Published in final edited form as:

J Neurosurg. 2010 March ; 112(3): 539–548. doi:10.3171/2009.7.JNS09787.

Comonitoring of adenosine and dopamine using the Wireless Instantaneous Neurotransmitter Concentration System: proof of principle

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Abstract

Object—The authors of previous studies have demonstrated that local adenosine efflux may contribute to the therapeutic mechanism of action of thalamic deep brain stimulation (DBS) for essential tremor. Real-time monitoring of the neurochemical output of DBS-targeted regions may thus advance functional neurosurgical procedures by identifying candidate neurotransmitters and neuromodulators involved in the physiological effects of DBS. This would in turn permit the development of a method of chemically guided placement of DBS electrodes in vivo. Designed in compliance with FDA-recognized standards for medical electrical device safety, the authors report on the utility of the Wireless Instantaneous Neurotransmitter Concentration System (WINCS) for real-time comonitoring of electrical stimulation–evoked adenosine and dopamine efflux in vivo, utilizing fast-scan cyclic voltammetry (FSCV) at a polyacrylonitrile-based (T-650) carbon fiber microelectrode (CFM).

Methods—The WINCS was used for FSCV, which consisted of a triangle wave scanned between −0.4 and +1.5 V at a rate of 400 V/second and applied at 10 Hz. All voltages applied to the CFM were with respect to an Ag/AgCl reference electrode. The CFM was constructed by aspirating a single T-650 carbon fiber ($r = 2.5 \mu\text{m}$) into a glass capillary and pulling to a microscopic tip using a pipette puller. The exposed carbon fiber (the sensing region) extended beyond the glass insulation by $\sim 50 \mu\text{m}$. Proof of principle tests included in vitro measurements of adenosine and dopamine, as well as in vivo measurements in urethane-anesthetized rats by monitoring adenosine and dopamine efflux in the dorsomedial caudate putamen evoked by high-frequency electrical stimulation of the ventral tegmental area and substantia nigra.

Results—The WINCS provided reliable, high-fidelity measurements of adenosine efflux. Peak oxidative currents appeared at +1.5 V and at +1.0 V for adenosine, separate from the peak oxidative current at +0.6 V for dopamine. The WINCS detected subsecond adenosine and dopamine efflux in the caudate putamen at an implanted CFM during high-frequency stimulation of the ventral tegmental

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area and substantia nigra. Both in vitro and in vivo testing demonstrated that WINCS can detect adenosine in the presence of other easily oxidizable neurochemicals such as dopamine comparable to the detection abilities of a conventional hardwired electrochemical system for FSCV.

Conclusions—Altogether, these results demonstrate that WINCS is well suited for wireless monitoring of high-frequency stimulation-evoked changes in brain extracellular concentrations of adenosine. Clinical applications of selective adenosine measurements may prove important to the future development of DBS technology.

Keywords

deep brain stimulation; neuromodulation; neurotransmitter; voltammetry; adenosine

Deep brain stimulation is an effective neurosurgical approach to the treatment of tremor, Parkinson disease, epilepsy, and refractory psychiatric illness.^{3,13,15,23–25,29} Despite a continuing increase in the number of individuals receiving this therapy, the cellular mechanisms involved in the therapeutic effects of DBS remain unknown. Bekar et al.² have previously shown that thalamic DBS is associated with a marked increase in the local efflux of ATP and extracellular accumulation of the neuromodulator catabolic product adenosine. It has further been demonstrated that intrathalamic infusions of A1 adenosine receptor agonists directly reduce tremor, a finding that implicates adenosinergic mechanisms in tremor control. Together, these findings suggested that DBS-mediated stimulation of endogenous adenosine efflux represents an important neurochemical mechanism of action of DBS in the treatment of thalamic-mediated tremor. Monitoring adenosine efflux elicited by DBS may thus serve to advance functional neurosurgery by providing a method for investigating the mechanism of action of thalamic DBS.^{18,22}

We have previously described the development of a new device, WINCS, for in vivo monitoring of the catecholamine neurotransmitter dopamine using FSCV in combination with a CFM⁴ as well as glutamate and adenosine using fixed potential amperometry in combination with enzyme-based electrochemical sensors.¹ Unfortunately, the response time to changes in adenosine concentrations is relatively slow (~ 2 seconds) for commercially available enzyme-based biosensors compared with CFMs (subsecond). This delay occurs because the amperometric detection of adenosine requires interaction with 3 active entrapped enzymes (adenosine deaminase, nucleoside phosphorylase, and xanthine oxidase) on the sensor surface.²¹ As an alternative, Venton's laboratory has recently demonstrated the in vitro and in vivo detection of adenosine at CFMs using FSCV.^{9,28} One major advantage of this state-of-the-art electroanalytical procedure is that subsecond temporal resolution can be achieved, making it the fastest method available for measuring changes in extracellular concentrations of adenosine. A second advantage is minimization of tissue damage and accompanying high spatial resolution as CFM electrode dimensions (typically 50–250 μm length of 5–10- μm outer diameter carbon fiber) are a fraction of the size of commercially available adenosine biosensors (typically 1–2 mm of 200–300- μm outer diameter enzyme-coated platinum wire).

The WINCS supports FSCV at a CFM to provide superior temporal and spatial resolution, including chemical specificity.⁷ In a series of proof-of-principle tests in the present study, we demonstrate the utility of an operating room-compatible wireless electrochemical monitoring system for both in vitro and in vivo codetection of adenosine and dopamine. Together, our results suggest the utility of WINCS as a versatile intraoperative tool for elucidating the role of adenosine, as well as a range of other electroactive neurotransmitters and neuromodulators involved in the therapeutic mechanism of action of DBS.

Methods

Animal Care

Ten adult male Sprague-Dawley rats (Harlan, Inc.), weighing 250–330 g, were used for in vivo testing. The rats were housed under standard conditions, with ad libitum access to food and water. Care was provided in accordance with the National Institutes of Health guidelines (publication 86-23) and approved by the Mayo Clinic Institutional Animal Care and Use Committee.

Fast-Scan Cyclic Voltammetry Using WINCS

We have previously described the development of the WINCS hardware that combines FSCV with digital telemetry to perform real-time electrochemical measurements at an implanted CFM.⁴ Briefly, WINCS incorporates front-end analog circuitry for FSCV, a microprocessor, and Bluetooth radio, all on a single rechargeable lithium-polymer battery-powered, multilayer, printed circuit board (Fig. 1A) that is hermetically sealed in a polycarbonate case, permitting sterilization using the Sterrad gas plasma process.

In FSCV mode, WINCS uses a transimpedance amplifier to convert current to voltage, and a difference amplifier to subtract a triangular waveform potential applied to a CFM prior to signal digitization. A digital-to-analog converter applies the FSCV waveform to the CFM, and an analog-to-digital converter samples FSCV at a rate of 100 kilosamples per second. Bluetooth 2.4–2.5 GHz digital telemetry is used to wirelessly communicate between WINCS and a base-station computer running Windows XP. Custom software controls WINCS parameters and operation, such as data acquisition and transmission, the applied potential waveform, and data-sampling rate. Data, in the form of a sequence of unsigned 2-byte integers, are saved to the base-station computer hard drive for offline processing either with MATLAB (The MathWorks, Inc.) or LabVIEW software (National Instruments Corp.).

As shown in Fig. 1B, FSCV consists of a linear potential ramp applied to the CFMs every 0.1 second between -0.4 and $+1.5$ V at a scan rate of 400 V/second.²⁸ Under these conditions, the duration of the potential waveform scan in which the analyte is measured is 9.5 msec. The CFM is held at a bias potential of -0.4 V between scans. The CFM was constructed by aspirating a single polyacrylonitrile-based carbon fiber (T650, Amoco, Inc.; 5- μ m diameter) into a borosilicate glass capillary and pulling it into a microscopic tip using a pipette puller.⁸ The exposed carbon fiber is then cut to a length of ~ 50 μ m with the aid of a dissecting microscope. The Ag/AgCl reference electrodes were prepared by chloridizing a 31-g Teflon-coated silver wire (World Precision Instruments, Inc.).¹²

Flow Injection Analysis

Flow injection analysis was used for in vitro FSCV calibration of the CFMs in solutions of adenosine and dopamine.¹⁶ Briefly, single CFMs were placed in a flowing stream of buffer solution (150-mM NaCl and 12-mM Tris-base buffer at a pH 7.4) pumped at a rate of 2 ml/minute, and 1 ml of analyte (adenosine, dopamine, or a combination) was injected as a bolus. An Ag/AgCl reference electrode was located in the bottom of the reservoir and immersed in buffer solution. A 1-ml electronic loop injector, locally fabricated, introduced the bolus of analyte for 5 or 10 seconds into the flowing stream at defined analyte test concentrations.

Chemicals Used

Adenosine and dopamine hydrochloride were purchased from Sigma-Aldrich. Adenosine was dissolved in pure water to make a 5-mM stock solution, and dopamine was dissolved with 1-mM perchloric acid to prevent air oxidation of the molecule. Adenosine and dopamine (10

mM) stock solutions were diluted with Tris-buffer solution right before the flow injection experiments were started. All dopamine solutions were kept in the dark.

In Vivo Experiments

The rats were anesthetized with intraperitoneal injections of urethane (1.6 g/kg) and placed in a stereotactic frame (David Kopf Instruments) with the incisor bar set at -3.3 mm in accordance with the rat brain atlas.²⁷ Body temperature was maintained at $36 \pm 0.5^\circ\text{C}$ using a temperature-regulated heating pad (TC-831, CWE, Inc.). Multiple craniectomies were performed for implanting the reference, stimulating, and recording electrodes. All coordinates, AP, ML, and DV, are given in millimeters, and the bregma, dura mater, and midline were used as reference points. A twisted bipolar stimulating electrode (insulated except for the tips, which were separated by 1 mm; MS 303/2, Plastics One, Inc.) was implanted into the region of the VTA/SN of the right hemisphere: AP -5.3 ; ML $+1.2$; DV -7.5 . The CFM was implanted ipsilaterally into the dorsomedial CPu: AP $+1.2$; ML $+2.5$; DV -4.5 . The Ag/AgCl reference electrode was placed in superficial cortical tissue contralateral to the stimulating electrode. The CFM was allowed to equilibrate in the brain for 30 minutes postimplantation to obtain a stable background charging current at the CFM.

Electrical stimulation was governed by an external programmable pulse generator and delivered by an optical isolation unit (Master-8/ISOFlex, AMPI). A stimulation train of 130 cathodal 300- μA monophasic square current pulses (2-msec pulse width) at 130 Hz was used. The potential waveform was applied via WINCS to the CFM around the time of stimulation (30 seconds before and 60–90 seconds after stimulation) every 10 minutes, and data were stored on a computer for offline analysis. From each stored data file, the peak current for adenosine at $+1.5$ V and for dopamine at $+0.6$ V was converted to a concentration using the averaged calibration values obtained for each CFM before or immediately after the experiment using flow injection analysis and known adenosine and dopamine standard solutions. At the completion of the experiments, the rats were killed by decapitation while under deep anesthesia.

Results

Detection of Adenosine by WINCS In Vitro

To demonstrate the ability of WINCS to measure rapid changes in adenosine concentrations in vitro and in vivo, we used FSCV and T-650 CFMs.¹ Figure 2 shows the ability of WINCS-applied FSCV at a CFM to measure adenosine at a known concentration (5 μM) via a bolus injection through the flow injection analysis system (in 20 trials). A pseudocolor plot was generated with the WINCS software in which time is plotted on the x axis and the applied electrode potential is plotted on the y axis. The resulting color gradient represents the current detected at the CFM (Fig. 2A). At $\sim +1.5$ and $+1.0$ V, a light-brown color representing background current can be seen to change to green and purple, respectively, for 10 seconds. This color change represents the peak oxidative currents generated by the injection of adenosine into the flow cell. Figure 2B shows that the initial cyclic voltammogram in the absence of adenosine produced a large background charging current due to double-layer capacitance at the CFM tip, while the faradaic (oxidation) current occurring immediately after injection of 5 μM adenosine at $+1.5$ V increased slightly above this background current (Fig. 2B *inset*).

Adenosine is known to undergo a series of 2-electron oxidations.¹¹ Each product formed is easier to oxidize, so oxidative potentials are less positive for each successive step. The following oxidation scheme has been identified previously,²⁸ with the first 2-electron oxidation of adenosine resulting in a peak near the switching potential, $+1.5$ V, and the second subsequent 2-electron oxidation product oxidizing near $+1.0$ V. As predicted by this oxidation series, the cyclic voltammogram obtained by WINCS revealed 2 oxidative peaks for adenosine: the first

peak near +1.5 V, and the second near +1.0 V. A current versus time plot of the first and second adenosine oxidative peak currents measured during adenosine injection into the flow cell is shown in Fig. 2C, and a representative background-subtracted cyclic voltammogram of these 2 oxidations is shown in Fig. 2D (based on 10 trials).

As shown in Fig. 3A, closer inspection of the current versus time plot collected with WINCS in Fig. 2C provided confirmation of a delay in the detection of the second oxidative peak for adenosine with respect to the first oxidative peak at +1.5 V. The current from the first peak (Fig. 3B) at the point of the *blue line* in Fig. 3A was already significantly elevated at least 0.5 seconds before the second oxidative peak, +1.0 V, achieved a maximal increase (Fig. 3C). When the scan was taken to +1.3 V, neither the first nor second adenosine oxidative peaks were observed (3 trials; data not shown), indicating that the appearance of the second peak is dependent on the formation of an adenosine oxidation product from the first peak. These findings are highly consistent with previous reports of FSCV recordings of adenosine oxidation at polyacrylonitrile-based T-650 CFMs.^{9,28}

Figure 4A shows the WINCS-recorded FSCV pseudocolor plots for increasing adenosine concentrations (0.5, 1.0, 5.0, and 10.0 μ M). As shown in Fig. 4B, a plot of the first and second peak oxidative currents versus adenosine concentration revealed a linear relationship over the range of adenosine concentrations tested (0.5–10 μ M; 3 injections/concentration) with linear correlation coefficients of 0.99 and 0.96, respectively.

Codetection of Adenosine and Dopamine by WINCS In Vitro

A particular advantage of FSCV at CFMs over amperometry at commercially available enzyme-coated biosensors (Sarissa Biomedical Ltd.; see Agnesi et al.¹) is that electroactive molecules exhibiting significantly different oxidation–reduction potentials can be simultaneously detected in a single voltammogram. As shown in Fig. 5A, flow cell analysis of dopamine (1.5 μ M) with WINCS-based FSCV resulted in a pseudocolor plot showing a typical oxidative peak at +0.6 V and reductive peak at –0.2 V.^{4,12} The cyclic voltammogram depicted in Fig. 5A clearly shows the oxidation–reduction potentials for dopamine using the present scanning parameters. In contrast, the pseudocolor plot and voltammogram for adenosine (5.0 μ M) show 2 oxidative currents at both +1.5 and +1.0 V (Fig. 5B).²⁸ In a pseudocolor plot and representative cyclic voltammogram of a combined injection of dopamine and adenosine at these concentrations, 3 distinct peaks corresponding to dopamine oxidation and the oxidation of adenosine (first peak) and its oxidation product (second peak) were clearly evident (Fig. 5C; 6 injections). In contrast to adenosine, the oxidation–reduction reaction of dopamine is somewhat reversible, with a reductive peak for dopamine occurring at –0.2 V (Fig. 5A and C). It is important to note that dopamine at higher concentrations could partially interfere with the detection of the second oxidative peak for adenosine.

Codetection of Adenosine and Dopamine by WINCS In Vivo

An example of VTA/SN stimulation-evoked adenosine and dopamine coefflux in the CPu of anesthetized rats is shown in the pseudocolor plot of Fig. 6A and the current versus time plot in Fig. 6B depicting a large stimulation artifact occurring at the time of stimulation, followed by an increase in adenosine efflux that reached peak values ($\sim 1.8 \mu$ M) within 1–1.5 seconds after stimulation and recovery to prestimulation baseline levels within 7–8 seconds after stimulation. As shown in Fig. 6C, a representative cyclic voltammogram taken at 1.4 seconds poststimulation clearly indicates a significant separation between peak oxidative potentials for dopamine and adenosine. A current versus time plot of dopamine oxidation also illustrates the stimulus time-locked dependency of dopamine efflux (Fig. 6D). However, of the total number of 87 stimulations applied to the VTA/SN in 10 rats, coefflux of adenosine and dopamine did not occur with every stimulation, but occurred less frequently (in 24 [28%] of 87 stimulations;

mean peak concentration $0.24 \pm 0.07 \mu\text{M}$ for dopamine and $1.91 \pm 0.12 \mu\text{M}$ for adenosine), compared with efflux of adenosine alone (51 [58%] of 87 stimulations; mean peak concentration $1.56 \pm 0.08 \mu\text{M}$). Likewise, sole evoked dopamine efflux occurred less frequently (in 12 [14%] of 87 stimulations; mean peak concentration $0.41 \pm 0.11 \mu\text{M}$) compared with the efflux of adenosine alone.

As shown in Fig. 7, VTA/SN stimulation-evoked adenosine occasionally exhibited a biphasic pattern of efflux (in 9 of 24 dopamine-adenosine codetected cases and in 22 of 51 cases with adenosine efflux alone, overall 41.3%). An example of this biphasic adenosine response is shown in Fig. 7, where the initial response was observed to begin ~ 0.5 seconds poststimulation, reach a maximal value ($\sim 1.4 \mu\text{M}$) within 1.5 seconds poststimulation (Fig. 7C and D), and decrease to $0.3 \mu\text{M}$ within 6 seconds of stimulation. Thereafter, a second slower and less intense increase in adenosine efflux appeared 8 seconds after stimulation, achieved a second maximal increase ($\sim 0.6 \mu\text{M}$) within 25 seconds poststimulation, and recovered to prestimulation baseline levels within ~ 40 seconds of stimulation (Fig. 7A and B).

Discussion

In the present study, we reported on the use of WINCS for wireless real-time spatially and chemically resolved monitoring of adenosine at a CFM using FSCV. We have previously proposed the WINCS device as a portable, multipurpose, and versatile research tool capable of intraoperative neuromonitoring.⁴ An important hypothesis emerging for the therapeutic mechanisms of DBS is that electrical stimulation releases neurotransmitters, as well as neuromodulators such as adenosine, locally at the target site and within interconnected pathways.^{19,20,26} The utility of the WINCS device we have described, and the concept of intraoperative neurochemical monitoring during functional neurosurgery, in general, are based on this neurotransmitter/modulator release hypothesis. For example, Bekar et al.² have previously shown that thalamic DBS is associated with a marked increase in the local efflux of ATP and extracellular accumulation of its catabolic product adenosine, and its relationship to tremor control. Therefore, by supporting real-time chemical measurements at an implanted microsensor, WINCS provides a practical tool for use in clinical investigations of the central mechanisms of DBS. Clinical application of WINCS-based adenosine measurements may prove important in achieving a better understanding of the neurochemical mechanisms of action of DBS.

Wireless Instantaneous Neurotransmitter Concentration System–Based FSCV of Adenosine and Dopamine In Vitro

Flow injection analyses demonstrated the capability of WINCS-based FSCV at polyacrylonitrile (T-650) CFMs to reliably quantify linear changes in adenosine concentrations in vitro. In pH 7.4 buffer solutions containing $0.5\text{--}10 \mu\text{M}$ adenosine, an initial scan revealed a cyclic voltammogram containing a single peak at $+1.5 \text{ V}$ corresponding to the oxidation of adenosine. On subsequent scans a second oxidative peak appeared at $+1.0 \text{ V}$, corresponding to oxidation of the first product of adenosine oxidation. These findings are consistent with adenosine undergoing a series of 2-electron oxidations, as described in the recent study by Swamy and Venton²⁸ utilizing similar fast-scan recording procedures and T-650 CFMs in vitro.

Similar to the in vitro findings of Swamy and Venton,²⁸ the T-650 CFM was also found to be insensitive to the adenosine metabolite inosine, and to be ~ 7 times more sensitive to adenosine than its metabolic precursors ATP and adenosine monophosphate (data not shown). As those investigators previously noted, this confers considerable advantage of FSCV coupled with CFMs over existing amperometric recording techniques using enzyme-based sensors that exhibit equivalent sensitivity to adenosine and inosine.²¹ Together, these findings demonstrate

that WINCS-based FSCV at the T-650 CFM is capable of relatively selective wireless detection of adenosine in an aqueous buffer medium.

Swamy and Venton²⁸ were the first to characterize the mechanism of adenosine oxidation at polyacrylonitrile-based T-650, as well as pitch-based P-55, CFMs, and flow injection analysis. Similar to the present study, oxidation of adenosine at T-650 CFMs resulted in only 2 oxidative peaks at +1.5 and +1.0 V. However, in the case of P-55 CFMs, 3 oxidative peaks (+1.5, +0.9, and +0.5 V) and 2 reductive peaks (+0.25 and -0.05 V) were detected. The third oxidative peak at +0.5 V was attributed to an additional series oxidation of the first oxidation product of adenosine, while the shift in the second peak potential and appearance of reductive peaks were considered to be the result of intrinsic differences in electron transfer properties between the 2 types of carbon fibers. This latter finding is of particular significance with respect to the detection of dopamine in the presence of adenosine using FSCV at specific CFMs in vivo, as the additional oxidation of adenosine at P-55 CFMs would probably interfere with the measurement of dopamine at the peak oxidative potential of +0.6 V.

Detection of Adenosine and Dopamine In Vivo With WINCS-Based FSCV

Given the potential interference of adenosine oxidation with the detection of dopamine at P-55 CFMs, we evaluated the capability of the WINCS device to codetect subsecond changes in adenosine and dopamine concentrations in vivo, utilizing a similar animal preparation to that described by Cechova and Venton.⁹ This consisted of implantation of a single T-650 CFM into the CPu of anesthetized rats and FSCV recordings during brief electrical stimulation of the ipsilateral VTA/SN. Under these conditions, 87 bouts of VTA/SN stimulation applied in 10 animals resulted in coefflux of adenosine and dopamine in the CPu 28% of the time, compared with only adenosine (58%) or dopamine efflux (14%). Unfortunately, we often observed a stimulation artifact during the stimulation period (Fig. 6A and B), making it difficult to observe the precise onset of the evoked adenosine and dopamine efflux. However, as demonstrated in Fig. 6, the peak dopamine and adenosine efflux appeared after the stimulation period, making it possible to discriminate the coefflux of these neurochemicals. A future version of WINCS is planned to contain an onboard electrical stimulator capable of stimulation pulses interleaved with the FSCV so that such stimulation artifacts can be eliminated.

Similar to the in vivo findings of Cechova and Venton,⁹ we observed a biphasic adenosine response in some cases, with evoked adenosine attaining an initial and relatively rapid peak increase, followed by a second slower peak increase and gradual recovery back to prestimulation baseline. Although the temporal pattern of the second increase in adenosine was comparable to that reported by Venton and coworkers (peak at ~ 25 seconds and recovery in 40 seconds), the initial response in adenosine we observed in the present study exhibited a more rapid time course (peak 0.5–1.5 vs 2–5 seconds, and recovery ~ 7 vs ~ 15 seconds), and on average a 2-fold greater peak increase in adenosine concentration than that reported by Cechova and Venton. These differences may be accounted for by differences in stimulation parameters used in the present study (130 pulses at 130 Hz; 2 msec wide at 300 μ A) compared with those used by these investigators (60 pulses at 60 Hz; 2 msec wide at 300 μ A).

Regardless, our findings in the present study confirms those of Cechova and Venton⁹ in that extracellular adenosine can be transiently increased by short bursts of neuronal activity in concentrations that can activate central adenosine receptors (see Latini and Pedata¹⁷). These results suggest the possibility that extracellular levels of adenosine may increase both locally and at sites distal to implanted DBS electrodes in human patients, although this requires further investigation. Taken together, these results demonstrate the utility of WINCS-based FSCV for wireless combined monitoring of adenosine and dopamine efflux in the brain, and highlight its capacity to assess the role of neurochemical efflux in the mechanisms of action of DBS.

Neuromonitoring and WINCS

Adenosine acting as an endogenous anticonvulsant⁶ has been further supported by recent research suggesting that gliosis-related neuronal cell loss may be causally linked to dysfunction of central adenosine systems that in turn provoke seizure activity.^{6,14} There are ongoing innovative studies developing adenosine-augmenting cell and gene therapies^{5,10} that could result in new, improved treatment options for patients suffering from intractable epilepsy. The WINCS in combination with FSCV at implanted CFMs could play a role in assessing the effectiveness of these novel interventions during trials because of its ability to render highly sensitive measurements of adenosine.

Conclusions

In the present study, we reported on the use of WINCS-based FSCV for wireless, real-time, spatially and chemically resolved monitoring of adenosine at a CFM. The WINCS provided reliable, high-fidelity FSCV measurements of extracellular concentrations of adenosine both in vitro and in vivo. With the present FSCV parameters, adenosine oxidation at a CFM occurred at +1.5 V making it possible to detect even in the presence of other electroactive neurochemical substances, such as catecholamines, that oxidize at lower potentials. These results demonstrate that WINCS is well suited to in vivo monitoring of adenosine, and clinical application of adenosine measurements may prove important in achieving a better understanding of the neurochemical mechanisms of action of DBS.

Acknowledgments

The authors acknowledge the contribution of the engineers of Division of Engineering of Mayo Clinic, April E. Horne, David M. Johnson, Kenneth R. Kressin, Justin C. Robinson, Sidney V. Whitlock, and Bruce A. Winter for their invaluable efforts in the realization of the WINCS device and software.

Disclosure: This work was supported by the NIH (K08 NS 52232 award to Dr. Lee), the Mayo Foundation (2008–2010 Research Early Career Development Award for Clinician Scientists award to Dr. Lee), and the Mathews Foundation (John T. and Lillian Mathews Professorship in Neuroscience). Dr. Tye was supported by an American Australian Sir Keith Murdoch Fellowship and a NARSAD Young Investigator Award.

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Abbreviations

AP	anteroposterior
ATP	adenosine triphosphate
CFM	carbon fiber microelectrode
CPu	caudate putamen
DBS	deep brain stimulation
DV	dorsoventral
FSCV	fast-scan cyclic voltammetry
ML	mediolateral
VTA/SN	ventral tegmental area and substantia nigra
WINCS	Wireless Instantaneous Neurotransmitter Concentration System

Fig. 1.
A: Photograph of WINCS printed circuit board. **B:** Diagram of an FSCV waveform for measuring adenosine and dopamine, where the applied potential is from -0.4 V to $+1.5$ V and back to -0.4 V at a rate of 400 V/second every 100 msec.

Fig. 2.

Plots showing wireless detection of adenosine using WINCS at a CFM in vitro. **A:** Pseudocolor plot obtained during a 10-second flow cell injection of 5- μ M adenosine, exhibiting 3D information. The x axis, y axis, and color gradient indicate time, voltage applied at the CFM, and current detected from the CFM, respectively. The FSCV waveform was applied from -0.4 V to +1.5 V and back to -0.4 V at 400 V/second every 100 msec. The *green oval* surrounded by a *purple ring* appears first around +1.5 V after the adenosine injection, and this represents the first oxidative peak of adenosine. A second oxidative peak (*purple oval*) around +1.0 V occurs after the appearance of the first oxidative peak. *Black* and *red solid vertical lines* refer to FSCV taken in the absence or presence of adenosine, respectively. *Black* and *red dotted horizontal lines* indicate first and second peak oxidative currents, respectively, for adenosine versus time. **B:** Graph showing that a large background current is present in normal Tris-buffered solution at a CFM by the rapid scanning of the potential (*black solid line*). Addition of 5- μ M adenosine increased this background current only slightly (*red solid line*, see inset). **C:** Graph showing current versus time traces for the first and second peak oxidative currents. **D:** A representative background-subtracted unfolded voltammogram of adenosine (*lower plot*), which is averaged over 10 voltammograms. The corresponding time versus voltage plot (*gray line, upper plot*) also represents the potential waveform used during the FSCV.

Fig. 3.

Graphs showing that the appearance of the second adenosine oxidative peak follows the appearance of the first peak. **A:** Close-up of a portion in Fig. 2C, showing the current versus time traces for the first oxidative peak (*square*) and second peak (*triangle*) for 5- μ M adenosine in a flow injection analysis experiment. **B:** Cyclic voltammogram obtained when adenosine was first detected. The *black line* indicates the forward-going potential from -0.4 V to +1.5 V, and the *red line* the reverse-going potential from +1.5 V back to -0.4 V. Note the presence of only the first adenosine oxidative peak near +1.5 V (*blue dashed line* in panel A). **C:** Cyclic voltammogram obtained 0.5 seconds later (*green dashed line* in panel A), demonstrating the appearance of the second adenosine oxidative peak near +1.0 V in addition to the first oxidative peak near +1.5 V.

Fig. 4.

Concentration-dependency of the electrochemical response to adenosine. **A:** Four FSCV pseudocolor plots of increasing adenosine concentrations (0.5, 1.0, 5.0, and 10.0 μM) using the flow cell analysis system. **B:** Calibration curves for adenosine. The amplitude of adenosine peak oxidative current was measured from the baseline to the maximum point after injection of each concentration of adenosine. Each line describes the best linear fit for the first (*square*, $r^2 = 0.99$) and second (*triangle*, $r^2 = 0.96$) peak oxidative currents recorded at a single CFM (*error bars* represent the SEM).

Fig. 5.

Codetection of adenosine and dopamine using WINCS-based FSCV at a CFM in the flow cell analysis system. The pseudocolor plots illustrate 1.5- μ M dopamine (**A**), 5.0- μ M adenosine (**B**), and a mixture of 1.5- μ M dopamine and 5.0- μ M adenosine (**C**). To measure both dopamine and adenosine, the applied potential waveform was scanned from -0.4 to $+1.5$ V and back at 400 V/second every 100 msec. The bottom graphs show representative cyclic voltammograms from each flow cell injection. The *black line* indicates the current recorded by the forward-going potential from -0.4 to $+1.5$ V, and the *red line* by the reverse-going potential from $+1.5$ to -0.4 V.

Fig. 6.

In vivo dopamine and adenosine efflux measured with WINCS-based FSCV at CFMs in the CPU of anesthetized rats. **A:** Electrical stimulation (130 pulses at 130 Hz, 0.3 mA, 2 msec pulse width) of the VTA/SN evoked both dopamine and adenosine efflux in the CPU. The pseudocolor plot shows the appearance of dopamine efflux immediately during and after the stimulation, while the peak corresponding to adenosine efflux was delayed by a few seconds after cessation of stimulation. **B:** Current versus time plot at +1.5 V (yellow dashed line in panel A) demonstrating efflux of adenosine following electrical stimulation. **C:** Background-subtracted cyclic voltammogram demonstrates simultaneous measurement of dopamine and adenosine efflux (red dashed line in panel A). The black and red lines indicate the currents generated by the forward- and reverse-going potential, respectively. **D:** Current versus time plot at +0.6 V (white dashed line in panel A) demonstrating efflux of dopamine following electrical stimulation.

Fig. 7.

In vivo adenosine efflux measured with WINCS-based FSCV at CFMs in the CPu of anesthetized rats. **A:** Electrical stimulation (130 pulses at 130 Hz, 0.3 mA, 2 msec pulse width) of the VTA/SN evoked adenosine efflux in the CPu. A representative background-subtracted cyclic voltammogram shows the first and second oxidative peaks at +1.5 V and +1.0 V, respectively at 2.5 seconds after the start of stimulation (red dashed line). **B:** Current versus time plot at +1.5 V, demonstrating a biphasic pattern of adenosine efflux after stimulation; the first increase occurring at 2 seconds (gray box) and the second increase (peak at 25 seconds after stimulation) with a more prolonged efflux. **C:** Pseudocolor plot with the expanded time

scale of panel A. **D:** Expanded view of the *gray box* in panel B, demonstrating the time course of initial adenosine efflux.