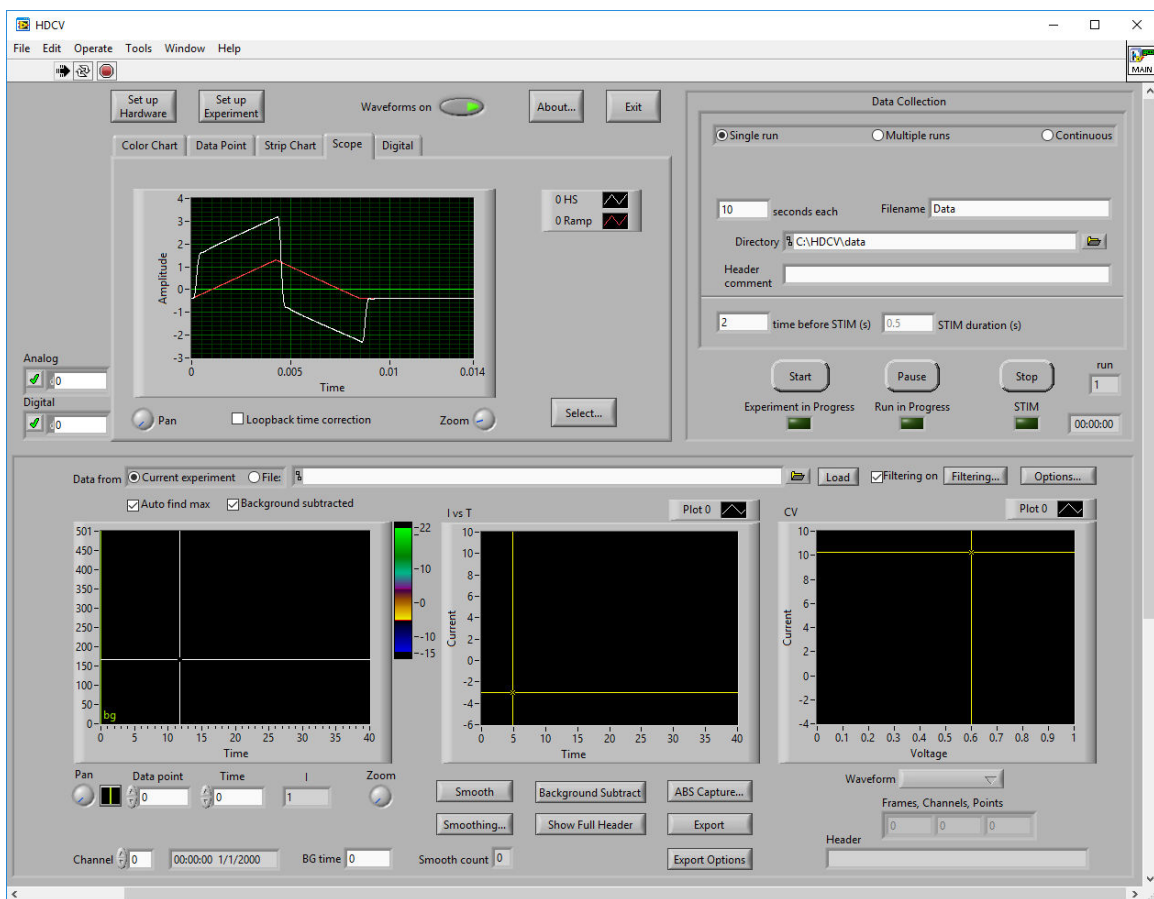


Figure 6-5: One Channel Scope Response in HDCV

Turn off the waveform and connect the headstage cable to the desired electrodes and place them in the desired system.

6.2 Two Channel Operation

6.2.1 Two Channel WaveNeuro 4 Hardware Setup

Connect two headstage cables to HEADSTAGE 0 and HEADSTAGE 1 connectors on the front panel of the WaveNeuro 4 (see: Figure 6-1). STIM OUT may be used by pressing the “Select AO3 Mode” button until “STIM OUT ACTIVE” LED illuminates. Review Sections 2.7.5 and 5.1.3 for details on using stimulus.

6.2.2 Two Channel HDCV Software Setup

Refer to the two channel hardware setup screenshot (see: Figure 6-6). In HDCV, click “Set up Hardware” button. Select the Default Device (typically, Dev0, if this guide has been followed). Enter “2” for the Number of CV inputs and the Number of CV outputs. If you wish to use stimulus, select “Analog” under STIM setting. If you wish to use any digital triggers, enter the desired number in the “Optional digital outputs” field. Press Done.

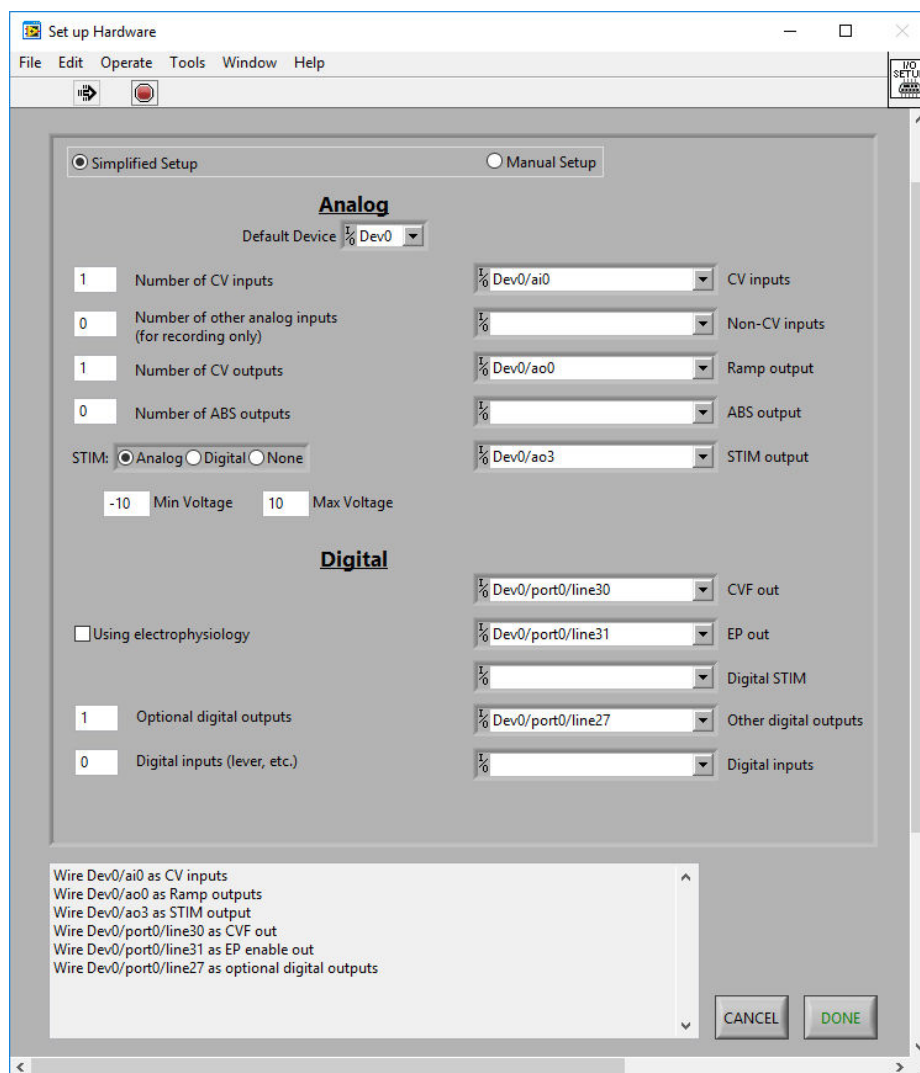


Figure 6-6: Two Channel CV Ramp Setup in HDCV

Once the channel mapping (hardware setup) has been completed, click “Set up Experiment” button to enter your ramp parameters, headstage gain, and other optional controls such as flow cell digital trigger and/or stimulus settings.

Design your waveform on the “CV Ramp” tab (see: Figure 6-7). Shown is the typical dopamine waveform. Pine Research recommends to name the ramp with the channel number as the first character (e.g., “1 Ramp” as in Figure 6-7), which is important when the WaveNeuro 4 is used in multichannel mode. Be certain the multiplier is set to 3.

Below, you will see that the Scan Rate is different between channel 0 and channel 1. This was only done for informative purposes and do visually see the difference between individual channel CV ramp and responses.

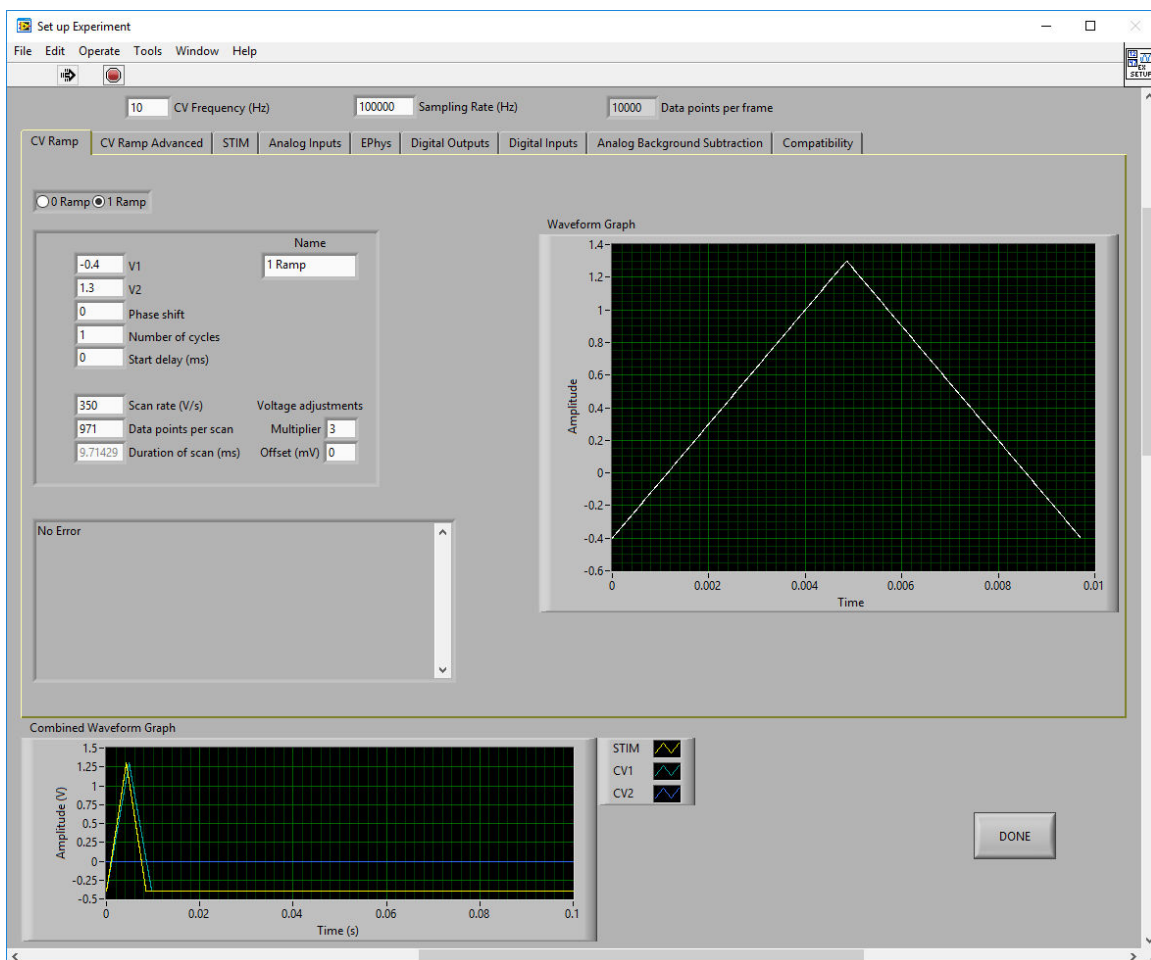


Figure 6-7: Two Channel CV Ramp Setup in HDCV

Next, enter the headstage amplifier gain details on the “Analog Inputs” tab (see: Figure 6-8). Pine Research recommends that a name be provided, starting with the channel number (e.g., “1 HS” as in Figure 6-8), which is important when the WaveNeuro 4 is used in multichannel mode.

Enter any optional digital triggers information on the “Digital Outputs” tab (see: Section 5.1.3). Enter any optional stimulus parameters on the “STIM” tab (see: Section 5.1.3). Press Done. Click the button to toggle the Waveform to the ON position.

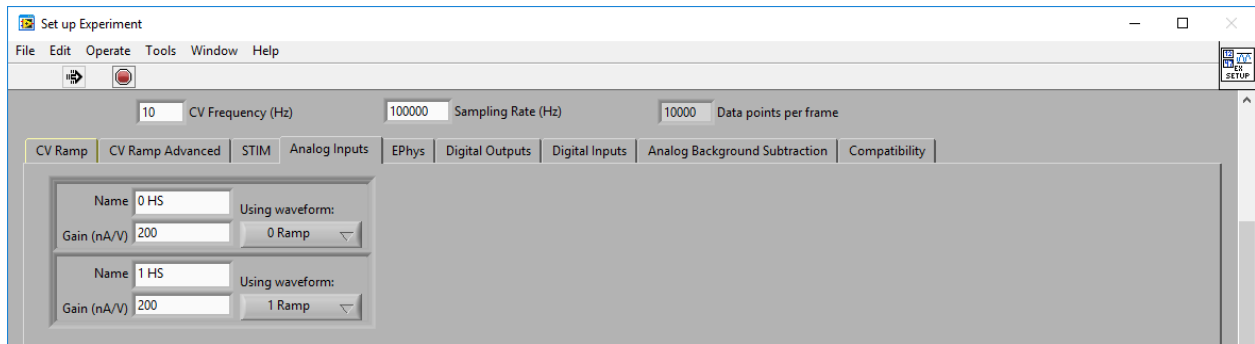


Figure 6-8: Two Channel Analog Inputs in HDCV

By default, the "Scope" tab will be shown in the main window (see: Figure 6-9). When all plots are visible (right click the legend and select "Plot Visible"), the CV ramp and current response (from the HEADSTAGE 0 and HEADSTAGE 1 connectors) will be shown (even when not connected to electrodes/in solution, as the system is reference grounded/working driven by default, see: Section 3.1.1 for more details).

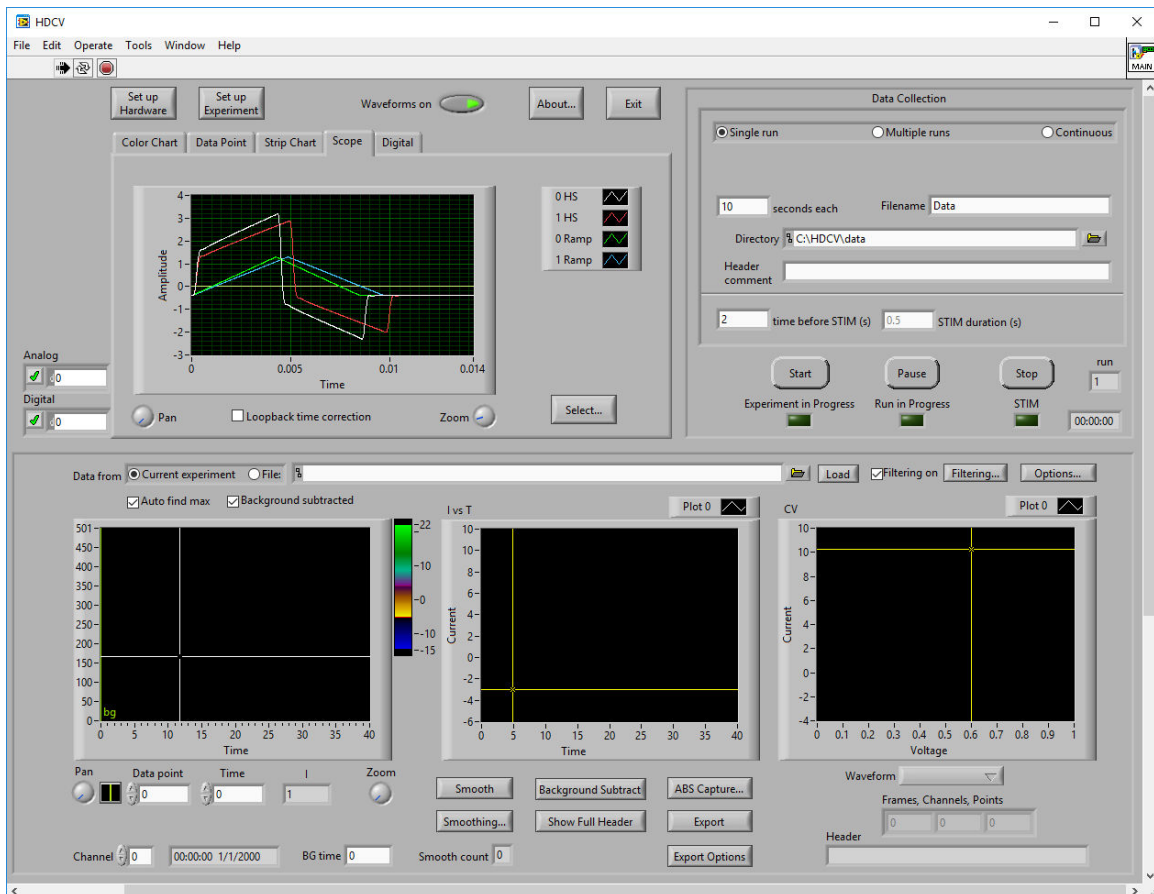


Figure 6-9: Two Channel Scope Response in HDCV

Turn off the waveform and connect the headstage cable to the desired electrodes and place them in the desired system.

6.3 Three Channel Operation

6.3.1 Three Channel WaveNeuro 4 Hardware Setup

Connect three headstage cables to HEADSTAGE 0, HEADSTAGE 1, and HEADSTAGE 2 connectors on the front panel of the WaveNeuro 4 (see: Figure 6-1). STIM OUT may be used by pressing the “Select AO3 Mode” button until “STIM OUT ACTIVE” LED illuminates. Review Sections 2.7.5 and 5.1.3 for details on using stimulus.

6.3.2 Three Channel HDCV Software Setup

Refer to the triple channel hardware setup screenshot (see: Figure 6-10). In HDCV, click “Set up Hardware” button. Select the Default Device (typically, Dev0, if this guide has been followed). Enter “3” for the Number of CV inputs and the Number of CV outputs. If you wish to use stimulus, select “Analog” under STIM setting. If you wish to use any digital triggers, enter the desired number in the “Optional digital outputs” field. Press Done.

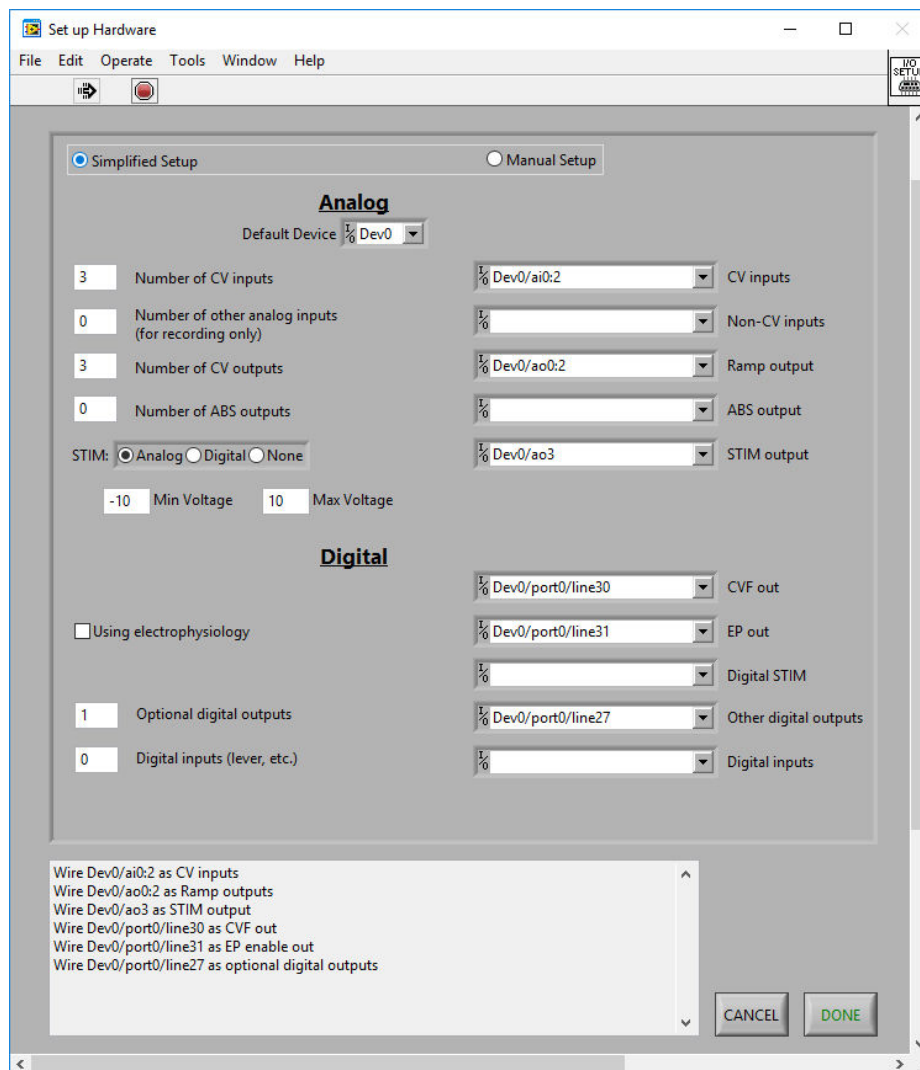


Figure 6-10: Three Channel CV Ramp Setup in HDCV

Once the channel mapping (hardware setup) has been completed, click “Set up Experiment” button to enter your ramp parameters, headstage gain, and other optional controls such as flow cell digital trigger and/or stimulus settings.

Design your waveform on the “CV Ramp” tab (see: Figure 6-11). Shown is the typical dopamine waveform. Pine Research recommends to name the ramp with the channel number as the first character (e.g., “2 Ramp” as in Figure 6-11), which is important when the WaveNeuro 4 is used in multichannel mode. Be certain the multiplier is set to 3.

Below, you will see that the Scan Rate is different between channels 0, 1 and 2. This was only done for informative purposes and do visually see the difference between individual channel CV ramp and responses.

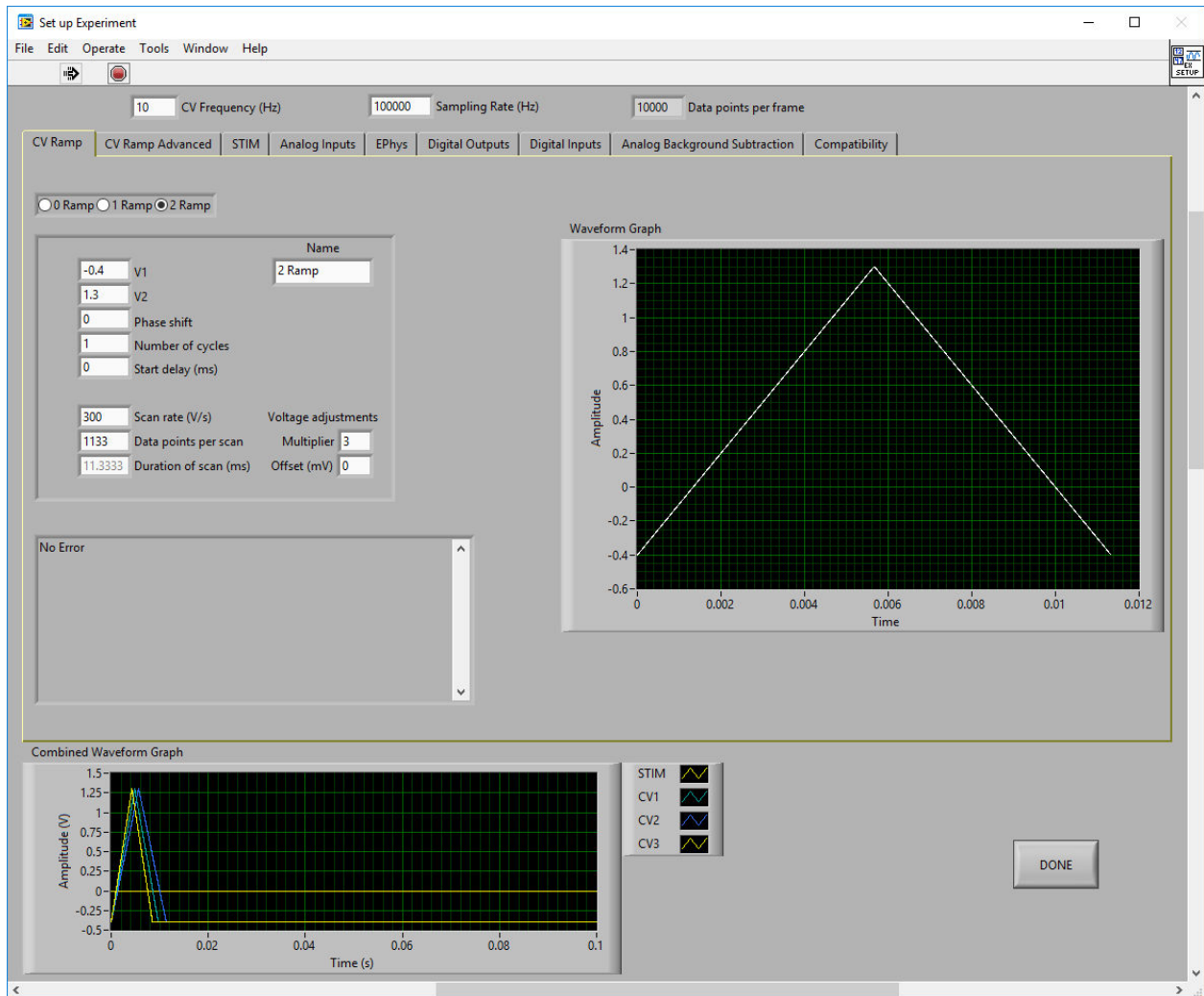


Figure 6-11: Three Channel CV Ramp Setup in HDCV

Next, enter the headstage amplifier gain details on the “Analog Inputs” tab (see: Figure 6-12). Pine Research recommends that a name be provided, starting with the channel number (e.g., “2 HS” as in Figure 6-8), which is important when the WaveNeuro 4 is used in multichannel mode.

Enter any optional digital triggers information on the “Digital Outputs” tab (see: Section 5.1.3). Enter any optional stimulus parameters on the “STIM” tab (see: Section 5.1.3). Press Done. Click the button to toggle the Waveform to the ON position.

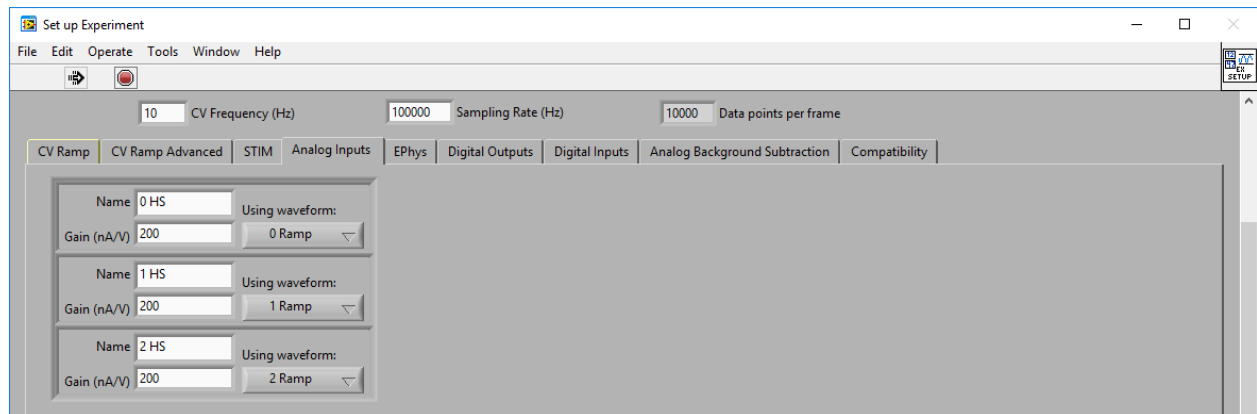


Figure 6-12: Three Channel Analog Inputs in HDCV

By default, the “Scope” tab will be shown in the main window (see: Figure 6-13). When all plots are visible (right click the legend and select “Plot Visible”), the CV ramp and current response (from the HEADSTAGE 0, HEADSTAGE 1, and HEADSTAGE 2 connectors) will be shown (even when not connected to electrodes/in solution, as the system is reference grounded/working driven by default, see: Section 3.1.1 for more details).

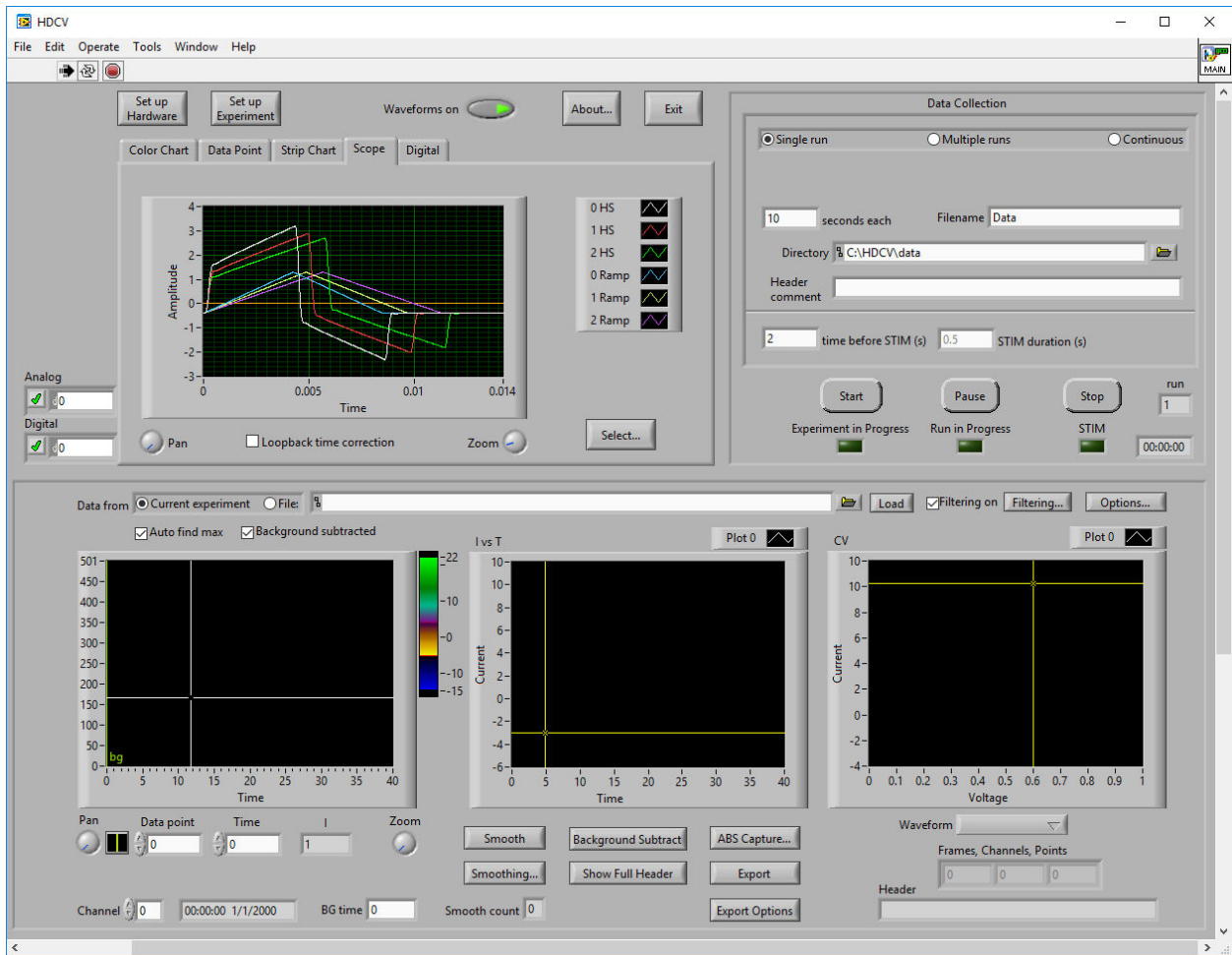


Figure 6-13: Three Channel Scope Response in HDCV

Turn off the waveform and connect the headstage cable to the desired electrodes and place them in the desired system.

6.4 Four Channel Operation

6.4.1 Four Channel WaveNeuro 4 Hardware Setup

Connect four headstage cables to HEADSTAGE 0, HEADSTAGE 1, HEADSTAGE 2, and HEADSTAGE 3 connectors on the front panel of the WaveNeuro 4 (see: Figure 6-1). STIM OUT cannot be used in four channel FSCV mode (see: Figure 6-14). Four FSCV channels may be used by pressing the "Select AO3 Mode" button until "INDEPENDENT HS3 RAMP" LED illuminates.



INFO:

Up to three independent FSCV channels and stimulus output are possible; however, use of four independent FSCV channels disallows the use of stimulus output, as HEADSTAGE3 and STIM OUT share the same analog I/O on the PCIe-6363.

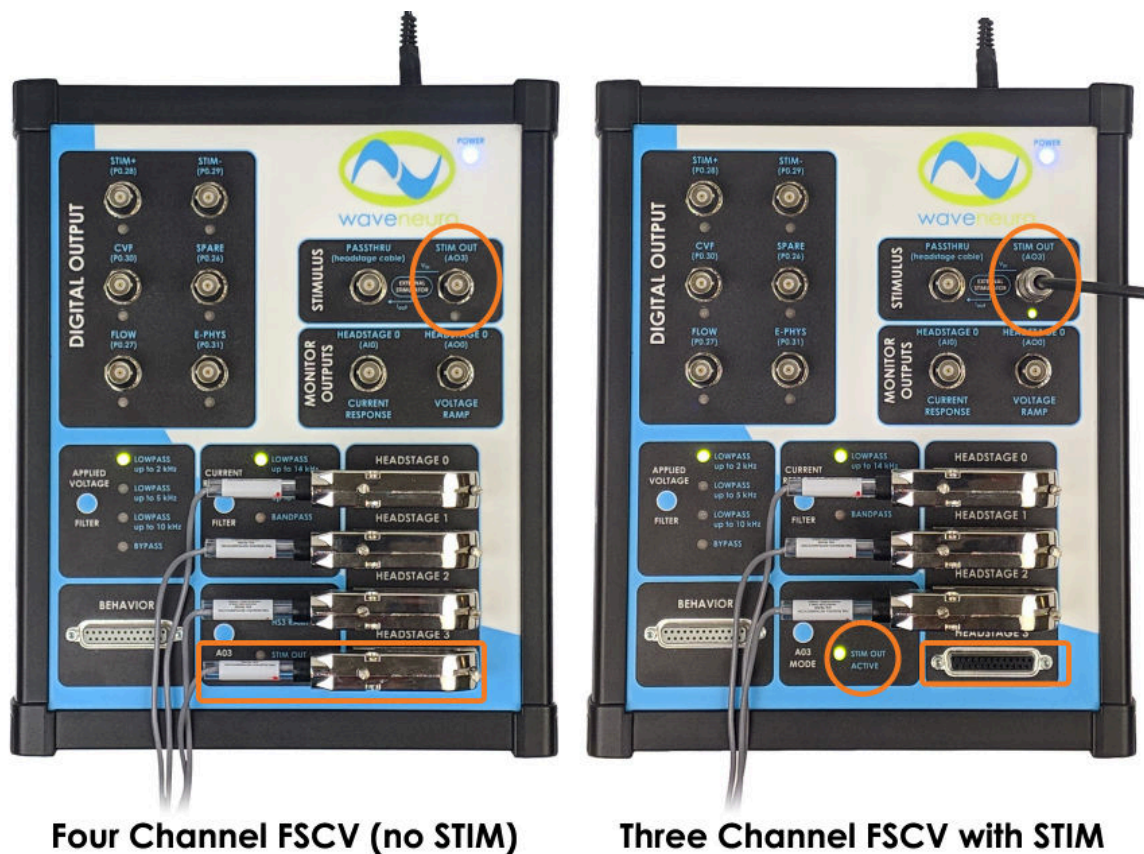


Figure 6-14: Unique Multichannel Configurations of the WaveNeuro 4

6.4.2 Four Channel HDCV Software Setup

Refer to the triple channel hardware setup screenshot (see: Figure 6-15). In HDCV, click “Set up Hardware” button. Select the Default Device (typically, Dev0, if this guide has been followed). Enter “4” for the Number of CV inputs and the Number of CV outputs. You must select “None” under STIM setting, as stimulus cannot be achieved with HDCV when four FSCV channels are in use. If you wish to use any digital triggers, enter the desired number in the “Optional digital outputs” field. Press Done.



INFO:

Up to three independent FSCV channels and stimulus output are possible; however, use of four independent FSCV channels disallows the use of stimulus output, as HEADSTAGE3 and STIM OUT share the same analog I/O on the PCIe-6363.

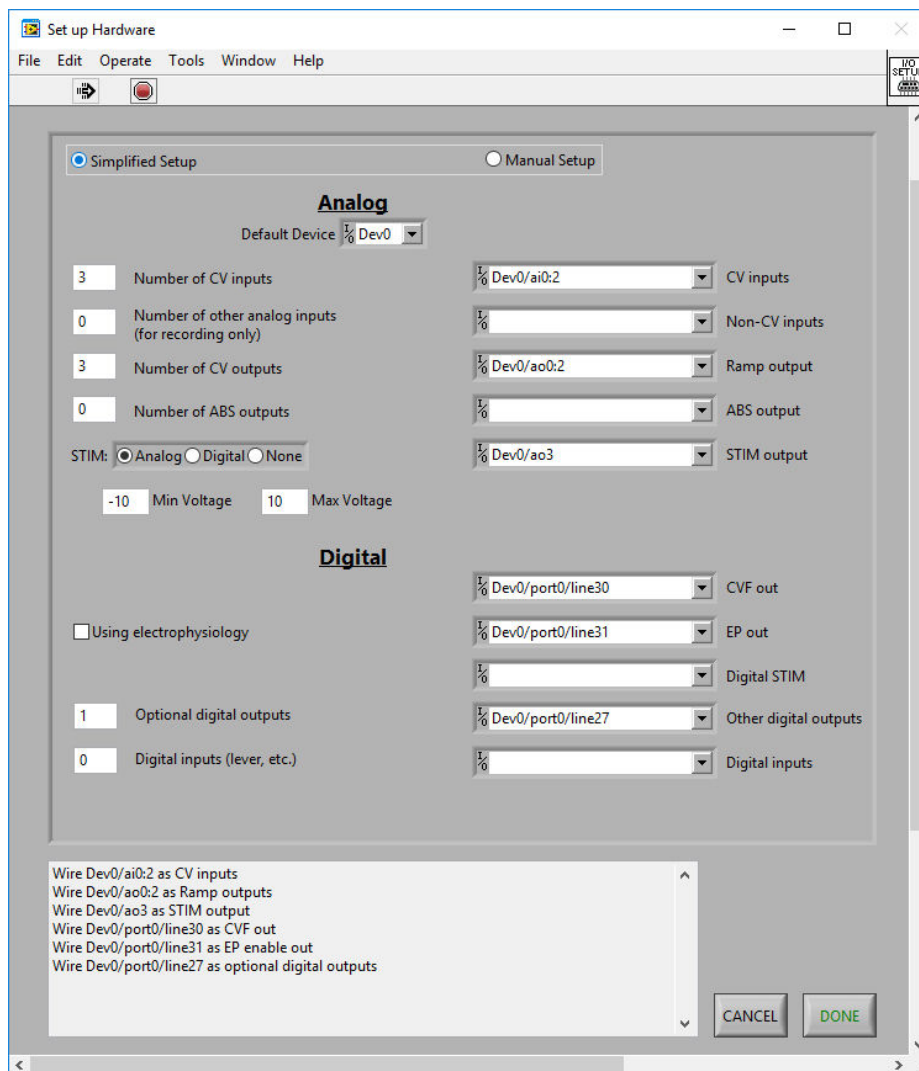


Figure 6-15: Four Channel CV Ramp Setup in HDCV

Once the channel mapping (hardware setup) has been completed, click “Set up Experiment” button to enter your ramp parameters, headstage gain, and other optional controls such as flow cell digital trigger and/or stimulus settings.

Design your waveform on the “CV Ramp” tab (see: Figure 6-16). Shown is the typical dopamine waveform. Pine Research recommends to name the ramp with the channel number as the first character (e.g., “3 Ramp” as in Figure 6-16), which is important when the WaveNeuro 4 is used in multichannel mode. Be certain the multiplier is set to 3.

Below, you will see that the Scan Rate is different between channels 0, 1, 2 and 3. This was only done for informative purposes and do visually see the difference between individual channel CV ramp and responses.

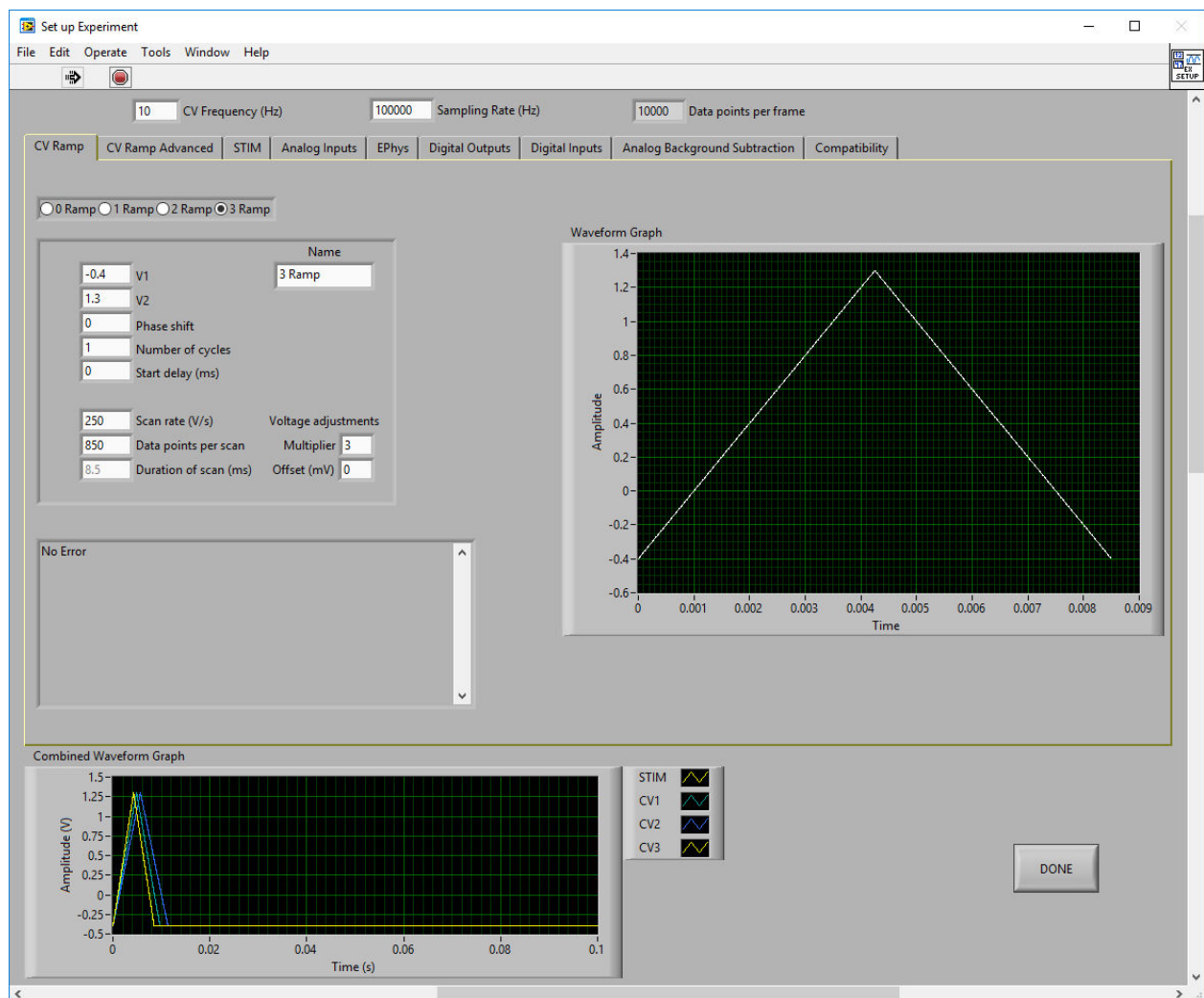


Figure 6-16: Four Channel CV Ramp Setup in HDCV

Next, enter the headstage amplifier gain details on the “Analog Inputs” tab (see: Figure 6-17). Pine Research recommends that a name be provided, starting with the channel number (e.g., “3 HS” as in Figure 6-17), which is important when the WaveNeuro 4 is used in multichannel mode.

Enter any optional digital triggers information on the “Digital Outputs” tab (see: Section 5.1.3). Press Done. Click the button to toggle the Waveform to the ON position.

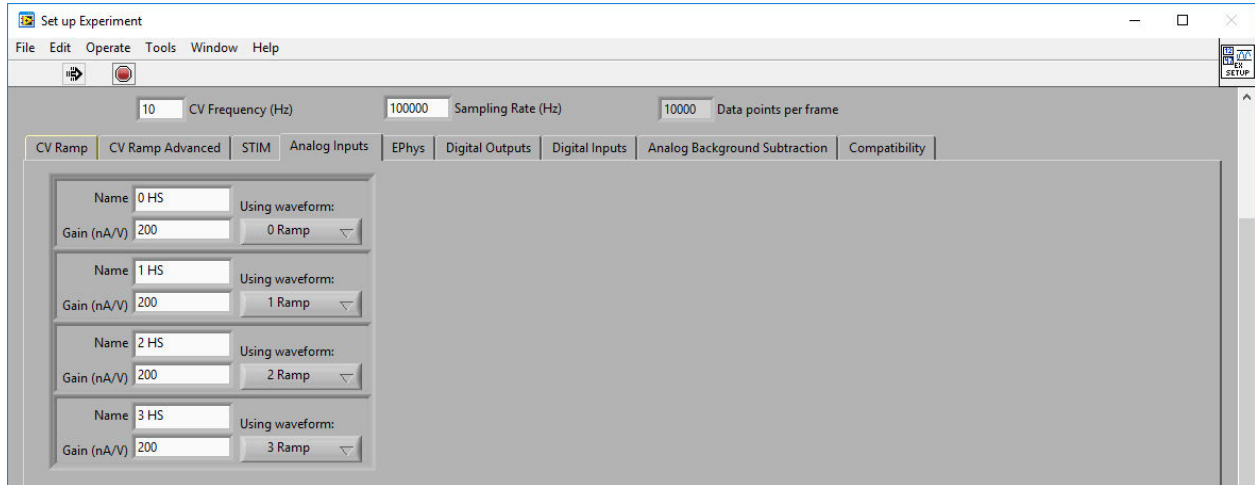


Figure 6-17: Four Channel Analog Inputs in HDCV

By default, the “Scope” tab will be shown in the main window (see: Figure 6-18). When all plots are visible (right click the legend and select “Plot Visible”), the CV ramp and current response (from the HEADSTAGE 0, HEADSTAGE 1, HEADSTAGE 2, and HEADSTAGE 3 connectors) will be shown (even when not connected to electrodes/in solution, as the system is reference grounded/working driven by default, see: Section 3.1.1 for more details).

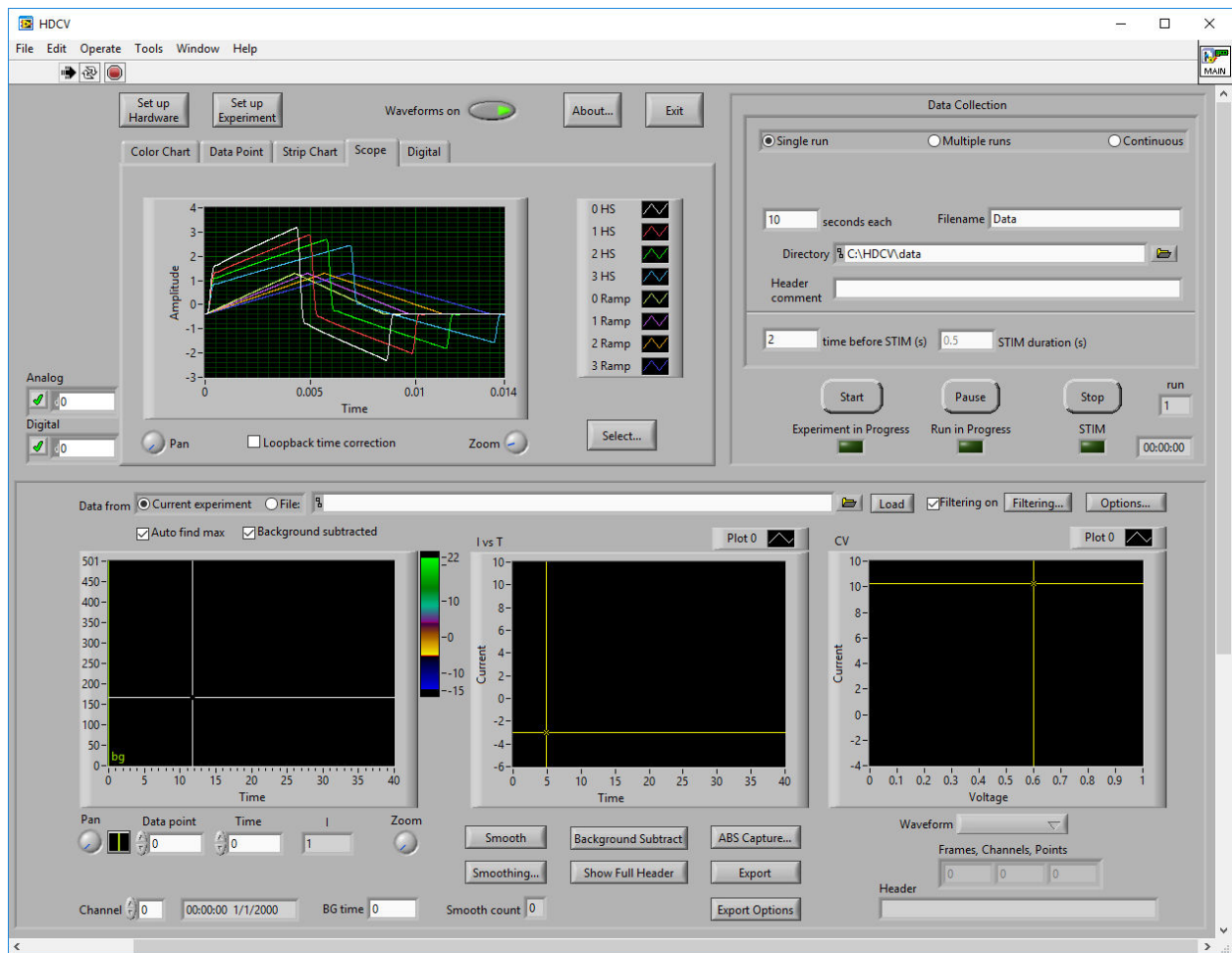


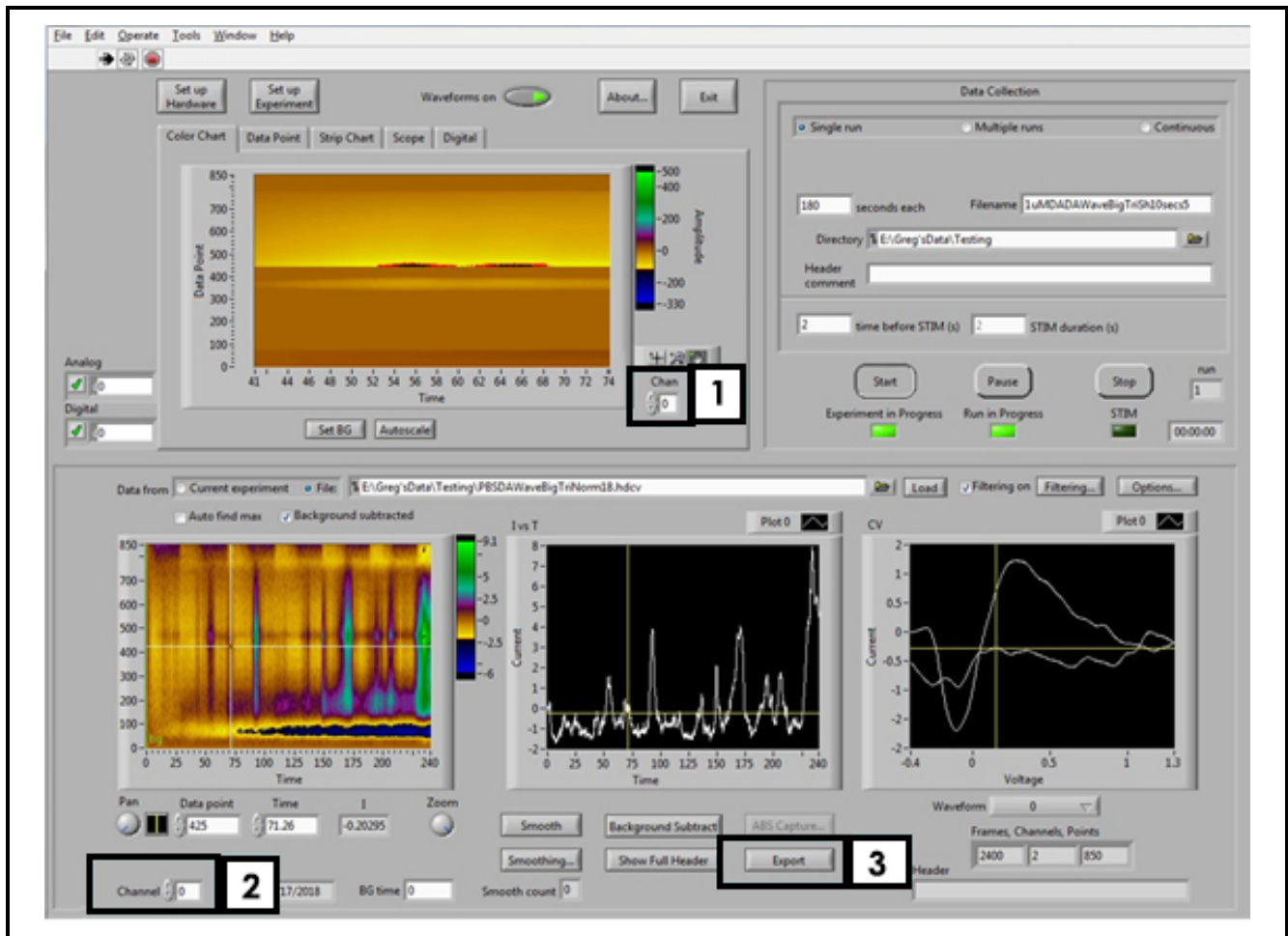
Figure 6-18: Four Channel Scope Response in HDCV

Turn off the waveform and connect the headstage cable to the desired electrodes and place them in the desired system.

6.5 Multichannel Differences in HDCV Software

6.5.1 Viewing Multichannel Data in HDCV

While HDCV software is able to collect multiple channels, it only displays the Color Chart and experimental results for one channel at a time. In both HDCV and HDCV Analysis, the data channel that displays can be defined using the “Chan” or “Channel” select function (see: Figure 6-19 for HDCV Acquisition and Figure 6-20 for HDCV Analysis). Scrolling through the channels will enable each channel to be displayed and manipulated independently in the software package.



#	Label	Description
1	Chan	Toggle button to show Live Color Chart for Channel <i>n</i>
2	Channel	Toggle button to show Saved Experiment Color Chart for Channel <i>n</i>
3	Export	Export Saved Data Button

Figure 6-19. HDCV Application Primary Panels and Buttons

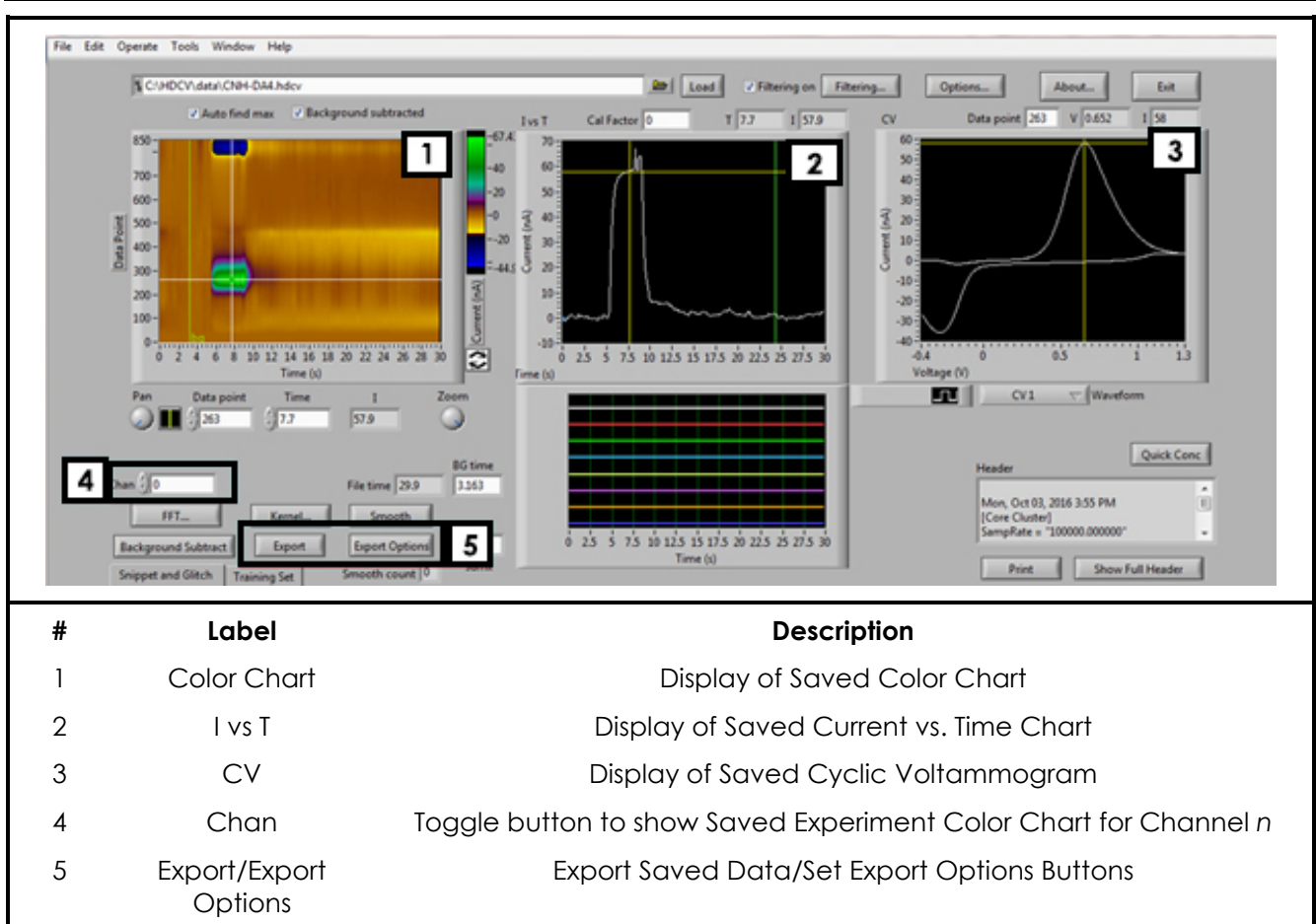


Figure 6-20: Main Analysis Page with Features Highlighted

6.5.2 Multichannel Mode Filter Settings in HDCV

The applied and analog signals can be filtered with hardware (electronic) filters inside the potentiostat that are selected on the front panel of the WaveNeuro 4 (see: Figure 6-21).

There are two sets filters, one for the applied voltage (ramps) and another for the measured current responses. The filter setting selected, for either applied voltage or current response, apply to all FSCV channels in use. You cannot use different filters on a per channel basis.

Each filter set has four possible settings including a bypass option, which does not engage a hardware filter. HDCV is agnostic to these hardware filters, so be advised you cannot know their selections in HDCV software, nor can you control them via HDCV software. They can only be changed manually by pressing the blue button on either section of the top panel of the WaveNeuro 4.

The default applied voltage filter is "LOWPASS up to 2 kHz" and the default current response filter is "LOWPASS up to 14 kHz." These default filters are selected when the WaveNeuro 4 is powered on.

Be advised, HDCV software also provides additional filtering capabilities. These filters are software filters. Information on the software filters is found in the HDCV Acquisition and Analysis guides.

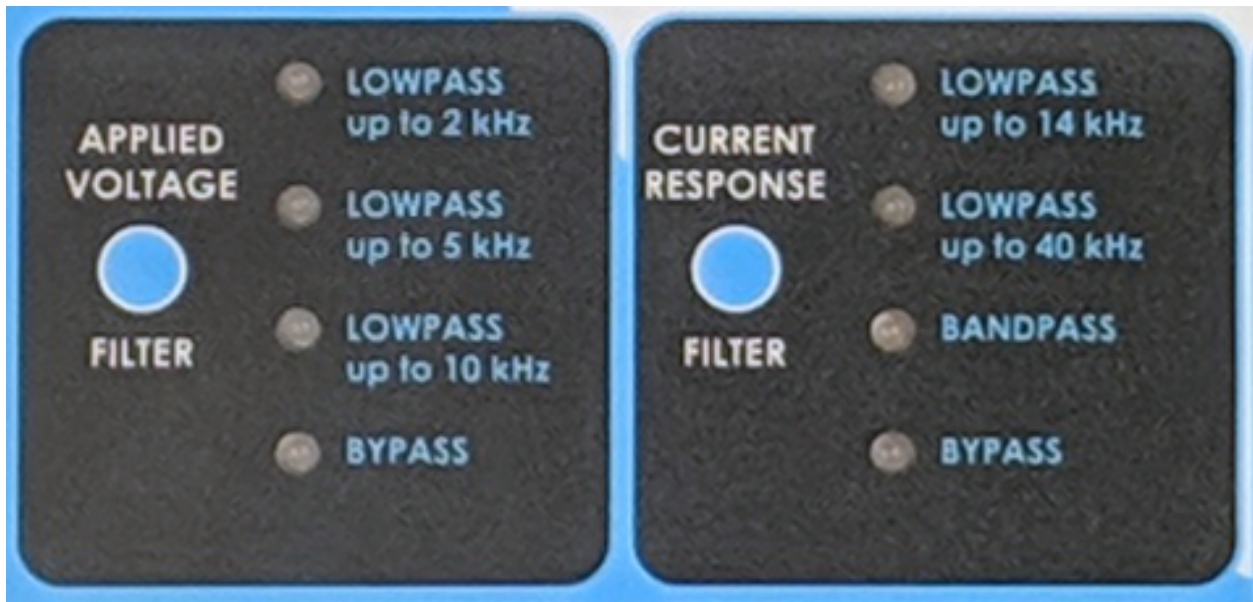


Figure 6-21: WaveNeuro 4 Hardware Filter Settings (Top Panel)

6.5.3 Multichannel Data Export in HDCV

When data is collected with the WaveNeuro 4 using HDCV in multichannel mode, only one channel can be displayed and manipulated at a time. To navigate through the collected channels the Channel feature in the software is used (see: Figure 6-19 and Figure 6-20). The display channel can be manipulated independently without altering the other data channels that were collected.

After manipulating the data, researchers often want to export the data for further analysis or plotting. HDCV enables data to be exported to .txt files. When working with multiple data collection channels only data from the live channel, the channel being actively manipulated, is exported. When the “Export” button is pressed, the active color plot, I-T trace, and CV export into the current working directory (the directory with the data file that is being analyzed).



NOTE:

The entire time course of the file will be exported for the IvT trace and the color plot even if, at the time of export, the limits have been redefined in the active windows.

The “Export Options” menu provides some control over what data is exported (see: Figure 6-19 and Figure 6-20). Pressing the Export Options generates a pop-up menu of options (see: Figure 6-22). The exported data is always saved as files in .txt format. The type of data files, color plot, IvT, and/or CV, can be selected to be written when the “Export” button is pressed. In addition the precision, number of digits after the decimal point, to include in the exported data can be specified.

The CV data is saved in two columns, potential in volts and current in nanoamps. The IvT trace is exported as two columns, time in seconds and current in nanoamps. The Color Chart will be exported as a series of columns. Each column is the current data in nanoamps from a single CV, no potential data is saved.

Stated another way, if 10 CVs are collected per second (each CV has 850 points) and the frame is 10 seconds, the text file export will have 100 columns (10 CV per second for 10 seconds) with each column having 850 data points. No potential data is saved into the color plot data. The potential ramp values can be extracted from the exported CV .txt file.

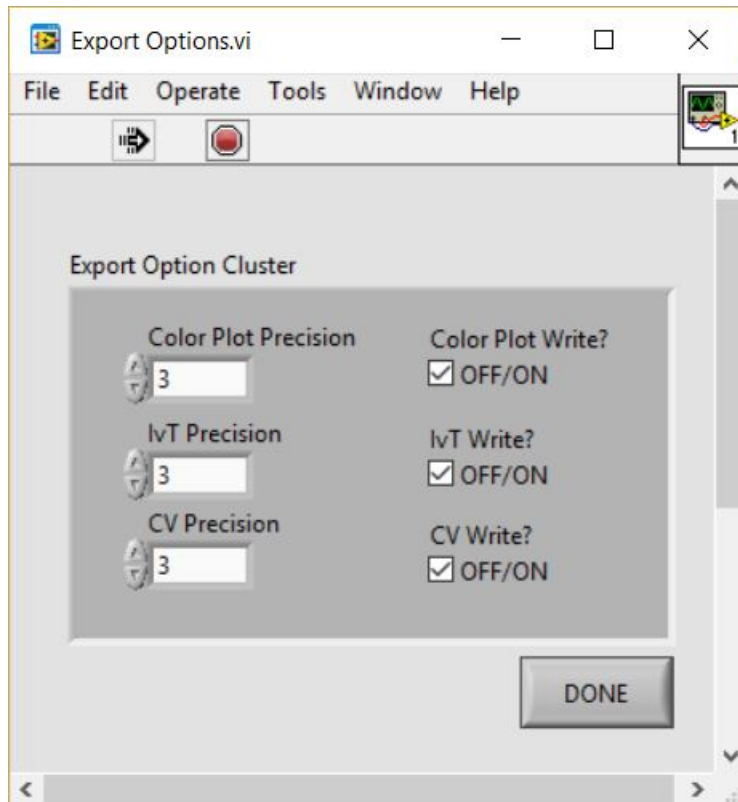


Figure 6-22: Export Options Window in HDCV and Analysis

7. GROUNDING INFORMATION

To avoid issues with signal noise when making electrochemical measurements, it is important to properly take precautions with proper grounding. The most common grounding setup is to tie the Chassis of the WaveNeuro 4 to the Faraday cage, which contains the electrochemical system, electrodes, and headstage cables.

7.1 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 4 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 4 should be physically located outside the Faraday cage. The headstage cable (or/and DB-25 extension cable) should extend from the WaveNeuro 4 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 7-1).

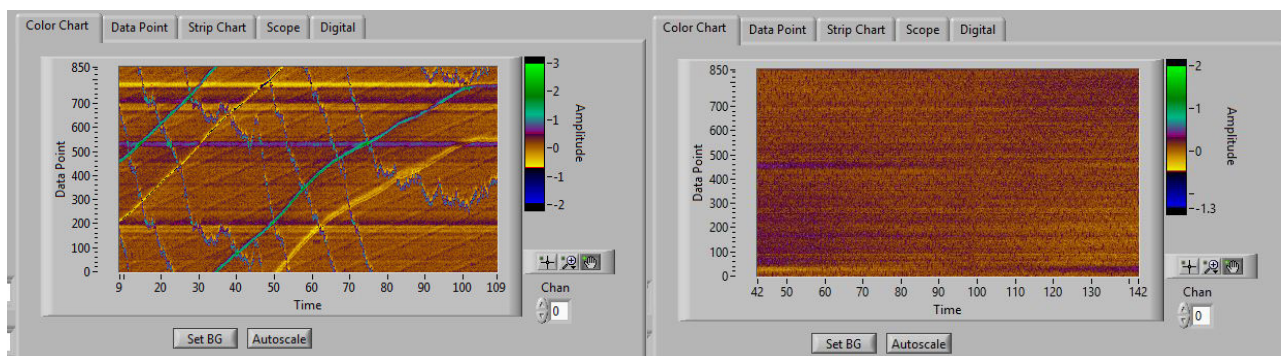


Figure 7-1: Live Color Chart Displays in HDCV with Noise (left) and without Noise (right)

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 4. When such a piece of equipment goes through a power cycle, it may induce noise or a glitch in the electrochemical measurement. The WaveNeuro 4 is designed to be connected to a personal computer (tower, desktop, or laptop) via a cable. The cables are long enough that the WaveNeuro 4 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.

7.2 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.

7.2.1 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 4 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 4 chassis is tied to earth ground through the NI cable connector.

7.2.2 Chassis



CHASSIS:

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

The metal case that contains the WaveNeuro 4 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 4 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.

7.2.3 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 4 potentiostat is the zero volt reference point used by the instrument circuit board.

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 8-1 and Table 8-2). 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.

Power Cord (USA) EWM18B7



This cord is for use in continental Europe, Russia, and Indonesia.

Power Cord (Europe) EWM18B8EU



This cord is for use in the United Kingdom, Ireland, Oman, Hong Kong, and Singapore.

Power Cord (UK) EWM18B8UK



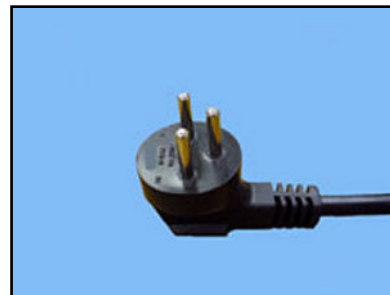
This cord is for use exclusively in China.

Power Cord (China) EWM18B8CN



This cord is for use in India and South Africa.

Power Cord (India) EWM18B8IN



This cord is for use exclusively in Israel.

Power Cord (Israel) EWM18B8IL

Table 8-1: 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



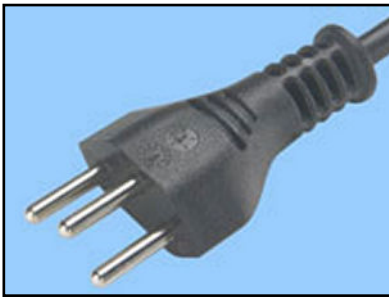
This cord is for use exclusively in Denmark.

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 8-2: 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

