Published in final edited form as:

IEEE Int Symp Med Meas Appl. 2017 May ; 2017: 349–354. doi:10.1109/MeMeA.2017.7985901.

Multifunctional System for Observing, Measuring and Analyzing Stimulation-Evoked Neurochemical Signaling

Christopher J. Kimble [Senior Member, IEEE],

Division of Engineering, Mayo Clinic, Rochester, Minnesota

Joshua B. Boesche,

Division of Engineering, Mayo Clinic, Rochester, Minnesota

Diane R. Eaker,

Division of Engineering, Mayo Clinic, Rochester, Minnesota

Kenneth R. Kressin,

Division of Engineering, Mayo Clinic, Rochester, Minnesota

James K. Trevathan,

Mayo Graduate School, Mayo Clinic, Rochester, Minnesota

Seungleal Paek,

Mayo Graduate School, Mayo Clinic, Rochester, Minnesota

Anders J. Asp,

Mayo Graduate School, Mayo Clinic, Rochester, Minnesota

Malcolm B. McIntosh, and

Division of Engineering, Mayo Clinic, Rochester, Minnesota

J. Luis Lujan [Member, IEEE]

Department of Neurologic Surgery, Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, Minnesota

Abstract

The ability to measure neurotransmitter activity using implanted electrochemical sensors offers researchers a potent technique for analyzing neural activity across specific neural circuitry. We have developed a wirelessly controlled device, WINCS Harmoni, to observe and measure neurotransmitter dynamics at up to four separate sensors, with high temporal and spatial resolution. WINCS Harmoni also incorporates a versatile neurostimulator that can be synchronized with electrochemical recording. The WINCS Harmoni platform is thus optimally suited for probing the neurochemical effects of neurostimulation, and may in turn enable the development of personalized therapies for multiple brain disorders.

Keywords

voltammetry; stimulation; neurotransmitter; neuromodulation

I. Introduction

Fast-scan cyclic voltammetry (FSCV) presents a useful research tool for studying the dynamics of neurotransmitter release, with high spatial and temporal resolution [1]. We previously developed the Wireless Instantaneous Neurotransmitter Concentration Sensing (WINCS) system and employed it to study stimulus-evoked neurotransmitter release in animals and humans using FSCV [2,3]. The Mayo Investigational Neurostimulator Control System (MINCS) extended the capabilities of WINCS by synchronizing stimulation with FSCV recording, thereby mitigating artifacts from electrical stimulation that would otherwise obscure FSCV data [4]. The ad hoc coupling of WINCS and MINCS served as a prototype for a unified, wirelessly controlled device, WINCS Harmoni, that records neurotransmitter activity at multiple sensors, synchronizes neurochemical measurements with stimulation, and interoperates with MATLAB® to analyze data and to perform neurotransmitter-based closed-loop control of electrical stimulation.

II. WINCS Harmoni system overview

WINCS Harmoni is an advanced multifunctional platform to enable neuroscience research. It measures extracellular neurotransmitter activity at as many as four carbon fiber microelectrodes (CFMs) or boron-doped diamond (BDD) microelectrodes, and performs data acquisition in synchrony with neurostimulation. Originally designed for human use in IRB- or FDA-approved research studies, WINCS Harmoni incorporates many features intended to ensure patient safety.

Packaged in a 3D-printed, splash-resistant, electrostatic-dissipative enclosure (ABS-ESD7 acrylonitrile butadiene styrene, Stratasys, Eden Prairie MN), WINCS Harmoni (Figures 1, 2) is a rechargeable battery-powered instrument that supports electrochemical signal acquisition at any combination of four sensing electrodes, and electrical stimulation at up to four stimulating electrodes. WINCS Harmoni can perform FSCV or fixed-potential amperometry (FPA), measuring electrochemical activity in the form of reduction/oxidation reaction currents at sub-nanoampere resolution. Stimulation can be either voltage- or current-regulated, with ranges from 50 mV to 10 V (in 1 mV steps) and from 10 μ A to 10 mA (in 1 μ A steps). WINCS Harmoni communicates via a wireless Bluetooth link with a base station, a Windows-based computer running WincsWare—the core software that controls all hardware module functions (other than fault protection) and integrates all WINCS Harmoni operations.

The WINCS Harmon hardware incorporates two principal printed circuit board assemblies (PCBAs, also known as “boards”): the Primary and Stimulator PCBAs. These two boards are powered by separate rechargeable lithium-ion batteries, and they are controlled by separate LPC1769 ARM Cortex-M3 microcontrollers (NXP Semiconductors, Eindhoven, Netherlands). Both boards incorporate battery recharging and fuel gauge circuits (bq24073 and bq27500, respectively; Texas Instruments, Dallas TX). An array of LEDs provides feedback to the user regarding device status (e.g., battery charging, power on, stimulation in progress, and fault status). A third PCBA serves as a simple connector interface for the

electrode leads on the front panel, which also houses a pushbutton on-off switch and charging port.

The WINCS Harmoni system includes an auxiliary hardware module, the Harmoni Optical Interface and Synchronization Transceiver (HOIST), that communicates with WINCS Harmoni via nonconductive plastic optical fibers at an approximate rate of 80,000 samples/second (more than double that of Bluetooth). HOIST connects to the base station via USB (Universal Serial Bus). HOIST allows data synchronization with signals from magnetic resonance imaging (MRI) scanners, thereby mitigating scanner-generated electromagnetic noise that would otherwise obscure the recorded neurochemical data. HOIST can receive synchronization signals from instruments other than MRI scanners. A synchronization signal can also be transmitted by HOIST to auxiliary equipment (e.g., electromyographic sensors) to designate the onset of a stimulus pulse pattern or FSCV scan.

III. Primary PCBA hardware subsystems

Primary Microcontroller

The 32-bit ARM Cortex-M3 LPC1769 microcontroller features a high level of integration and relatively low power consumption. The LPC1769 offers a variety of digital peripherals, 64 kB of static random-access memory (SRAM), 512 kB of flash programming memory, an 8-channel 12-bit analog-to-digital converter (ADC) and up to 70 general purpose input/output (GPIO) lines. An external 12-MHz crystal enables system operation at 120 MHz. The LPC1769 operates on 3.3 V, and draws approximately 100 mA (typical) during FSCV. The LPC1769 microcontroller on the Primary board directly controls electrochemical data acquisition, handles all communication with the base station, and serves as the intermediary between the base station and the microcontroller on the Stimulator board via an electrically isolated interface.

Bluetooth Communications

A class-1 WT41-A Bluetooth transceiver module (Bluegiga, Espoo, Finland) provides the wireless link between the hardware module and the base station. With a 35×14 mm footprint, it integrates a Bluetooth radio, an antenna and a microcontroller that implements the lower-level functions of the Bluetooth protocol. The WT41-A communicates with the primary microcontroller via a 4-wire serial Universal Asynchronous Receive/Transmit (UART) interface. The FCC-certified WT41-A can transmit at power levels as high as 19 dBm, facilitating data throughput in crowded electromagnetic environments with high wireless communication traffic. However, in a relatively uncongested RF environment, the WT41-A can support data rates of 500 kbps. The WT41-A supply current depends on data rate, distance to the base station and presence of other RF traffic; an average supply current of 50–80 mA is typical.

Data Acquisition

The Primary PCBA includes a custom, multichannel delta-sigma (Σ) analog-to-digital converter (ADC) integrated circuit (IC) designed by the Mayo Clinic Special Purpose Processor Development Group (SPPDG) and fabricated by IBM Microelectronics

specifically for WINCS Harmoni (Figure 1C). Each channel incorporates a third-order Σ modulator that delivers 1-bit samples of the input current at a rate of 12.8 megabits per second. A digital low-pass filter and decimation stage converts this digital output stream to 16-bit samples that are delivered serially to the microcontroller over an I2S (Integrated Inter-IC Sound) interface at rates of up to 100,000 16-bit samples per second (100 ksp/s). This maximum FSCV sampling rate is fast enough to measure neurochemical activity with good fidelity. The user can select individual sampling rates for each channel. The corner frequency of the Σ ADC's 5-stage digital low-pass filter is set to one-half the sampling rate (i.e., Nyquist frequency) by default. WINCS Harmoni software limits the maximum sampling rate for FPA to 6.25 ksp/s. Electrochemical sensing supports eight gain ranges. The nominal full-scale range for the most sensitive gain setting is $\pm 1 \mu\text{A}$. Taking noise into account, the effective resolution in this range is approximately 0.1 nA, when shielded in a Faraday cage. The nominal full-scale range for the least sensitive electrochemical gain setting is $\pm 8 \mu\text{A}$, wide enough to handle the background currents of typical sensors [5]. A high-resolution mode is also provided, for FPA only. The nominal full-scale range in this mode is $\pm 35 \text{ nA}$, and the sampling rate is restricted to 24.4 bits per second. The Σ ADC is powered by three separate power supply domains on the Primary board: 3.3 V for its analog circuitry, 1.2 V for its core digital circuitry, and 3.3 V for its i/o drivers and receivers. The Σ ADC is packaged in a 48-pin QFN (Quad Flat No-Leads) package, which incorporates a large die-attached ground pad. This center ground pad reduces chip-to-ground impedance.

Waveform Generation for Electrochemical Recordings

A Serial Peripheral Interface (SPI) bus operated by the microcontroller controls a serial string of five AD5541A 16-bit DACs (Analog Devices, Norwood, MA) to produce the time-varying waveforms or static potentials that are applied across the electrodes for FSCV or FPA, respectively. The ADC utilizes feedback action to impose the DAC-programmed potential upon each of its working-electrode inputs. An adjustable output of a fifth DAC is buffered by an ADA4528-1 op amp and distributed to the electrochemical counter electrodes, enabling the differential electrode voltage to be varied over a $\pm 2.5 \text{ V}$ range (although our implementation limits the span of an FSCV scan to 2 V), adequate to cover the required range for detecting neurotransmitters of interest using FSCV. An Analog Devices ADR441A voltage reference IC supplies a 3.00-V reference voltage to all five DACs. The microcontroller sequences DAC operation in synchrony with electrochemical data acquisition by the Σ ADC.

IV. Stimulator PCBA hardware subsystems

Stimulator Microcontroller, Interface with Primary PCBA

The stimulator board (Figures 1A and 4) generates user-programmed current- or voltage-regulated stimulus sequences, measures stimulus output amplitudes, and implements fault protection circuits for patient safety. The microcontroller on the WINCS Harmoni Stimulator board (NXP LPC1769) directly controls all stimulator activity. It communicates with the base station via the WINCS Harmoni Primary microcontroller. The Primary and Stimulator boards have separate batteries and are electrically isolated from one another to prevent unregulated current between the stimulating electrodes and the electrochemical

counter electrode that could result in tissue damage. A four-channel digital isolator (Si8441AB, Silicon Labs, Austin TX) on the Primary board isolates a UART interface between the microcontrollers on the Primary and Stimulator boards. The microcontrollers emit synchronization signals to coordinate stimulation with signal acquisition during FSCV, in order to eliminate stimulus artifact in the recorded FSCV data [4].

Voltage- and Current-Regulated Drivers

An AD5541A 16-bit DAC generates the inputs for the voltage- and current-mode stimulation drivers. The 2.5-V full-scale range of the DAC is determined by an ADR3425 voltage reference (Analog Devices, Norwood, MA). Voltage-mode stimulation is provided by an LM8261 op amp (Texas Instruments, Dallas, TX) which has a slew rate of 12 V/ μ s and low supply current (1 mA). Current-mode stimulation is provided by two LM8261 op amps configured as a modified Howland current pump [6]. Cathodic or anodic stimulation can be delivered through various combinations of electrode contacts via four pairs of high-side and low-side single-pole, single-throw (SPST) analog switches (ADG1401, Analog Devices, Norwood, MA) comprising four separate half-H bridge circuits. The switches are turned on or off by the microcontroller via quad AND gates that are also part of the stimulator's fault protection system. If a fault is detected, the switches are shut off. Resistance in the on state is less than 2 Ω , while leakage current for low drain/source voltages is in the sub-nanoampere range.

Fault Protection Circuits

The WINCS Harmoni Stimulator board employs redundant voltage sensors and redundant current sensors primarily for fault protection. Of secondary importance is the measurement and reporting of stimulus-pulse amplitudes (voltage and current), which can be used to estimate electrode impedance. Driver output voltage is sensed at the common input to the high-side switches, the farthest downstream node at which voltage can be sensed at a single point. Redundant op amps (OPA743, Texas Instruments, Dallas, TX) buffer the signal and deliver it to separate ADC inputs of the microcontroller, which reports the measurement to the base station. Driver output current is also sensed at the common input to the high-side switches. Redundant MAX9643UATA+ current-sense amplifiers (Maxim Integrated, San Jose, CA) multiply the voltage across a 4.7- Ω current-sense resistor by a factor of 10. Each current-sense amp supplies its output signal to a Texas Instruments OPA727 op amp, configured as a non-inverting amplifier with a gain of 5.6. The duplicate signals are then delivered to redundant over-current detection circuits, charge-density protection circuits, and ADC inputs on the microcontroller.

The WINCS Harmoni Stimulator board incorporates four types of fault-detection circuits: over-voltage, over-current, charge density and sustained output. Two instances of each circuit are implemented in hardware, ensuring single-fault safety without reliance on software or firmware. The over-voltage circuit compares a scaled version of the stimulus voltage to a threshold reference at an Analog Devices ADCMP341 comparator. In a similar fashion, the over-current circuit compares a voltage representing the stimulus current to a threshold reference. For any fault, if the fault threshold is exceeded, downstream circuitry latches off the eight SPST switches that gate stimulus output, and the user is notified about

the fault by the base station and by a fault LED on the hardware module. Charge density detectors ensure that charge density at the electrode-tissue interface does not exceed charge-transfer limits for safe stimulation, typically 30 μC per stimulus pulse per square centimeter of electrode surface area [7]. Here, a voltage representing the stimulus current is integrated, pulse by pulse, by an LM8261 op amp equipped with a 6.81-k Ω input resistor and a 0.01- μF feedback capacitor. The charge accumulating in the capacitor is discharged by an ADG1401 analog switch at the falling edge of each stimulus pulse. The integrator voltage (which is negative) is inverted and applied to an ADCMP341 comparator, which when tripped engages the fault latch. The sustained output detectors ensure that the charge density limit is not exceeded when the magnitude of the stimulus current is small, below the charge density detector's nominal 230- μA pulse-detection threshold. The sustained output detector is similar to the charge density detector. It can detect charge density faults at currents below the charge density detector's threshold (the nominal sustained output threshold for a 230- μA stimulus pulse is 7.7 ms). It employs a similar integrator, but the input to the integrator is a 1.25 V reference voltage instead of a signal proportional to the stimulus current, and the integration period is delimited by the on-time of the high-side output switches.

V. Wincs Ware

WincsWare is the cornerstone software that controls all high level WINCS Harmoni functions. A Microsoft Visual Studio C# .NET application, WincsWare provides a versatile user interface that simplifies setup of FSCV and stimulus sequences. WincsWare receives, records, processes, and displays data transmitted by the hardware module with minimal delay on the order of milliseconds. Additionally, WincsWare provides an application programming interface (API) with MATLAB to enable data analysis and implementation of complex control strategies such as closed-loop neuromodulation [8].

Standard FSCV waveforms (including paired-pulse FSCV [9]) and charge-balanced stimulus sequences can be defined by entering only a few parameters such as baseline voltage, peak voltage and scan rate. Arbitrary waveforms, for both FSCV and stimulation, can be defined manually within the user interface. Stimulus waveforms can be downloaded from an Excel spreadsheet. WincsWare facilitates synchronizing stimulation with FSCV scans to allow artifact-free data analysis. Data can be exported as a bitmap or in an Excel-compatible file format for further analysis and publication.

The WincsWare user interface displays electrochemical measurements in the form of cyclic voltammograms (CVs) for each FSCV recording channel, where the vertical axis represents current amplitude and the horizontal axis represents applied potential. Separate CVs depict raw and background-subtracted measurements. Background subtraction extracts the Faradaic current from the raw CV, which is dominated by much larger charging and discharging currents. The background-subtracted CV presents a recognizable signature for the analyte of interest (Figure 5C,D insets). For each channel, a current intensity plot (also known as pseudocolor plot) shows sequential CVs as a function of applied potential (vertical axis) over time (horizontal axis) (Figure 5A,B). WincsWare offers cursors for data visualization that can be manipulated by the user to control background subtraction and to display measurements acquired at specific potentials (Figure 5C,D).

FSCV waveforms, stimulus sequence patterns, multichannel acquisition settings and other parameters can be saved and recalled. WincsWare saves all acquired data, unfiltered, in files that can be retrieved for replay in WincsWare or exported for offline analysis (e.g., in MATLAB). During live acquisition or replay, stimulator activity is displayed concurrently with the recorded data by a series of flag annotations. Time-stamped text annotations can be inserted at any time during live acquisition or playback. An overview plot spanning the entire recording session facilitates replay. The software allows customization of the size, position, order and scale of all data displays. A variety of configurable software filters can be applied to the data displays during live acquisition or replay.

VI. Functionality Validation

Benchtop validation was performed largely by the WINCS Harmoni Automated Test, Calibration and Hardware Exerciser System (WATCHES), a computer-controlled system of electronic test instruments and switchable load resistors that calibrates the DACs and verifies the accuracy of measurement, stimulation and fault detection.

In vitro dopamine detection was performed using flow injection analysis. A CFM was placed in a flowing stream of artificial cerebrospinal fluid (aCSF) buffer solution with a pH value of 7.4, containing in mM: 140 NaCl, 3.25 KCL, 1.2 CaCl₂, 1.2 MgCl₂, 1.25 NaH₂PO₄, 15 Trizma, and 2 Na₂SO₄. Boluses of aCSF buffered dopamine solution (i.e., 0.1 μ M, 0.5 μ M, 1.0 μ M) were injected for five seconds at 3 mL/min. Flow rate was controlled using a FIALab 3200 injection system (FIALab Instruments, Seattle, WA). Flow injection dopamine measurements were obtained by sweeping the CFM potential from a resting potential of -0.4 V to a switching potential of 1.45 V and back to the resting potential, at a rate of 400 V/s every 100ms [3]. The CFM was held at the resting potential between scans.

In vivo stimulation-evoked dopamine release was measured in three rodents undergoing deep brain stimulation (DBS). All animal procedures were performed according to the guidelines of the Mayo Clinic Institutional Animal Care and Use Committee (IACUC). Animals were kept on a standard 12-hour light-dark cycle with access to food and water ad libitum in conventional housing in accordance with National Institutes of Health (NIH) and U. S. Department of Agriculture guidelines. Animals were anesthetized via an intraperitoneal injection of urethane (1000 mg/kg). Once anesthetized, the head was fixed in a stereotactic frame (David Kopf Instruments, California). Three small burr holes were made according to a brain atlas [10] to provide access to the medial forebrain bundle (MFB) (stimulating electrode), the striatum (FSCV electrode), and the contralateral cortex (reference electrode). All electrodes were inserted into the appropriate stereotactic target coordinates using a micromanipulator. A bipolar stimulating electrode was placed in the MFB, a recording CFM was implanted in the striatum, and a reference Ag/AgCL electrode was implanted in the contralateral cortex. The electrodes were connected to WINCS Harmoni for concurrent detection of stimulation-evoked dopamine release. Following implantation, the recording electrodes were allowed to stabilize within the tissue environment for twenty minutes. A comprehensive range of stimulation parameters (Table 1) was used to evoke a wide range of dopamine release responses. Stimulation was delivered for two seconds followed by a rest period of thirty seconds. Stimulation-evoked dopamine

measurements were obtained using the same paradigm used for in vitro measurements [3]. The magnitude of the stimulation-evoked dopaminergic responses was obtained by measuring oxidation and reduction current peaks. Each CFM was calibrated using the injection system to deliver a solution of 1 μ M dopamine and Tris buffer, and measuring the oxidation and reduction currents via WINCS Harmoni with respect to the Ag/AgCl reference electrode.

VII. Results and Discussion

WINCS Harmoni leveraged the spatial resolution and signal specificity of FSCV to allow identification of specific neurotransmitters such as dopamine (Figure 5). Flow injection analysis showed that WINCS Harmoni was capable of detecting in vitro dopamine (Figure 5A) at the expected oxidation and reduction peaks of approximately +0.53V and -0.2 V, respectively. Here, the magnitudes of the dopamine oxidation and reduction current peaks detected by WINCS Harmoni were proportional to the amount of dopamine undergoing redox reactions at the electrode surface. In addition to in vitro detection of neurotransmitters, WINCS Harmoni was capable of detecting striatal dopamine release evoked by electrical stimulation of the rodent MFB (Figure 5B). For this, WINCS Harmoni provided independent but synchronized electrical stimulation and measurement of neurochemical analytes. Expected dopamine oxidation and reduction current peaks were observed for 2-second bursts of high frequency (90 Hz) stimulation near 0.6 V and -0.2 V, respectively. The presence of dopamine was verified by administration of the dopamine reuptake inhibitor nomifensine (20 mg/kg).

The integration of the hardware for stimulation and neurochemical recording into a single system enabled the interleaving of stimulation with FSCV recording. As a result, the impact of stimulation artifact on the measurement and characterization of low-amplitude FSCV responses was minimized by controlling where the stimulation artifact appears on the voltammogram (Figure 5B). This ensures that stimulation artifacts do not overlap with the signals of interest.

Both in vitro and in vivo testing demonstrate that WINCS Harmoni is capable of providing subsecond detection of neurochemical release (Figure 5), which may provide new insight into the cellular and molecular mechanisms of therapeutic neuromodulation. The present proof of principle study demonstrates application of WINCS Harmoni as an investigational platform for wireless multichannel neurochemical sensing and synchronized stimulation. WINCS Harmoni provides a unique research platform capable of collecting and integrating neurochemical information to characterize and modulate neural activity, both locally and globally, for improving understanding of neural circuitry in both healthy and diseased states.

VIII. Concluding Remarks

Normal brain function relies on a precise and delicate balance of neurochemical signaling mediated by the release of specific neurotransmitters. When the neurochemical balance is disrupted as is the case in neurologic and psychiatric disorders, the results can be devastating. Conventional neuromodulation systems have successfully achieved therapeutic

outcomes in patients suffering from these disorders. However, limitations in the understanding of neurochemical signaling in normal and diseased states make reaching optimal benefits a difficult and expensive endeavor. WINCS Harmoni is a versatile research platform designed for studying neurochemical signaling in healthy and diseased conditions. Here, we have shown that WINCS Harmoni is capable of measuring neurochemical signaling in animal models of brain stimulation. This device is currently under evaluation for implementing closed-loop control of neurochemical responses to DBS. The pre-clinical, and eventually clinical, use of research platforms like the one described herein will enable the characterization of neurochemical processes underlying the therapeutic responses to stimulation therapies. In turn, this may lead to the development of advanced personalized therapies for the treatment of a wide range of neurologic and psychiatric disorders.

Acknowledgments

The development of WINCS Harmoni by the Mayo Clinic Division of Engineering (DOE) and the Mayo Clinic Neural Engineering Laboratories (NEL) was supported in part by The Grainger Foundation.

References

1. Bucher ES, Wightman RM. Electrochemical Analysis of Neurotransmitters. *Annu Rev Anal Chem.* 2015; 8:239–261.
2. Kimble CJ, et al. Wireless Instantaneous Neurotransmitter Concentration Sensing System (WINCS) for intraoperative neurochemical monitoring. *Conf Proc IEEE Eng Med Biol Soc.* 2009:4856–4859. [PubMed: 19963865]
3. Chang SY, et al. Wireless fast-scan cyclic voltammetry to monitor adenosine in patients with essential tremor during deep brain stimulation. *Mayo Clin Proc.* Aug; 2012 87(8):760–765. [PubMed: 22809886]
4. Chang SY, et al. Development of the Mayo Investigational Neuromodulation Control System: toward a closed-loop electrochemical feedback system for deep brain stimulation. *J Neurosurg.* Dec; 2013 119(6):1556–1565. Erratum in: *J. Neurosurg.*, vol. 120(5), p. 1258, May 2014. [PubMed: 24116724]
5. Bennet KE, et al. A diamond-based electrode for detection of neurochemicals in the human brain. *Front Hum Neurosci.* Mar.2016 10 art. 102.
6. Horowitz, P., Hill, W. *The Art of Electronics*. 2nd. Cambridge, MA: Cambridge University Press; 1989.
7. Kuncel AM, Grill WM. Selection of stimulus parameters for deep brain stimulation. *Clin Neurophysiol.* Nov; 2004 115(11):2431–2441. [PubMed: 15465430]
8. Lee KH, et al. WINCS Harmoni: Closed-loop dynamic neurochemical control of therapeutic interventions. *Scientific Reports.* submitted for publication.
9. Jang DP, et al. Paired pulse voltammetry for differentiating complex analytes. *Analyst.* 2012; 137(6):1428–1435. [PubMed: 22299131]
10. Paxinos, G., Watson, C. *The Rat Brain in Sterotaxic Coordinates*. 6th. Cambridge, MA: Academic Press; 2006.

Figure 1. Exploded view of the WINCS Harmoni hardware, highlighting the custom Σ ADC integrated circuit.

Figure 2.
WINCS Harmoni system functional/interface diagram.

Figure 3.
WINCS Harmoni hardware block diagram.

Figure 4.
WINCS stimulator block diagram.

Figure 5.

Dopaminergic responses measured in vitro and in vivo using WINCS Harmoni and fast-scan cyclic voltammetry (FSCV). (A) Pseudo-color plot measured for a 1 μ M dopamine injection. The black horizontal line shows the oxidation current at 0.53 V. (B) Pseudo-color plot showing dopamine oxidation and reduction evoked by electrical stimulation of the medial forebrain bundle (MFB) in a healthy rat. The black horizontal line shows the peak oxidation current at 0.6 V. (C) Peak oxidation current as a function of time measured at the potential described by the black dotted line in (A). Inset shows the background subtracted voltammogram (current vs. voltage) recorded at the vertical (grey) dotted line shown in (A). (D) Peak oxidation current as a function of time measured at the potential described by the black dotted line in (B). Inset shows the background subtracted voltammogram (current vs. voltage) recorded at the vertical (grey) dotted line shown in (B).

Table 1

Range of stimulation parameters used to evoke dopaminergic responses.

Frequency (Hz)	Pulse width (ms)	Amplitude (mA)	Duration (s)
60 – 100	0.1 – 2.0	0.01 – 0.80	1 – 2