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Wireless Fast Scan Cyclic Voltammetry for Rats

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Introduction

Fast-scan cyclic voltammetry (FSCV) is a method whereby *in vivo* monoamine levels are detected by rapidly alternating a voltage across an implanted microelectrode and measuring the subsequent current. The use of tethered FSCV systems is currently the best (and only) way to obtain *in vivo* voltammetric measurements. However, the forces applied to the skull by the tether are cumbersome, impair movement, and restrict the rat to a small, confined area. Development of a wireless system is necessary to measure spontaneous, sub-second neurotransmitter release events while conducting detailed behavioral studies currently not possible with tethered systems. A wireless system capable of measuring *in vivo* monoamine release allows researchers to monitor neurotransmitter release in situations where a traditional tether system is not practical such as mazes, enclosed behavioral or metabolic chambers or in combination with a force plate actometer for measurement of very small body movements (Fowler et al., 2001). The development of this technology will open new doors to research and discovery about the underlying neurochemical events associated with behavior and allow researchers to evaluate the complex relationships in ways that are currently not possible.

System Specifications

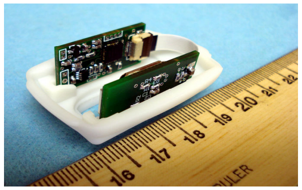
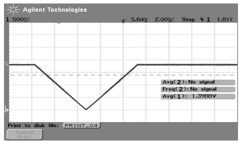


Figure 1: FSCV Electronics in Headmount and Battery Backpack

- Prototype battery life (5 hours – continuous acquisition)
- System weight (on head including rathat) – 9 grams
- Electronics size (two 0.56" x 1" boards)
- Three electrodes (1 working, 1 reference, and 1 counter)
- Integrated, bipolar user-controlled stimulus
- User-controlled sweep repeat rate
- User-controlled scan rate 0 – 400 V/s with a 1.2 mV voltage step; 1000 V/s can be achieved with a 2.4 mV voltage step.
- User-controlled voltage range of -1.0V -> +1.4 V
- User-controlled low pass filters
- Total samples per sweep - 1000
- 12-bit D/A resolution
- 16-bit A/D resolution (250 kHz sampling rate)
- Prototype limit of detection 0.35 µM DA
- Wireless (Bluetooth)
- FSCV acquisition and analysis software



Agilent Technologies
1 500mV/ 5.64ms 2.00ms/ Stop 1 1.81V
Avg(2):No signal
Freq(2):No signal
Avg(1): 1.3988V
Print to disk file: PRINT_04

Figure 2: Oscilloscope Capture - Single Voltage Sweep (inverted)

Methods and Results

Carbon-fiber cylinder microelectrodes were prepared as previously described using T-650 carbon fibers (Amoco, Greenville, SC) (Kawagoe et al., 1991). Carbon fibers were aspirated into glass capillaries (A-M Systems, Inc, Carlsborg, WA) and the capillaries were subsequently heated and pulled (Narishige, Long Island, NY). The extending carbon fiber was trimmed to 25 µm and sealed in place with epoxy (EPON resin 828, Miller-Stephenson, Danbury, CT; m-phenylenediamine, DuPont, Wilmington, DE). Electrodes were cured at 100 C for 12 h followed by 12 h at 150 C.

In Vitro

In vitro tests were carried out in a flow cell apparatus. The flow cell allows for easier background subtraction since the dopamine "front" is well defined. It also allows more accurate calibration as it is not necessary to wait for a long time in order for the solution to become homogeneous after adding dopamine.

The wireless fast scan cyclic voltammetry system was initially tested using a carbon fiber microelectrode inserted into a flow cell. Artificial cerebral spinal fluid (pH 7.4) was pumped through the system at 2.0 mL/min using a syringe pump. For comparison, measurements were first collected using the Tar Heel system. A triangular waveform, starting at -0.4 V, increasing to +1.4 V, and scanning back to -0.4 V (versus a Ag/AgCl electrode), was applied to the carbon-fiber microelectrode at a scan rate of 400 V/s and an update rate of 10 cycles/s. Dopamine (1.0 µM) was introduced to the system using a 2-position, 6-place valve. The current arising from dopamine oxidation (at about 0.6 V) in successive voltammograms was measured and plotted versus time. After successfully collecting a series of cyclic voltammograms of dopamine using this system, the carbon-fiber microelectrode and reference electrode were disconnected from the EI-400 and connected to the Pinnacle Technology wireless fast scan system. Again, dopamine was introduced to the system and a series of cyclic voltammograms were collected with the wireless system using the same waveform, scan rate, and update rate. The response to 0.25, 0.5, 1.0, and 6.0 µM dopamine was measured.

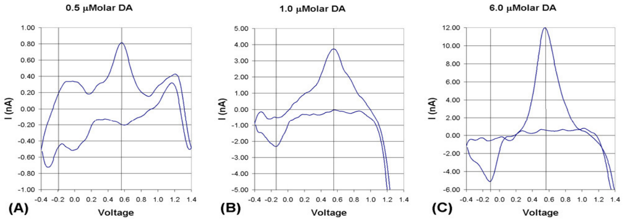
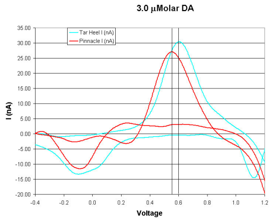


Figure 3: 400 V/s In Vitro Testing (A) 0.5 µM dopamine, (B) 1 µM dopamine, (C) 6 µM dopamine

Figure 4: Comparison of Tar Heel and Pinnacle In Vitro Response at 3.0 µM Dopamine



Support

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Brain Slice Testing

Brain slice tests were conducted to clearly demonstrate the system's ability to stimulate and detect dopamine release under physiologically relevant conditions. Leftover tissue using excess material from another study was used and no animal was specifically euthanized for this study. A single slice was submerged under a CSF maintained at 34 C and continuously flowing (2 mL/min) through a superfusion chamber (Warner Instruments, Hamden, CT). The mouse brain slice was equilibrated for 30 minutes prior to obtaining measurements. For comparison, measurements of stimulated dopamine release were first obtained using a Dagan Chem Clamp amplifier (Dagan Corporation, Minneapolis, MN) interfaced with a computer as described above. The carbon fiber was inserted until the tip was 100 µm below the surface of the brain slice in the dorsolateral caudate-putamen region of the striatum between the prongs of a bipolar stimulating electrode (FHC, Bowdoinham, ME), situated 200 µm apart. To evoke dopamine release, a single biphasic stimulating current pulse (2 ms each phase, 350 µA in amplitude), was applied to the stimulating electrode. Dopamine was detected using the -0.4 V → +1.4 V → -0.4 V waveform applied at a scan rate of 400 V/s and an update rate of 10 cycles/s. The current arising from dopamine oxidation (at about 0.6 V) in successive voltammograms was measured and plotted versus time. Once dopamine release was measured in the brain slice, the carbon-fiber microelectrode, reference electrode, and bipolar stimulating electrodes were disconnected from the Dagan Potentiostat and connected to the Pinnacle Technology wireless fast scan system. Similar files of dopamine release were obtained using this system, demonstrating its utility in obtaining physiologically relevant dopamine release measurements in the brain.

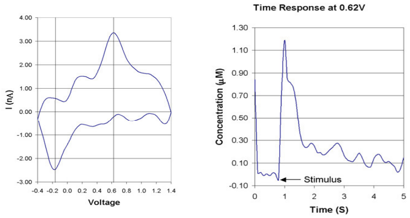

Figure 5: Brain Slice Results (400 V/s, 350 mA bipolar stimulus)

Conclusion

A miniaturized wireless fast scan system has been constructed that will easily fit on the head of a rat (Figure 1). The system was successfully tested *in vitro* and the results were compared to those obtained with a state-of-the-art FSCV system (Tar Heel). To test the efficacy of the stimulus, we tested for dopamine release on brain slices. The brain slice testing clearly demonstrates the system's ability to stimulate and detect dopamine release under physiologically relevant conditions. Future tasks include electronics, mechanical and software optimization, limit of detection optimization, microdrive incorporation, system validation and beta testing.

References

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