Chapter 16

Real-Time Chemical Measurements of Dopamine Release in the Brain

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Abstract

Rapid changes in extracellular dopamine concentrations in freely moving or anesthetized rats can be detected using fast-scan cyclic voltammetry (FSCV). Background-subtracted FSCV is a real-time electrochemical technique that can monitor neurochemical transmission in the brain on a subsecond timescale, while providing chemical information on the analyte. Also, this voltammetric approach allows for the investigation of the kinetics of release and uptake of molecules in the brain. This chapter describes, completely, how to make these measurements and the properties of FSCV that make it uniquely suitable for performing chemical measurements of dopaminergic neurotransmission in vivo.

Key words: Fast scan cyclic voltammetry, In vivo, Electrochemistry, Carbon fiber microelectrode

1. Introduction

Dopamine is a neurotransmitter of particular interest due to its involvement in motivated behavior and reward-driven learning (1, 2), as well as various neurological disorders such as Parkinson's disease (3), schizophrenia (4), and drug addiction (5, 6). Extracellular dopamine concentrations in the brain vary on a subsecond timescale due to phasic firing of dopamine neurons (7). These naturally occurring dopamine transients are enhanced upon administration of drugs of abuse (8, 9), and become time-locked to cues that predict reward availability (1, 9–12). The ability to characterize and provide real-time measurements of rapidly

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fluctuating dopamine in vivo requires an analytical method with rapid temporal resolution. To date, the most widely used technique to monitor in vivo neurotransmitter release is microdialysis (13). This method provides excellent chemical selectivity and is well suited for measuring dopamine levels averaged over the course of minutes to hours. However, microdialysis lacks the temporal resolution to detect phasic dopamine fluctuations that occur on the subsecond to second timescale (14). In contrast, electrochemical techniques are especially useful for monitoring rapid chemical changes resulting from discrete neurochemical events due to rapid sampling rates (micro-to-millisecond timescale). Broadly speaking, the techniques that involve current flow at an electrode under potential control can be divided into two groups: voltammetric and amperometric methods (15). Of these techniques, fast-scan cyclic voltammetry (FSCV) provides the best combination of temporal resolution, sensitivity, and chemical selectivity—features that are essential for the detection of rapid neurotransmitter fluctuations in vivo (16). Additionally, FSCV has been combined with other techniques including microinjection (7), iontophoresis (12, 17), and electrophysiology (12, 18, 19) to provide a great deal of new information regarding dopamine and its role in the brain.

1.1. Use of FSCV in Dopamine Detection

In FSCV, a dynamic potential is applied to a carbon fiber microelectrode (20). As the voltage is cycled through a triangular potential pattern, current is generated and recorded as a function of potential. The current generated over a single scan is plotted versus the applied potential. This resulting voltammogram is characteristic of the analyte, allowing it to be distinguished from many other electroactive species. This principle by which FSCV operates allows the identification of dopamine by the location of the potentials at which it oxidizes/reduces and the characteristic peak shape (Fig. 1). This also allows dopamine to be distinguished from many other interferents in the brain, such as ascorbic acid and changes in pH (16).

Although FSCV generates characteristic voltammograms that can serve as qualitative identifiers for a molecule of interest, selectivity is always a concern. Several criteria need to be followed in order to positively confirm the identity of a voltammetric signal (21). These steps include electrochemical, anatomical, physiological, and pharmacological verification.

1. The in vivo signal should match the standard voltammogram collected in vitro.
2. The signal must be collected in an anatomical region known to be analyte rich.
3. Stimulation of cell bodies should illicit analyte release.
4. Pharmacological manipulation should be used to reduce or/and increase analyte release.

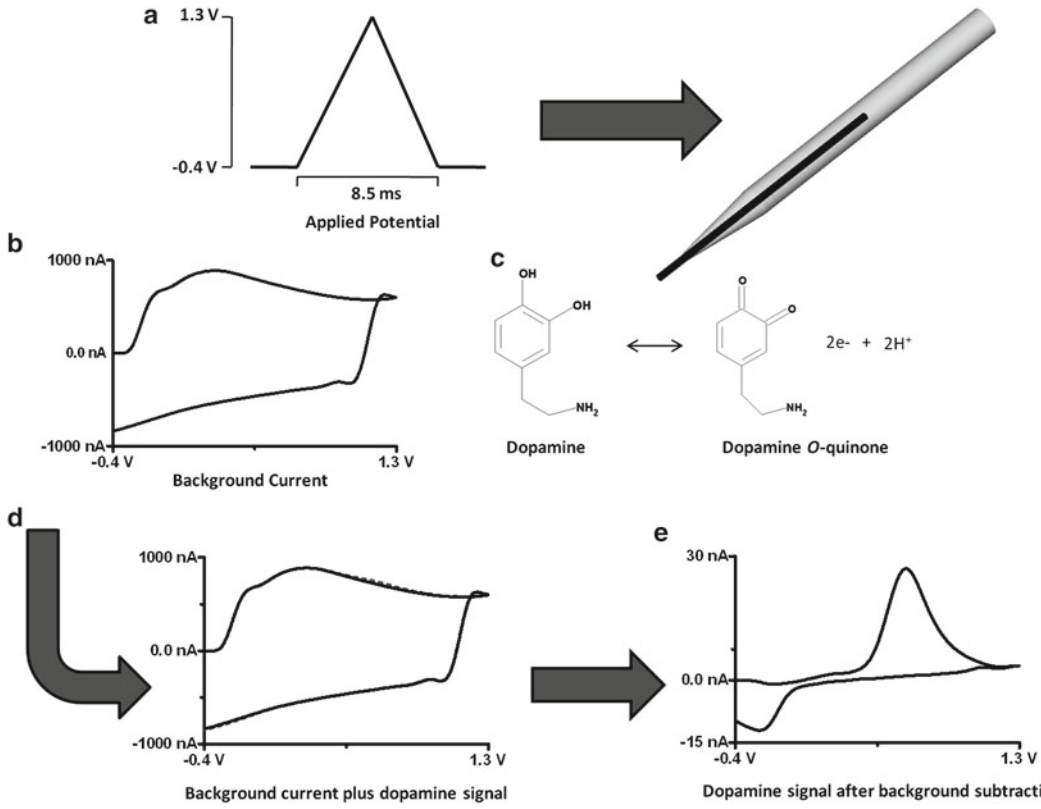

Fig. 1. Principles of background subtracted FSCV. (**a**) First, a potential waveform is applied to a working electrode. (**b**) This generates a stable non-faradaic background current. (**c**) The redox reaction will exchange electrons at the electrode surface, generating faradaic current. (**d**) At low analyte concentrations, the faradaic response (*dashed red line*) is small compared to the background current. (**e**) The stable non-faradaic background current can be subtracted from the faradaic current arising from the redox reaction. The resulting cyclic voltammogram is an electrochemical fingerprint of the analyte.

These steps must be taken in order to perform reliable electrochemical measurements and authenticate the identity of an analyte.

The microelectrodes typically used with FSCV are cylindrically-shaped carbon-fiber microelectrodes. With this approach, a 5–7 μm diameter carbon fiber electrode is sealed in a glass capillary with a portion of the fiber (75–125 μm) extending from the tip. This carbon fiber electrode is approximately 40-fold shorter and 50-fold smaller in diameter than a typical microdialysis probe, and thus it is particularly well suited to probe brain regions that have gradations in the density of neuronal terminals over these dimensions (22). This small size results in minimal tissue damage during in vivo experiments, allows for characterization of specific brain regions, and its cylindrical shape enables detection from all sides of the electrode by way of hemispherical diffusion to the recording surface, which enhances sensitivity.

2. Materials

2.1. Electrode Fabrication

1. Carbon fiber: T-650 (GoodFellow, Huntingdon, England).
2. Borosilicate capillary glass: 0.6 mm O.D., 0.4 mm I.D. for freely-moving experiments and 1.0 mm O.D., 0.5 mm I.D. for anesthetized experiments (A-M Systems, Sequim, WA).
3. Vertical electrode puller: PE-21 (Narishige, Tokyo, Japan).
4. Vacuum pump.
5. Optical microscope.
6. Surgical scalpel to cut the carbon fiber.
7. Micromanipulator: for freely-moving experiments (custom made, UNC-CH Chemistry, Machine Shop).
8. Silver paint: Silver Print II (GC Electronics, Rockford, IL).
9. Heat shrink tubing: EPS-200-1/8″ and FP-301-3/32″ (3 M Electronics, Austin, TX).
10. NORIT A® activated carbon is used for isopropyl alcohol purification (MP Biomedicals, LLC, Solon, OH).
11. Silver wire: 0.5 mm diameter for Ag/AgCl reference electrode (Sigma-Aldrich, St. Louis, MO).
12. Gold connector: PCB socket (Newark Electronics, Chicago, IL).
13. Insulated leads: 30 gauge (Squires Electronics, Inc., Cornelius, OR).

2.2. Surgery

1. Anesthetic: xylazine and ketamine for freely-moving experiments, urethane for anesthetized experiments, and 0.25% bupivacaine is used as a local anesthetic.
2. Heating pads.
3. Stereotaxic frame: such as, Model 900 Small Animal Stereotaxic (David Kopf Instruments, Tujunga, CA).
4. Guide cannula (Bioanalytical Systems, Inc., West Lafayette, IN).
5. Anchor screws (Gexpro, Indianapolis, IN).
6. Cranioplastic cement: Grip Cement (Dentsply International, Inc., Milford, DE).
7. Stimulating electrode: 20 mm long bipolar stainless steel (Plastics One, Roanoke, VA).

2.3. Electrochemistry

1. Multifunction input/output cards: PCI-6251 and PCI-6711 (16 bit, 333 kHz) (National Instruments, Austin, TX).
2. Software for data collection and analysis: TH-1 (ESA, Chelmsford, MA), or custom written in house using LabVIEW (National Instruments, Austin, TX).

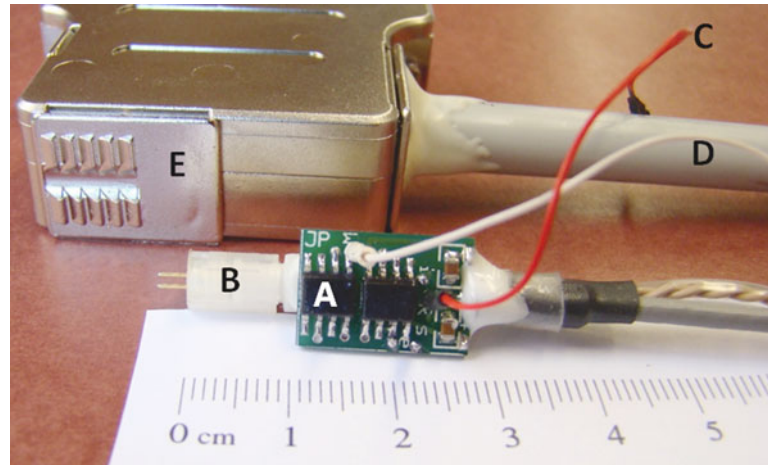

Fig. 2. Headstage (UNC-CH Electronics Design Facility). A miniaturized current-to-voltage convertor that consists of (**a**) operational amplifier, (**b**) threaded connection to stimulating electrode, (**c**) lead for reference electrode, (**d**) lead for working electrode, (**e**) DB-25 connector.

3. Potentiostat. One of the following is appropriate: EI-400 bio-potentiostat (Cypress Systems, Lawrence, KS), Universal Electrochemistry Instrument (UEI, UNC-Chapel Hill, Electronics Design Facility), or Universal Headstage Controller (United World Domination, Mebane, NC).
4. Headstage: miniaturized current-to-voltage converter (UNC-CH Electronics Design Facility; or United World Domination, Mebane, NC) (Fig. 2). A larger version can be used for anesthetized experiments and postexperiment calibration.
5. Commutator: 25 channel (Crist Instruments, Hagerstown, MD).
6. Screened behavioral chamber: custom made for in vivo experiments (Med Associates Inc., St. Albans, VT).
7. Optional equipment: TV, DVD-R, and video character generator (for monitoring, recording, and time-stamping animal behavior).

2.4. Stimulation

1. Multifunction input/output card: PCI-6711 (National Instruments, Austin, TX).
2. Bi-phasic stimulus isolator: DS4 (Digitimer, Ltd, Hertfordshire, England).

2.5. Electrode Postcalibration

1. Dopamine HCl: 1 mM dopamine in 0.1 N $HClO_4$ for stock solutions, and dilutions are made in buffer (Sigma-Aldrich, St. Louis, MO).

2. Flow injection apparatus: six-port, two-position high-performance liquid chromatography (HPLC) valve, with air actuator, and digital valve interface (VICI, Houston, TX).
3. Grounded Faraday cage: custom built in house.
4. Tris buffer: 3.25 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 2.0 mM Na_2SO_4, 1.25 mM NaH_2PO_4, 145 mM NaCl, and 15 mM Trizma ® HCl at pH 7.4 (Sigma-Aldrich, St. Louis, MO).

3. Methods

3.1. Electrochemistry, Instrumentation, and Software

Dopamine release in brain tissue can be monitored in real-time with high spatial and temporal resolution when micron-scale electrodes and low-noise instrumentation are implemented. Dopamine is electrochemically detected at carbon-fiber microelectrodes by applying a potential sufficient to liberate two electrons from dopamine to form dopamine ortho-quinone. This provides a current that can be converted to a voltage and measured using a current transducer. Instrumentation includes the Universal Electrochemical Instrument, the Universal Headstage Controller, or a EI-400 biopotentiostat. These instruments are generally used with computer-controlled interface boards and locally written software (LabVIEW, National Instruments, Austin, TX). Software is commercially available from ESA. The instrument provides all inputs and supplies power to the headstage, and usually consists of two main components: a low-pass filter and a headamp module. The computer-generated waveform contains digitization noise that must be smoothed by a low-pass filter before the signal reaches the working electrode. The output voltage from the current transducer is further amplified and conditioned by the headstage amplifier. The interface boards are responsible for the digital-to-analog and analog-to-digital conversions that are transmitted to and from the headstage, respectively.

The use of FSCV for the electrochemical detection of dopamine at carbon-fiber microelectrodes requires a waveform that optimizes peak currents, response time, and chemical selectivity. The most commonly used waveform holds the working electrode at −0.4 V vs. Ag/AgCl with periodic ramping to +1.3 V and back at a rate of 400 V/s and a frequency of 10 Hz (Fig. 1). The time between scans when the working electrode is held at a negative potential allows positively-charged dopamine to preconcentrate at the electrode surface (23). Due to the fast scan rate, scanning generates a large capacitive charging current at the electrode surface (24), which is significantly larger than faradaic currents resulting from redox processes at the microelectrode surface.

These background currents are stable over tens of seconds. This allows for subtraction, revealing the interesting faradaic responses. The resulting background-subtracted cyclic voltammograms provide information on the analyte identity, redox potentials, reversibility, and electron transfer kinetics. The shape of the peaks allows for the discrimination of multiple species (however all catecholamines produce similar voltammograms) and can be used to assess the role of mass transfer. The amplitude of the peaks can be correlated to the concentration of the analyte at the electrode surface. Under the conditions described, the cyclic voltammograms for dopamine should have a peak for the oxidative current at around +0.6 V.

3.2. Bipolar Electrical Stimulation

Electrical stimulation of dopaminergic cell bodies evokes dopamine release from the terminals in a time-locked manner, enabling the experimenter to monitor the kinetics of dopamine release and uptake with FSCV (25). The computer controlled stimulation is delivered with a biphasic stimulus isolator to the stimulating electrode. The device must be calibrated before use to ensure proper function. The waveform applied to the stimulating electrode is a biphasic square wave that is applied with a frequency, amplitude, pulse width, and number of pulses consistent with the experimental goals. Typical stimulation parameters for dopamine neuron cell bodies are 125 biphasic pulses, 60 Hz, ±125–150 μA, and 2 ms/phase. This stimulation must be applied between the ramps of the electrochemical waveform, such that the electrochemical data is not disturbed by the current stimulation (Fig. 3).

3.3. Electrode Fabrication

3.3.1. Carbon Fiber Microelectrode

1. A single carbon fiber is placed on a flat and clean surface that is well illuminated. The fiber is then aspirated into a borosilicate glass capillary, so that it extends from both ends.
2. The filled capillary is tapered in an electrode puller. This forms two electrodes from a single filled capillary. Each is inspected

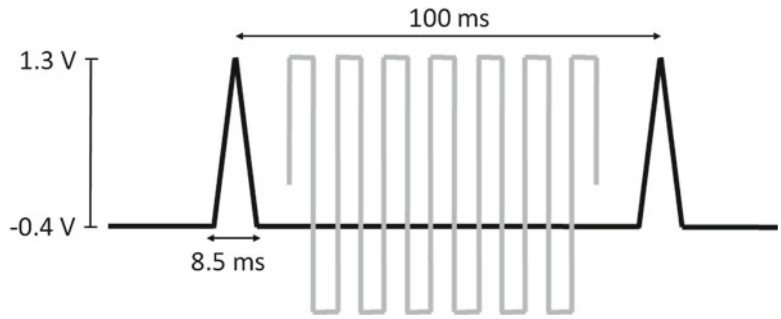

Fig. 3. Electrical stimulation. The bipolar electrical stimulation (*gray*), must not overlap with the applied electrochemical waveform (*black*).

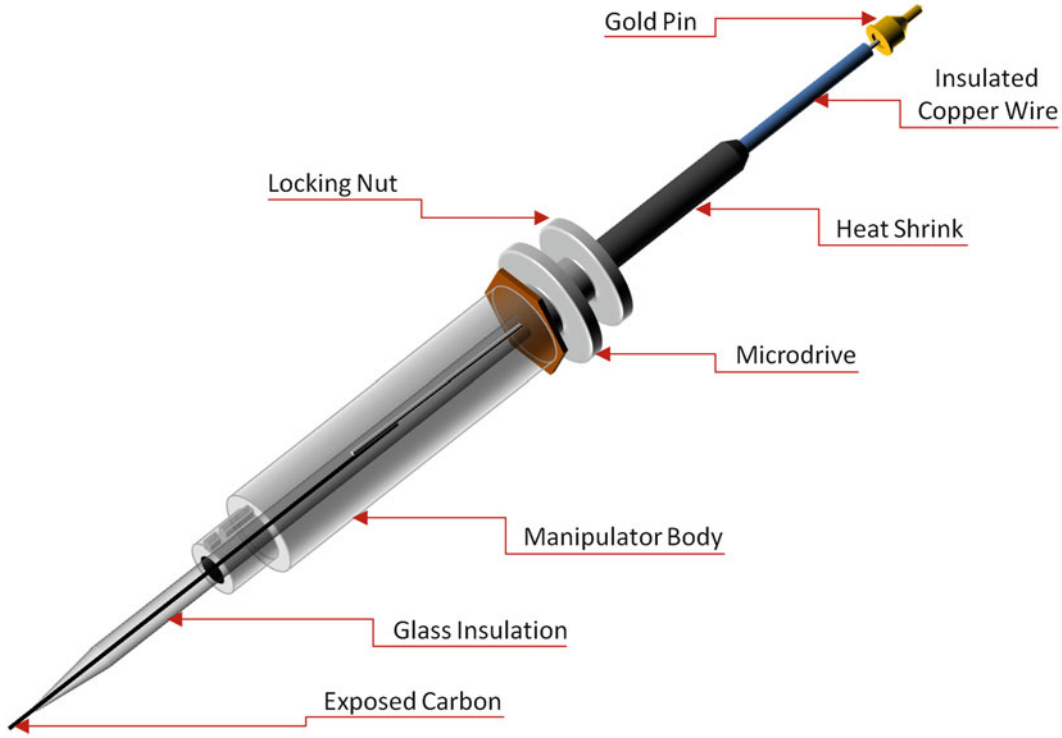

Fig. 4. Micromanipulator (UNC-CH Machine shop). An illustration of a loaded micromanipulator, ready for an experiment.

under the microscope to ensure a tight glass seal around the carbon fiber.

3. The exposed carbon fiber is then cut to length (~100 μm) with a sharp scalpel under a microscope using a magnification of 10×. The electrode should also be inspected under the microscope using a higher magnification of at least 40× for visible cracks or abnormalities in the fiber or glass seal, and discarded if any are present (see Note 1).
4. In freely-moving experiments:
   (a) An inspected 100 μm carbon fiber microelectrode is loaded into a custom micromanipulator and secured with heat shrink tubing (Fig. 4).
   (b) A small diameter insulated wire is painted with silver paint, and fed into the back of the capillary to make an electrical connection with the carbon fiber. A slight rotation of the wire ensures connectivity with the carbon fiber. The wire is secured to the micromanipulator with additional heat shrink tubing.
   (c) All loaded manipulators are stored with the exposed carbon in purified and filtered isopropyl alcohol.

5. In anesthetized experiments:
   (a) Larger diameter glass capillaries can be used.
   (b) The carbon fiber microelectrode is backfilled using a saturated solution of 150 mM potassium chloride and 4 M potassium acetate. A small diameter insulated wire is fed into the back of the capillary to make electrical connection.

3.3.2. Ag/AgCl Reference Electrode

1. A piece of silver wire is cut to approximately 10 mm, inserted into the socket of a gold connector, and soldered in place.
2. The solder is then covered with quick dry epoxy to avoid the contact of the soldering material with tissue.
3. On the day of surgery, the reference is chlorinated by connecting the positive terminal of a 2.5 V power supply to the gold pin on the silver wire and the negative terminal to a wire, with both leads immersed in 0.1 M hydrochloric acid. Chlorination is performed for about 1 min until the surface of the silver wire turns slightly white.

3.4. Surgery

3.4.1. Anesthetized Preparation

1. The rat is anesthetized with urethane (3 g/kg i.p.), the top of the head is shaved, and the animal is placed in a stereotaxic frame.
2. The scalp is locally anesthetized with a subcutaneous injection of 0.25% bupivacaine. An incision is made in the scalp, and the skin retracted to expose a 15–20 mm longitudinal and 10–15 mm lateral area of cranium.
3. Holes are drilled through the skull for stereotaxic placement of electrodes (stimulating, working, reference) (Fig. 5). The stimulating electrode can be positioned either in regions

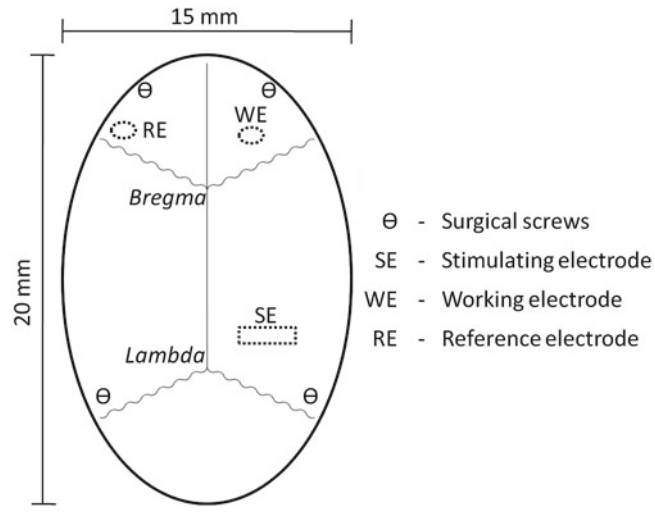

Fig. 5. A top view illustration of a rat skull, highlighting the general placement of holes (*dotted lines*) for electrode and surgical screw placement.

containing dopaminergic cell bodies (substantia nigra/ventral tegmental area) or at the ascending fibers of the medial forebrain bundle. The hole for the working electrode is drilled above the target terminal region (e.g., 1.3 mm lateral and 1.3 mm rostral from bregma for the caudate-putamen and the core of the nucleus accumbens, and +1.7 mm anterior and +0.8 mm lateral for the nucleus accumbens shell). The hole for the reference electrode is drilled in the contralateral hemisphere, opposite the working electrode.

4. Electrodes are lowered and secured in select areas of the brain using micromanipulators mounted on the stereotaxic frame. The reference electrode is secured with cranioplastic cement. Mild electrical stimulations are applied to evoke neurotransmitter release that is monitored at the microelectrode using FSCV. The rat is maintained on a heated pad for the duration of the experiment.

3.4.2. Freely-Moving Preparation

1. The rat is anesthetized with intramuscular or intraperitoneal ketamine (100 mg/kg) and intramuscular xylazine (10 mg/kg), the top of the head is shaved, and the animal is placed in a stereotaxic frame.
2. The scalp is locally anesthetized with a subcutaneous injection of 0.25% bupivacaine. An incision is made in the scalp, and the skin retracted to expose a 15–20 mm longitudinal and 10–15 mm lateral area of cranium.
3. Holes are drilled for the working electrode guide cannula, stimulating and reference electrodes (Fig. 5). In addition, four holes are drilled at a 45° angle into which anchor screws are secured.
4. Reference electrode and guide cannula are lowered using micromanipulators mounted on the stereotaxic frame.
5. Once the components are in place, they are secured with cranioplastic cement, leaving the stimulating electrode hole exposed.
6. The stimulating electrode is modified in order to provide adequate space between the plastic hub of the stimulating electrode and the guide cannula (for the working electrode) on the animal's head cap (Fig. 6). The stimulating electrode wires are bent at a 90° angle from the plastic hub and then bent back down at another 90° angle, to give a horizontal distance of ~5 mm between the hub and the main axis of the wires. Next, the tips are separated by 0.8–1.0 mm and carefully cut to a uniform length without disturbing the insulation. Dura mater is thoroughly cleared and the electrode is stereotaxically lowered into the tissue, oriented so that the tips of the electrode are splayed on the coronal plane. The electrode is lowered to 1 mm above the target brain region.

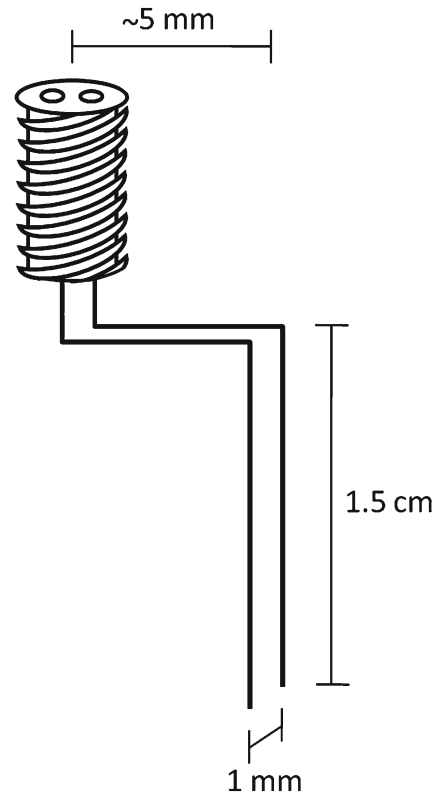

Fig. 6. Stimulating electrode.

7. The stimulating electrode is connected to the stimulator and a mild electrical stimulation is applied through the stimulating electrode. The animal's tail should respond to this stimulation by rapidly rising and then slowly falling back to the resting position. The stimulating electrode is lowered in 0.2 mm increments until this response is diminished. It is then lowered further in 0.1 mm increments until this tail response is almost non-detectable.
8. Finally, cranioplastic cement is applied to the exposed cranium, carefully covering the stimulating electrode and lower half of its plastic hub.
9. Immediately following surgery, the animal is placed on a warm heating pad until fully recovered. Once fully awake, soft food and fresh water are offered with a fruit-flavored analgesic, such as acetaminophen (0.1–0.3 g/kg) that the rat will readily lick.
10. The animal is monitored daily and gently handled to facilitate experimental procedures. While handling, the stylet should be gently removed from the guide cannula, cleaned with an alcohol wipe, and reinserted. Experiments can be conducted within 2–5 days of surgery.

3.5. Freely-Moving Rat Experiment

3.5.1. Making the Connections

Two to five days after surgery, depending on the rat's postsurgical recovery, the animal is prepared for the experiment. The animal is placed in the behavioral chamber, tethered using the stimulator cable on which the headstage is mounted (Fig. 2) and allowed to acclimate for about 10 min. Before the loaded micromanipulator is placed into the guide cannula, the electrode is inspected once again under a microscope to double check the condition of the seal. The electrode is retracted inside the micromanipulator as the tip of the electrode is monitored. Once the electrode tip disappears, each turn is counted until the electrode is fully retracted. This protects the electrode integrity as it is loaded into the cannula and allows the experimenter to index the tip location inside the manipulator. All connections are cleaned, and the guide cannula stylet is removed and replaced with the micromanipulator containing the retracted microelectrode. The manipulator is locked in place and the working and reference electrodes are connected to the headstage.

3.5.2. Lowering the Carbon Fiber Microelectrode

The electrode is slowly lowered into tissue as its output is monitored on an oscilloscope. To do this, the waveform is applied. As soon as the carbon fiber electrode comes in contact with tissue, the non-faradaic background current appears, and is monitored for stability as the electrode is lowered through the tissue (Fig. 7).

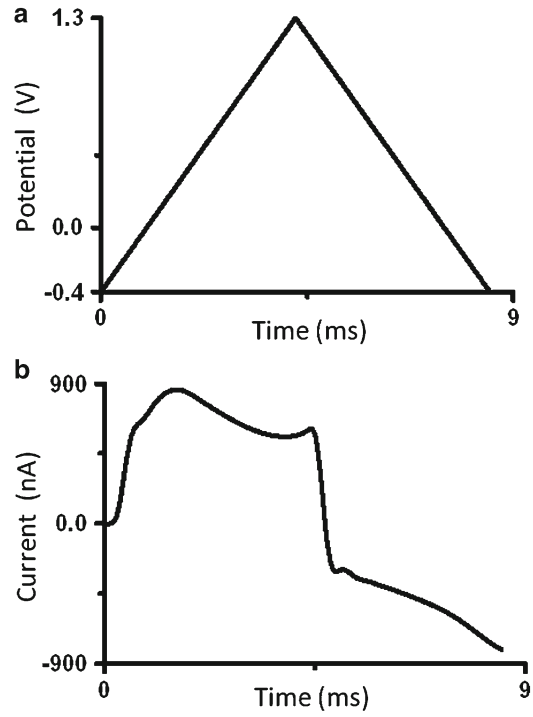

Fig. 7. Oscilloscope output. (**a**) Diagram of applied waveform. (**b**) Electrode response when circuit is completed in tissue or buffer.

A break in the electrode is evident by a sudden change in the shape of the background current to a more resistive profile (approximating a triangular wave), and it should be removed and replaced with a fresh carbon fiber electrode. Once in place at the target region, the electrode is conditioned for about 20 min to stabilize the signal. Electrochemical conditioning consists of applying the triangular waveform mentioned above for at least 10 min at a frequency of 60 Hz and then changing it to 10 Hz for 10 additional minutes of potential cycling.

A mild electrical stimulation is applied to the stimulating bipolar electrode while the current output is monitored at the carbon fiber microelectrode. If a dopamine signal is not obtained, the working electrode is lowered in small increments and stimulation repeated until electrically evoked dopamine release is observed. The electrode is then secured in position by a locking device on the micromanipulator and the experiment is initiated (see Note 2).

3.6. Anesthetized Rat Experiment

1. Immediately following surgery, the stereotaxic frame is placed into the grounded Faraday cage.
2. The electrodes (carbon fiber, stimulating, and Ag/AgCl reference) are lowered into the appropriate holes using the stereotaxic frame. There is no need to use screws and cranioplastic cement to secure the electrodes in an anesthetized experiment; however, the reference can be secured in place for stability.
3. The stimulating electrode is connected to the biphasic stimulus isolator and the working and reference electrodes are connected to the headstage.
4. As described above, the microelectrode is lowered in small increments (0.1 mm) into a brain region rich in dopamine terminals.
5. Dopamine neurons are electrically stimulated to illicit dopamine release at the terminals in a time-locked fashion (see Note 3).

3.7. After the Experiment

Upon completion of the experiment(s), there are two options depending on the objective of the experiment and the investigator's primary interest. These two options are described below.

3.7.1. Verification of Electrode Placement

The electrode tip is too small to leave a visible mark in tissue, thus an electrical lesion is made at the carbon fiber tip by applying a high current to the microelectrode. This unequivocally shows the location of the electrode in the tissue; however, this renders the electrode useless and it cannot be calibrated. The rat is transcardially perfused with 0.9% saline and 10% formalin solution to fix brain tissue. Finally, the animal is decapitated and the brain is removed from the skull and stored in formalin solution at 4°C, until it is sliced for histology.

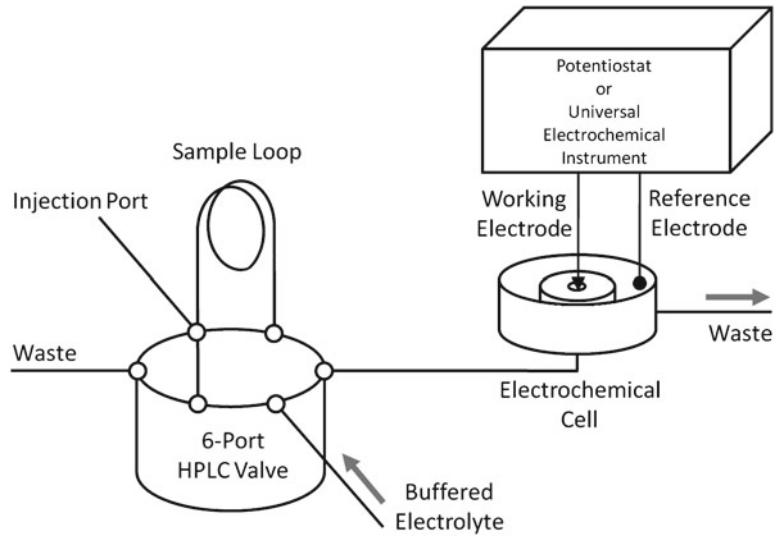

Fig. 8. Flow-injection analysis system. A syringe pump supplies a constant buffer flow across the working and reference electrodes. An HPLC valve controls the introduction of an analyte to the working electrode surface.

3.7.2. Electrode Postcalibration

Alternatively, the microelectrode is carefully removed from the brain, replaced with a sacrificial carbon fiber microelectrode, and an electrical lesion is made as described above. The electrode for calibration is rinsed in water and calibrated in vitro on a flow-injection apparatus using known physiological concentrations of dopamine (usually between 200 and 1,000 nM). This system consists of a custom-made electrochemical cell and a sample loop by which small volumes of analyte are rapidly injected into the cell using a six-port HPLC valve and a computer-controlled pneumatic actuator. A syringe pump is used to continuously supply physiological buffer at a constant flow rate through the electrochemical cell (Fig. 8.). The working electrode is lowered with a micromanipulator into the stream of buffer flowing at 1–3 mL/min. The Ag/AgCl reference electrode is submerged in the buffer as well and both are connected to the headstage. The same waveform used for the in vivo experiment is applied for the calibration of the electrode. Concentrations of the analyte of interest are loaded into the sample loop and introduced into the electrochemical cell with the digitally-controlled pneumatic actuator. The injection is software controlled. Each concentration of dopamine is sampled at least in triplicate and the averaged peak oxidative current is plotted against concentration. The resulting calibration plot is used to relate the current collected in vivo to corresponding dopamine concentrations.

3.8. Data Analysis

TH-1 (ESA, Chelmsford, MA) software is commercially available and can be used for data analysis. Additionally, custom software

written with Matlab (MathWorks, Inc., Natick, MA) can mathematically extract information from chemical data for quantitative analysis. The current method of multivariate statistical analysis involves the use of principle component regression (PCR) (16, 28). PCR has the ability to separate intensity based data into relevant components and noise, so that noise can be discarded. A training (calibration) set is used that includes individual cyclic voltammograms for the major species (typically dopamine and pH shifts, depending on the local microcircuitry) at various concentrations. Principle components that best describe the data are chosen. A principle component can be described as a vector that passes through the data that includes the most information. These principle components are then used to predict unknown concentrations from individual cyclic voltammograms collected in vivo, as long as the unknown concentrations fit within the training set.

3.9. FSCV Combined with Electrophysiology

FSCV can be combined with more traditional neuroscience tools such as electrophysiology, a technique that uses an electrode to measure action potentials (12, 18, 29). With this combined approach, the microelectrode employed for electrochemical detection is also used to monitor local synaptic activity. Between scans the holding potential is abbreviated and the electrode is allowed to float, thereby adopting the potential of its local environment, which is digitally recorded. The use of this method has allowed dopamine release to be correlated with changes in the firing of specific neurons in the vicinity of the electrode, shedding light on dopamine function in discrete brain microcircuits.

3.10. FSCV Combined with Intracranial Self-Stimulation

The behavioral paradigm of intracranial self-stimulation (ICSS) is an intensely rewarding experimental model (30). It has also been combined with FSCV (10, 12, 31). In ICSS a stimulating electrode is implanted into a specific brain nucleus and the animal is taught to use a lever to deliver a mild electrical stimulation to the chosen region. This serves as a powerful operant reinforcer and is often used in studies of motivated behavior. The use of ICSS in combination with FSCV has led to the association of rapid dopamine signaling with learned cues (such as audio or visual cues) that precede an electrical stimulation or reward availability (10, 12). This technique reveals information on the chemical mechanisms underlying reward based learning.

3.11. FSCV and Methods of Localized Pharmacological Manipulation

Microinjection and iontophoresis are two methods that have been implemented for administering small quantities of a compound into a specific region of the brain. While systemic application of drugs affects global brain circuitry, localized drug delivery techniques allow the experimenter to pharmacologically manipulate one discrete brain region. Microinjection involves the placement of a small needle into the desired brain location, and the

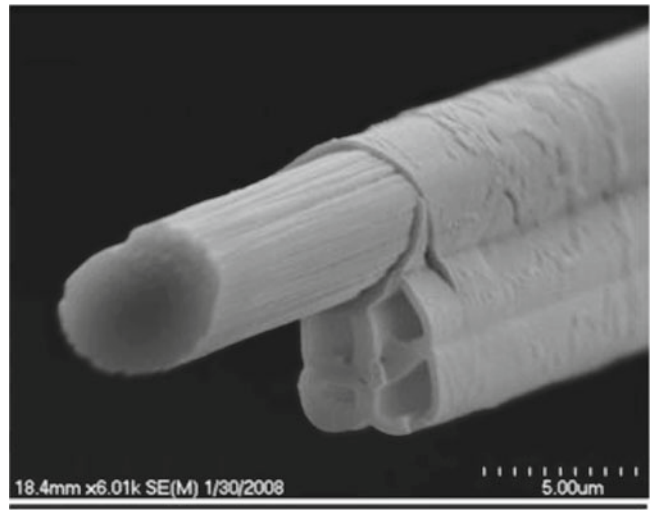

Fig. 9. Iontophoresis probe. Scanning electron micrograph of a five-barrel probe coupling FSCV with iontophoresis, using a carbon fiber microelectrode. Reprinted with permission from ref. (36). Copyright 2008 American Chemical Society.

subsequent pressure-driven infusion of a compound. Intracranial self-administration using microinjection has been used to elucidate the reinforcing action of specific agents in precise brain nuclei (32–34). Additionally, microinjection combined with FSCV has established that dopamine transients recorded in the nucleus accumbens shell require phasic neuronal activity in the ventral tegmental area (7), linking the activity of these two regions.

Iontophoresis can be used to locally apply compounds in an ionic solution using an applied current (35). When combined with FSCV (17, 36), capillary barrels are attached to a working electrode to deliver small quantities of a compound into tissue (Fig. 9). This approach enables drug administration to the same site as the working electrode. While it has its advantages, iontophoresis is a largely nonquantitative technique. However, recent advances have allowed researchers to accurately quantify the amount of drug delivered during an iontophoretic ejection by the use of an electroosmotic flow marker (17, 36). This marker allows quantitative analysis by taking into account the variability due to inconsistent barrel dimensions that affect electroosmosis, which in turn affects the observed iontophoretic ejection.

3.12. Recent Advances

One drawback to the use of FSCV in freely-moving experiments has been the cable which tethers the animal. The introduction of wireless integrated circuits has created new opportunities for studying dopamine function in freely-moving animals (37). Advantages of this technology include the ability to perform measurements during multiple animal social interactions, investigation of more natural behaviors and more complex environments, and fewer artifacts introduced during movement of electrical connections. Another

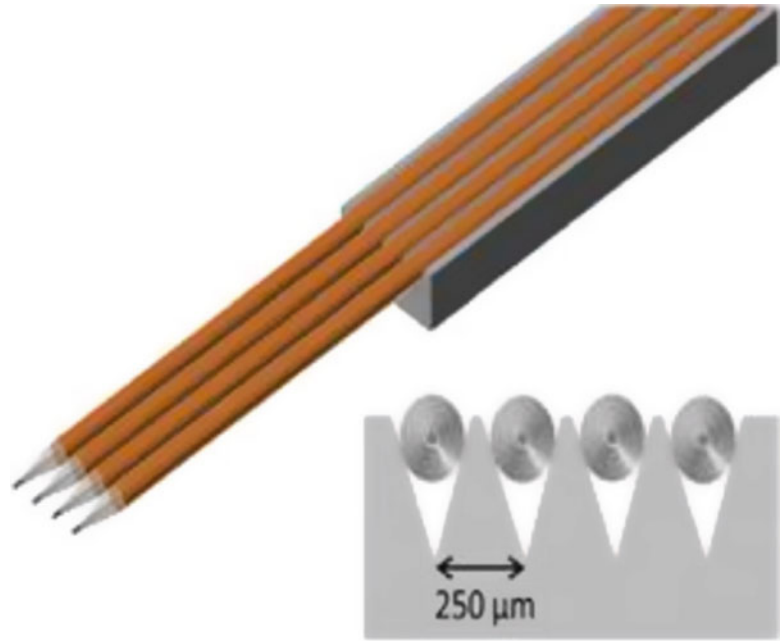

Fig. 10. Microelectrode array. An array of four carbon fiber microelectrodes, with fused silica insulation, secured with a fixed separation of 250 µm. Reprinted with permission from ref. 41 Copyright 2010 Elsevier.

recent development has been the incorporation of analog background subtraction to enable recordings over 30 min time intervals before distortion of dopamine signals occurs due to background drift (38). Other developments, such as microelectrode arrays (Fig. 10), allow multiple electrodes to be used in a single experiment (39–41). This has opened up the opportunity for researchers to simultaneously measure dopamine release at spatially discrete brain locations (39, 41). This approach also allows for the simultaneous detection of multiple signaling agents at various locations (40) and allows for more representative data to be obtained because of the increased number of recordings that can be acquired in a given experiment. Another recent advance has enabled chronic implantation of microelectrodes, enabling recording at the same electrode over months, rather than hours (42, 43). Finally, as an alternative to electrical stimulation, optogenetics can be used to stimulate specific neuronal populations using light-activated ion channels (44).

4. Notes

1. Optimal electrode length is determined by instrument limitations and experimental goals.
2. Many experiments monitor naturally-occurring dopamine fluctuations or transients. These dopamine release events are

evident at some but not all sites that support electrically-evoked dopamine release (26). Also, after several days of implantation, the reference electrode may drift by about 0.2 V, requiring the applied potential to be offset by 0.2 V.

3. Naturally-occurring transient dopamine release events are not generally detected in anesthetized animals, unless pharmacologically evoked (27).

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