31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Wireless Instantaneous Neurotransmitter Concentration Sensing System (WINCS) for Intraoperative Neurochemical Monitoring

Christopher J. Kimble, *Senior Member, IEEE*, David M. Johnson, *Member, IEEE*, Bruce A. Winter, Sidney V. Whitlock, Kenneth R. Kressin, April E. Horne, Justin C. Robinson, *Student Member, IEEE,* Jonathan M. Bledsoe, Susannah J. Tye, Su-Youne Chang, Filippo Agnesi, Christoph J. Griessenauer, Daniel Covey, Young-Min Shon, Kevin E. Bennet, Paul A. Garris, Kendall H. Lee

***Abstract*—The *Wireless Instantaneous Neurotransmitter Concentration Sensing System* (WINCS) measures extracellular neurotransmitter concentration *in vivo* and displays the data graphically in nearly real time. WINCS implements two electroanalytical methods, fast-scan cyclic voltammetry (FSCV) and fixed-potential amperometry (FPA), to measure neurotransmitter concentrations at an electrochemical sensor, typically a carbon-fiber microelectrode. WINCS comprises a battery-powered patient module and a custom software application (WINCSware) running on a nearby personal computer. The patient module impresses upon the electrochemical sensor either a constant potential (for FPA) or a time-varying waveform (for FSCV). A transimpedance amplifier converts the resulting current to a signal that is digitized and transmitted to the base station via a Bluetooth® radio link. WINCSware controls the operational parameters for FPA or FSCV, and records the transmitted data stream. Filtered data is displayed in various formats, including a background-subtracted plot of sequential FSCV scans—a representation that enables users to distinguish the signatures of various analytes with considerable specificity. Dopamine, glutamate, adenosine and serotonin were selected as analytes for test trials. Proof-of-principle tests included *in vitro* flow-injection measurements and *in vivo* measurements in rat and pig. Further testing demonstrated basic functionality in a 3-Tesla MRI unit. WINCS was designed in compliance with consensus standards for medical electrical device safety, and it is anticipated that its capability for real-time intraoperative monitoring of neurotransmitter release at an implanted sensor will prove useful for advancing functional neurosurgery.**

This work was supported in part by The Grainger Foundation, NIH (K08 NS 52232 award to KHL), Mayo Foundation (2008-2010 Research Early Career Development Award for Clinician Scientists award to KHL), the American Australian Association (Sir Keith Murdoch Fellowship to SJT), and the Austrian Academy of Sciences (neurosurgery fellowship award to CJG).

C. J. Kimble is with the Division of Engineering, Mayo Clinic, Rochester, MN 55905 USA (507-284-9972; ckimble@mayo.edu).

D. M. Johnson, B. A. Winter, S. V. Whitlock, K. R. Kressin, A. E. Horne, J. C. Robinson and K. E. Bennet are with the Division of Engineering, Mayo Clinic. J. C. Robinson is a student in the Department of Electrical and Computer Engineering, Iowa State Univ., Ames, IA 50011.

J. M. Bledsoe, S. J. Tye, S. Chang, C. J. Griessenauer, D. Covey, Y. Shon, P. A. Garris and K. H. Lee are with the Department of Neurologic Surgery, Mayo Clinic. Y. Shon is on leave from Catholic Medical Center, Seoul, South Korea. P. A. Garris is on sabbatical from the Department of Biological Sciences, Illinois State University, Normal, IL 61790 USA, where D. Covey is a graduate student.

F. Agnesi is with the Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN 55905 USA.

I. Introduction

A Wireless Instantaneous Neurotransmitter Concentration Sensing System (WINCS) has been developed at Mayo Clinic to measure the extracellular concentration of certain neurotransmitters *in vivo*. Although WINCS is expected to find use in animal research, it has been designed with patient safety in mind, for intraoperative use in a future study (wireless connectivity facilitates patient safety and minimizes clutter in the crowded operating room). WINCS comprises a compact "patient module" (Fig. 1) and custom software (WINCSware) running on a nearby PC.

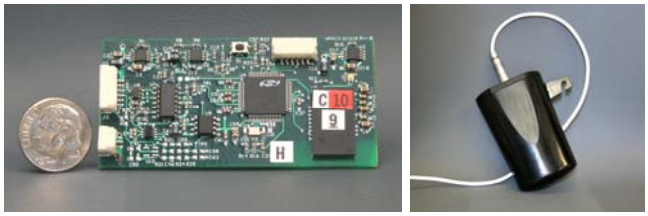

Fig. 1. WINCS Patient Module printed circuit board & sterilizable case.

WINCS measures neurotransmitter concentrations by employing two electroanalytical methods—fast-scan cyclic voltammetry (FSCV) and fixed-potential amperometry (FPA)—with an electrochemical sensor, typically a carbon-fiber microelectrode (CFM). Enzyme-linked microelectrodes can be used for neurotransmitters that are not electroactive (e.g., glutamate). In FPA, a constant potential is impressed upon the electrochemical sensor (with respect to a reference electrode), whereas in FSCV, as shown in Fig. 2, a time-varying waveform is applied, such as a sequence of pyramidal excursions (or "scans") from a baseline potential [1]. Oxidation and reduction of a given electroactive species adjacent to the surface of the working electrode (i.e., the electrochemical sensor) are supported at specific applied potentials. For example, dopamine is oxidized at an applied potential of approximately +0.6 V, whereas its oxidation product, dopamine-ortho-quinone, undergoes reduction back to dopamine at approximately -0.2 V [1]. During oxidation, dopamine molecules each release two electrons, producing a current proportional to the concentration of the dopamine molecules adjacent to the surface of the working electrode.

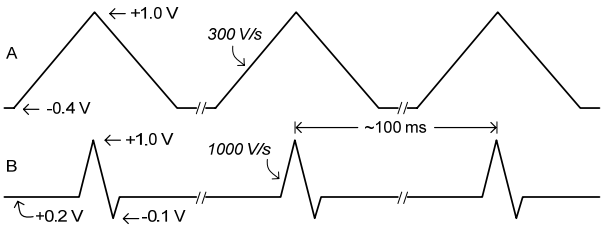

Fig. 2. Typical FSCV waveforms for dopamine (A) and serotonin (B).

Conversely, a reverse current is produced by the dopamine-ortho-quinone molecules undergoing reduction back to dopamine. The accurate measurement of these currents is the essential function of electroanalytical instruments such as WINCS.

Although FPA and FSCV are both highly sensitive techniques, FSCV offers greater specificity when it comes to resolving the electroactive chemical species adjacent to the working electrode. Whereas FPA can measure only oxidation current, FSCV measures oxidation current during the ascending sweep of the pyramidal scan, and reduction current during the negative sweep, creating two distinct current peaks in the resulting cyclic voltammogram (CV), a parametric plot of current versus applied potential, as illustrated by the plot on the right in Fig. 3. These peaks are clearly evident in a three-dimensional plot of an extended series of sequential voltammograms (Fig. 4), a plot which displays current (represented by a color scale) with respect to both applied potential (ordinate) and time (abscissa). Exquisite chemical resolution is afforded by these representations of the data.

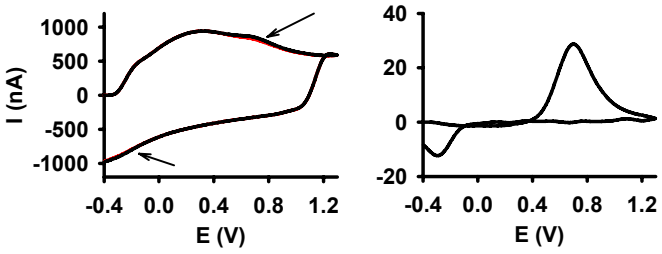

Fig. 3. Left: Cyclic voltammogram at a CFM, without background subtraction; the arrows indicate dopamine redox current components (shown in red). Right: Background-subtracted cyclic voltammogram for dopamine. Note the disparate ordinate scales.

Substantial capacitance can be associated with the electrochemical sensor, due to the double layer of charge at the electrode-electrolyte interface, and significant "background" current is produced when a time-varying potential is applied to such a capacitive sensor. As illustrated by the plot on the left in Fig. 3, the background current is overwhelmingly large. It must be disallowed, so that the electrochemical currents of interest can be discerned in the CV and color plot. Background subtraction is thus a critical function implemented by the WINCSware application.

Due to the small size of the electrochemical sensor, *in vivo* spatial resolution is a useful attribute of FPA and FSCV by WINCS.

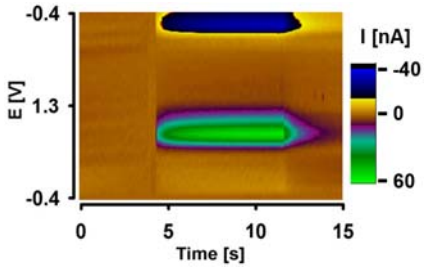

Fig. 4. Three-dimensional plot of sequential background-subtracted CVs at a CFM in a flow cell, for an ~8-second dopamine injection.

II. WINCS Hardware

The WINCS patient module incorporates front-end analog circuitry, a mixed-signal microcontroller and a Bluetooth® radio, all packaged on a small (3.3 cm × 6.5 cm) printed circuit board (PCB). The PCB and a 740-mAh Ultralife UBP005 lithium-ion battery (Ultralife Batteries, Inc., Newark, NY 14513 USA) are housed in a hermetically sealed polycarbonate enclosure that can be safely sterilized by the Sterrad gas plasma process (Advanced Sterilization Products, Irvine, CA 92618 USA).

Figure 5 presents a functional block diagram of the WINCS patient module. The WINCS front-end analog circuitry is similar to that of an instrument developed for neurochemical studies of laboratory rats [2]. An LMV751 low-noise op amp (National Semiconductor Corp., Santa Clara, CA 95052 USA) serves as a current-to-voltage transducer, effecting a transimpedance gain of 2E6 (the gain can be changed by replacing a chip resistor). The potential to be impressed upon the electrochemical sensor—a sequence of pyramidal excursions for FSCV, or a fixed potential for FPA—is applied to the noninverting input of the transimpedance amplifier. Feedback action causes the inverting terminal to follow this voltage, thereby also impressing it upon the electrochemical sensor.

The applied potential also shows up as a component of the transimpedance amplifier output, but it is subtracted from this signal by the second-stage, unity-gain INA2132U differential amplifier (Texas Instruments Inc., Dallas, TX 75266 USA), whose output is digitized after lowpass filtering by a 19.4-kHz RC filter. The INA2132U incorporates a laser-trimmed precision resistor network.

The C8051F061 mixed-signal 8-bit microcontroller (Silicon Laboratories, Inc., Austin, TX 78701 USA) generates the potential applied to the electrochemical sensor, digitizes the transimpedance signal and controls the flow of data to the base station. The microcontroller has 64 kB of nonvolatile (flash) memory and 4352 bytes of data RAM.

So that a wider range of applied potentials can be applied to the electrochemical sensor, a digital potentiometer (Analog Devices, Inc., Norwood, MA 02062 USA) has been employed to adjust the voltage of the reference electrode. One of the C8051F061's two 12-bit digital-to-analog converters (DACs) is now being utilized for this purpose, permitting the reference electrode voltage (with respect to

analog common) to be adjusted over a 2.5-V range. A fixed reference electrode voltage would limit the extent of the FSCV potentials that could be applied without saturating the transimpedance amplifier or exceeding the output capability of the DAC that produces the FSCV waveform or amperometric potential.

One of the microcontroller's two 16-bit analog-to-digital converters (ADCs) is used for FPA and FSCV signal acquisition. Noise limits the effective resolution of the ADC: nominally the smallest measurement increment for the 16-bit ADC would be 19 pA across the full-scale range of ±625 nA for FSCV, but under ideal conditions WINCS achieves an effective resolution of 14 bits, equivalent to a noise σ of ~75 pA.

The microcontroller communicates with the LMX9838 Bluetooth serial port module (National Semiconductor Corp., Santa Clara, CA 95052 USA) via a 921,600-baud UART (universal asynchronous receiver transmitter) connection. The LMX9838 is an FCC-licensed, single-chip Bluetooth-2.0 implementation. Crystal and antenna are both integrated. Bluetooth technology operates in the unlicensed Industrial, Scientific and Medical (ISM) band at 2.400 GHz to 2.485 GHz, using a spread-spectrum, frequency-hopping, full-duplex signal. A USB-connected Bluetooth adapter on the base-station computer completes the telemetry link.

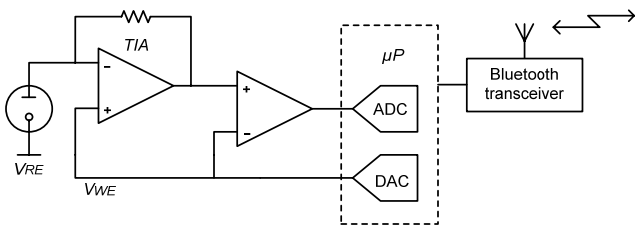

Fig. 5. WINCS patient module functional block diagram.

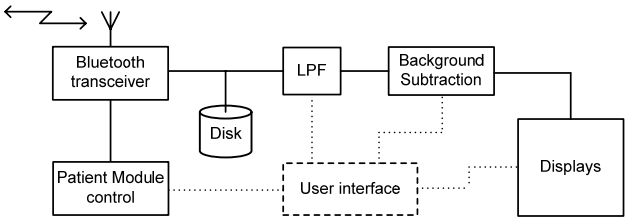

Fig. 6. WINCSware and base station functional block diagram.

III. WINCS Software

The base-station computer is a Windows-XP laptop or workstation running custom software—WINCSware—that controls the parameters and operation of the WINCS patient module, such as starting and stopping data acquisition and transmission, modifying the applied potential waveform, and changing the sampling rate.

In addition to controlling the patient module, WINCSware is responsible for saving, conditioning and displaying the transmitted data, all in nearly real time. All controls and configuration options are presented by the WINCSware graphical user interface. A simplified block diagram is presented in Fig. 6.

The user is afforded considerable flexibility in defining the applied waveform for FSCV. The baseline can be set at any 10-mV step within a range of -1 V to +1 V, and the waveform peak can be likewise set within its range of 0 V to +2 V. The ramp rate (slope) range is 100 V/s to 1000 V/s. For serotonin studies, a three-segment "N" waveform (Fig. 2) may be selected [3]. The sampling rate range for FSCV is 50 ksps to 100 ksps. Impractical combinations of parameter selections are disallowed by the user interface. For FPA, the user may select an applied potential between -1 V and +1 V. A 1-ksps sampling rate is typically used for FPA.

To filter the incoming data stream, WINCSware incorporates class libraries from National Instruments Measurement Studio (National Instruments Corp., Austin TX 78759 USA), an integrated suite of measurement and automation controls, tools, and class libraries. Selectable lowpass filter parameters include filter family (Butterworth, Chebyshev, elliptic), order (1 to 8) and cutoff frequency (1 Hz to 10 kHz). Filtering is done for display purposes only; unfiltered raw data are saved to the computer hard drive as a sequence of unsigned two-byte integers in a binary file.

A key process required for intelligible data display is background subtraction [1]. The current produced by a time-varying voltage that is impressed upon the capacitive sensor would overwhelm the relatively tiny oxidation-reduction currents that we seek to measure, so it must be removed. WINCSware enables the user to select a particular scan (one of the pyramidal excursions of the applied potential) as the background-subtraction locus. The array of data samples acquired during this scan is then subtracted from the arrays of data samples produced by all successive scans.

Optionally, several scans preceding the background-subtraction locus may be averaged together to generate the background-subtraction subtrahend. Sensor properties tend to drift during an experiment due to the ongoing dynamics of CFM surface chemistry, so users find it advantageous to select a scan immediately preceding a stimulus event as the background-subtraction locus.

Perhaps the most notable capability of WINCSware is its nearly real-time graphical display of the live data stream. For FSCV, four useful plots are presented:

1) Raw cyclic voltammogram (CV): A parametric plot of the "raw" sensor current versus applied voltage during an individual scan, displayed on a scan-by-scan basis (Fig. 3, plot on the left).
2) Background-subtracted CV: A parametric plot of the background-subtracted current versus applied voltage, displayed on a scan-by-scan basis (Fig. 3, right plot).
3) Sequential CV: A three-dimensional plot of sequential scans, with current represented by a color scale (Figs. 4 and 7). Each vertical column of pixels presents the data

acquired during one scan, with respect to the applied potential (ordinate) and time (abscissa). Researchers skilled in the art can readily recognize the distinct signatures of various chemical species in the colorful sequential-CV plot. The plot also offers the user a convenient interface for defining the background-subtraction locus, selected by positioning the cursor at the desired location and depressing a mouse button. The color map may be rescaled to accommodate the full current range for a particular scan by a similar cursor operation.

4) Strip chart: A rolling plot of the background-subtracted current measurements acquired during successive scans at a user-specified voltage on each ascending FSCV ramp is under development. Generally the oxidation voltage of the neurotransmitter under study is selected (by means of a control on the sequential-CV plot); the strip chart then displays the transient changes in the concentration of that neurotransmitter. For FPA, the strip chart simply displays the sequential sensor current measurements.

A current-versus-time strip chart is the only plot that is presented for FPA. Various configuration options are offered for the plots. Future features will facilitate replaying saved data and exporting plot contents (e.g., the sequential CV plot can be exported as a bitmap file for publication or presentation purposes).

WINCSware is a multithreaded .NET application written in C# with Microsoft Visual Studio .NET 2005 (Microsoft Corp., Redmond, WA 98052 USA) and Franson BlueTools (Franson Technology AB, Stockholm, Sweden). The embedded patient module application was written in C with the Keil µVision integrated development environment (Keil Software Inc., Plano, TX 75074 USA).

IV. In vitro and in vivo Testing of WINCS

WINCS has produced reliable, high-fidelity FSCV measurements of dopamine, serotonin and adenosine using flow-injection analysis with a CFM (Figs. 4 and 7). Moreover, WINCS has detected subsecond striatal dopamine and adenosine release at an implanted sensor during high-frequency stimulation of ascending fibers in the *in vivo* rat, and it has detected serotonin release in the raphe nucleus using rat brain slice [unpublished results]. WINCS has also performed amperometric measurements of adenosine and glutamate (Fig. 8) using biosensors [4].

In vitro and *in vivo* testing has demonstrated comparable signals to a conventional electrochemical instrumentation for both FSCV and amperometry [5]. Notably, we have also demonstrated the basic functionality of WINCS in the bore of a 3-Tesla MRI unit, suggesting the potential to integrate electrochemical and anatomic information in nearly real time [unpublished results]. Preliminary results indicate that median filtering effectively cleans up the RF artifact.

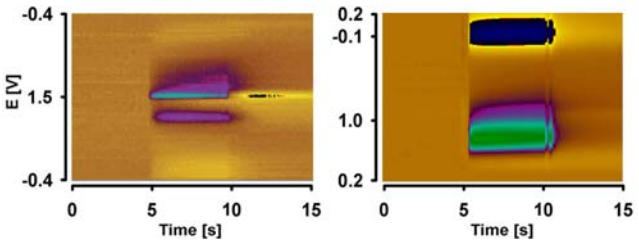

Fig. 7. Left: Three-dimensional plots of sequential background-subtracted CVs for adenosine (right) and serotonin (left). The color scale spans -13 nA to +20 nA for adenosine, and -100 nA to +160 nA for serotonin.

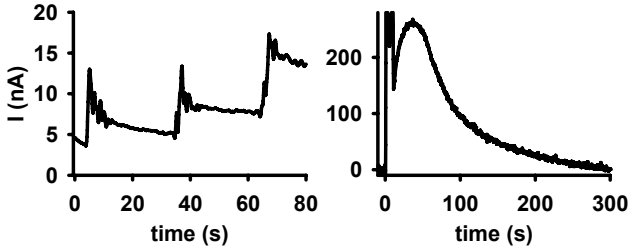

Fig. 8. Left: Calibration sequence for glutamate amperometry; the plateaus correspond to increasing concentrations of the analyte. Right: evoked glutamate response in pig motor cortex.

V. Conclusion

WINCS has demonstrated its effectiveness as a versatile research tool for *in vivo* neurotransmitter studies. Its ability to present live data graphically in nearly real time enhances its utility for both research and clinical applications.

Continuing development efforts at Mayo Clinic focus on improving performance and adding functionality, including electrophysiology recording and stimulation capability. We hope that a future version of WINCS will facilitate the placement of deep-brain stimulating (DBS) electrodes, or even provide feedback for DBS neuromodulation [6].

References

[1] D. L. Robinson, B. J. Venton, M. L. Heien, R. M. Wightman, "Detecting subsecond dopamine release with fast-scan cyclic voltammetry in vivo," *Clinical Chemistry*, vol. 49, no. 10, pp. 1763-1773, Oct. 2003.

[2] P. A. Garris, R. Ensman, J. Poehlman, A. Alexander, et al., "Wireless transmission of fast-scan cyclic voltammetry at a carbon-fiber microelectrode: proof of principle," *J. Neuroscience Methods*, vol. 140, pp. 103-115, 2004.

[3] B. P. Jackson, S. M. Dietz and R. M. Wightman, "Fast-scan cyclic voltammetry of 5-hydroxytryptamine," *Analytical Chemistry*, Vol. 67, No. 6, pp. 1115-1120, March 15, 1995.

[4] F. Agnesi, S. J. Tye, J. M. Bledsoe, et al., "Wireless Instantaneous Neurotransmitter Concentration System (WINCS)-based amperometric detection of dopamine, adenosine, and glutamate for intraoperative neurosurgical monitoring," *J. Neurosurgery* (in press).

[5] J. M. Bledsoe, C. J. Kimble, D. P. Covey, C. D. Blaha, et al., "Development of Wireless Instantaneous Neurotransmitter Concentration System (WINCS) for intraoperative neurochemical monitoring using fast-scan cyclic voltammetry," *J. Neurosurgery* (in press).

[6] K. H. Lee, C. D. Blaha, P. A. Garris, P. Mohseni, et al., "Evolution of deep brain stimulation: human electrometer and smart devices supporting the next generation of therapy," *Neuromodulation*, Vol. 12, No. 2, April, 2009 (in press).