Video Article

Presynaptic Dopamine Dynamics in Striatal Brain Slices with Fast-scan Cyclic Voltammetry

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Abstract

Extensive research has focused on the neurotransmitter dopamine because of its importance in the mechanism of action of drugs of abuse (e.g. cocaine and amphetamine), the role it plays in psychiatric illnesses (e.g. schizophrenia and Attention Deficit Hyperactivity Disorder), and its involvement in degenerative disorders like Parkinson's and Huntington's disease. Under normal physiological conditions, dopamine is known to regulate locomotor activity, cognition, learning, emotional affect, and neuroendocrine hormone secretion. One of the largest densities of dopamine neurons is within the striatum, which can be divided in two distinct neuroanatomical regions known as the nucleus accumbens and the caudate-putamen. The objective is to illustrate a general protocol for slice fast-scan cyclic voltammetry (FSCV) within the mouse striatum. FSCV is a well-defined electrochemical technique providing the opportunity to measure dopamine release and uptake in real time in discrete brain regions. Carbon fiber microelectrodes (diameter of ~ 7 µm) are used in FSCV to detect dopamine oxidation. The analytical advantage of using FSCV to detect dopamine is its enhanced temporal resolution of 100 milliseconds and spatial resolution of less than ten microns, providing complementary information to *in vivo* microdialysis.

Video Link

The video component of this article can be found at https://www.jove.com/video/3464/

Protocol

1. Experimental essentials

Electrode Fabrication

- There are numerous carbon fiber microelectrodes fabrication methods since most are made in-house. Typically what dictates the electrode fabrication details is the electrochemical technique that is applied to the electrode (e.g. amperometry vs. FSCV). For FSCV, microelectrodes can be made in-house using the following three-step procedure. For a more complete description of carbon fiber electrode fabrication, see a recent JOVE article [1]. However, note that the electrodes described below are cylindrical carbon fiber microelectrodes, which require fewer steps to fabricate versus the amperometric carbon fiber microelectrodes from the above-mentioned protocol. This simplified protocol does not require boiling the carbon fiber in acetone, fire-polishing the glass capillaries, or using epoxy to seal the glass-fiber junction.
- Using vacuum suction aspirate a carbon fiber (diameter 7 µm; Goodfellow Oakdale, PA) into a borosilicate glass capillary with microfilament (length 10 cm, o.d. 1.2 mm, i.d. 0.68 mm; A-M systems, Carlsborg, WA).
- Place the threaded capillary into the electrode puller (Narishige, Tokyo, Japan) where the capillary is pulled in half. Output settings for the electrode puller do vary from lab to lab. For reference, our output settings for the puller are 90.7 main magnet, 23.2 sub-magnet, and 53.4 for the heater. The output settings should be empirically refined to generate a glass taper that is approximately 4.4 mm in length, with a tight seal around the carbon fiber.
- Under a microscope (Olympus, Tokyo, Japan), trim the carbon fiber (using a scalpel blade) extending from the glass tip allowing approximately 50-200 µm of the carbon fiber to protrude from the tightly sealed interface.

Solution Preparation

Three types of artificial cerebrospinal fluid (aCSF) solutions need to be prepared in advance, all in ultrapure (18 MΩ cm) water.

- Sucrose-aCSF can be prepared at least one day before the slicing. The sucrose-aCSF buffer consists of (in mM): 180 sucrose, 30 NaCl, 4.5 KCl, 1.0 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, and 10 D-glucose (pH 7.4) and should be oxygenated using 95% O_2/5% CO_2 for 15 minutes [2]. If prepared ahead of time, the solution can be kept refrigerated at 4 °C for up to 1 week.

- ACSF solution for voltammetric recordings should be prepared the day of the experiment. The aCSF solution consists of (in mM): 126 NaCl, 2.5 KCl, 2.4 $CaCl_2$, 1.2 $MgCl_2$, 25 $NaHCO_3$, 1.2 NaH_2PO_4, 11 D-glucose, 0.4 ascorbic acid (pH 7.4). During the course of the experiment, oxygenate by bubbling with 95% O2/5% CO_2 at room temperature.
- Modified-aCSF solution for electrode calibrations contains (in mM): 2.5 KCl, 126 NaCl, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 1.2 $MgCl_2$, and 25 $NaHCO_3$ (pH 7.4) and can be kept for up to one week, without refrigeration or oxygenation.

Electrode Calibration

- Electrode viability and sensitivity is determined by pre- and post-calibration, respectively, using a flow "t-cell" containing 3 ports with a sealed reference electrode (Ag/AgCl). The fabricated microelectrode is lowered into the flow "t-cell". The inlet port is connected to a syringe pump, allowing for a continuous flow of modified-aCSF at a flow rate of 2 mL/min. The third port of the "t-cell" is connected to a syringe filled with 3 µM dopamine, made in modified-aCSF.
- To pre-calibrate, allow modified aCSF solution to flow for about 3 - 7 seconds, and then manually inject 1-2 mL of the 3 µM dopamine standard. For each electrode to be pre-calibrated, repeat the calibration at least 3 times and average the maximal currents obtained from each.
- Immediately after the slice voltammetry experiment (see section 3), the electrode is post-calibrated in the same manner as described above.
- The calibration factor (later used during data analysis) is determined by dividing the average dopamine oxidation current (nA) by the concentration of the dopamine standard. For example, the current response is divided by 3 when using a 3 µM dopamine standard.

2. Slice preparation

1. Pour 10 mL of sucrose aCSF into a small beaker and place in an ice bucket. Additionally, place instant adhesive (Loctite 404) in ice bucket, within reach.
2. Prepare a razor blade and the necessary tools required for dissection, such as forceps, spatula, and scissors, by wiping them clean with an alcohol pad.
3. Sacrifice mouse by CO_2 asphyxiation in a small gas chamber, followed by immediate decapitation using sharp scissors. Quickly remove the entire brain. Place the brain in beaker of ice-cold sucrose aCSF for approximately 10 minutes.
4. In the meantime, prepare Vibratome for slicing. First, place some crushed ice in the specimen bath. Position the specimen chamber and tighten to hold it firmly in place. Add more ice around the specimen chamber to fill in the gaps, making sure no ice gets into the chamber. Place the cleaned razor blade in the blade holder on the Vibratome and fill the specimen chamber with ice-cold sucrose aCSF.
5. To set up a working surface for preparing the brain, pour some cold sucrose aCSF on a piece of paper towel that has been placed on an upturned Petri dish. Using forceps transfer the brain onto the prepared Petri dish. For coronal slices, cut the cerebellum along the medial-lateral axis with a razor blade and discard. This creates a flat base that can be affixed to the Vibratome stage.
6. Place a drop of the Loctite adhesive (placed in the ice bucket during Step 1) on the specimen stage. Immediately, affix the flat end of the prepared brain on the stage, keeping it as upright as possible. Place the stage in the specimen chamber and tighten the screw, ensuring that the brain is completely immersed in the sucrose aCSF in the chamber.
7. Using the controls on the front of the Vibratome adjust the stage so that the razor blade lines up with the top of the brain. Optimal parameters for the Vibratome are obtained by setting the frequency and speed to low. Lower speeds are preferred to minimize the force of the blade from squashing the brain, which is observed at higher speeds. Slice thickness is set to 400 µm.
8. The first few slices will not contain the striatum. Repeat the slicing until slices containing the striatum are obtained. Once the striatal region is reached (affirmed by anatomical landmarks), use a paint brush to lift the slice and place in a beaker with oxygenated, room-temperature aCSF. Typically, one can obtain 3 to 4 slices containing the striatal complex so that the caudate-putamen and nucleus accumbens are included.
9. Allow the slices to acclimate in oxygenated aCSF at room temperature for at least 1 hour before using for experiments.

3. Voltammetric recordings from slices

While the slices are incubating, the slice recording chamber can be prepared.

1. Take tubing connected to the submersion recording chamber (Custom Scientific, Denver, CO) and place in oxygenating, room temperature aCSF. Set the perfusion pump (Watson Marlow Limited, Falmouth, England) to a flow rate of 1 mL/min. Set the temperature controller to 32 °C. After aCSF fills the custom-built slice holder (modified mesh disc stage), prepare it for the slice by removing any air bubbles using a needle syringe (BD spinal needle).
2. Prime the slice bath by drawing a vacuum on the outflow tubing (leading to glass liquid waste bottle) using a syringe to start flow. Place a kimwipe to act as a wick on the edge of the slice holder to control buffer overflow.
3. After 1 hour incubation, transfer the slices to the slice holder in the recording chamber, which is continuously perfused with 95% O_2/5% CO_2 room temperature aCSF.
4. Submerge the Ag/AgCl reference electrode in the slice holder (taped to lid of slice chamber) and connect the electrode using an alligator clip to the head stage.
    - The Ag/AgCl reference electrode can be made in house by anodizing (+1 V) a 250 µm silver wire (A-M Systems, Carlsborg, WA) in 1 M HCl for 5 minutes to deposit a thin layer of AgCl on the surface of the silver wire.
5. Lower the tungsten stimulating electrode (Plastics One, Roanoke, VA) to the surface of the striatal brain slice. The stimulating electrode makes contact with the slice as it rests on top of it, but should not puncture the slice. In our experimental set-up, the stimulation is generated by a Neurolog stimulator.
6. The carbon fiber working microelectrode is back-filled with a 150 mM KCl solution using a BD spinal needle. A lead wire is then inserted (Squires Electronics, Cornelius, OR), which is connected to the head stage of the low-noise ChemClamp potentiostat (Dagan Corporations, Minneapolis, MN) using an alligator clip. The working electrode is placed approximately 100 - 200 µm away from the bipolar stimulating electrodes, about 75 µm deep into the slice.

7. Electrical stimulations, using either single (monophasic, 350 µA, 60 Hz, and 4 ms pulse width) or multiple (e.g. 5 pulses, 350 µA, 20 Hz, and 4 ms wide) pulses, are delivered by the stimulating electrode to evoke neurotransmitter release [3].
8. To measure electrically evoked dopamine using FSCV, a triangle waveform is applied to the electrode. Typical parameters for dopamine detection: potential of the carbon fiber microelectrode is held at -0.4 V versus a Ag/AgCl reference electrode, ramped to a positive limit of +1.2 V, then brought back down to -0.4 V at a scan rate of 400 V/s.
9. The slice is electrically stimulated every 5 minutes and voltammetric measurements of the resulting dopamine efflux are made for 15 seconds.
10. After at least three stable electrically stimulated dopamine release recordings (difference between peak height is < 10%), aCSF containing pharmacological agent of interest is perfused at a flow rate of 1 mL/min over the slice for 30 minutes to obtain maximal effect. Electrically stimulated dopamine recordings are made every 5 minutes during the pharmacological perfusion.

4. Data analysis

Resulting current versus time traces obtained from the slice can be fit by nonlinear regression to a set of Michaelis-Menten based equations, as described by Wightman and colleagues in software written in LabVIEW (National Instruments, Austin, TX) [4-6]. In this software, current versus time traces can be fitted by varying two parameters, V_{max} (nM/s; corresponding to the rate of uptake by the dopamine transporter as demonstrated by the descending phase of the trace) and dopamine concentration per pulse (nM; corresponding to the peak height maximum). The K_m value, reflective of the affinity of dopamine for the dopamine transporter, is set to 160 nM and not changed. The electrode's post-calibration factor that is determined after the experiment is required prior to fitting. The LabView software contains the coefficient of determination (R^2) parameter to determine the goodness of fit (R^2 values > 0.8 are used).

5. Representative Results

FSCV was used to examine single-pulse, electrically stimulated dopamine release and uptake in the caudate-putamen (CPu), nucleus accumbens (NAc) core, and NAc shell in mice. Representative results shown in Figure 1A demonstrate current (or concentration) versus time plots. The red arrow indicates when electrical stimulation is applied to the slice followed by a corresponding rise in the amount of current attributable to changes in dopamine concentration at the carbon fiber microelectrode. The predominant process during electrical stimulation is dopamine release, but other processes such as uptake and diffusion contribute to the overall observed peak height as well. The descending phase of the peak is mainly attributed to reuptake of the neurotransmitter by its transporter since neuronal stimulation has been stopped [7]. However, the peak decay is not limited to reuptake, as diffusion and metabolism also contribute to the decrease in current. It has been postulated that since the time scale of electrochemical measurements is a matter of seconds, FSCV is too fast to measure contributions from metabolism [7]. In these current versus time traces, the y-axis is converted to concentration (µM) using the post-experiment calibration factor. The inset for Figure 1A is the respective background subtracted cyclic voltammograms for the current versus time traces. Plotting the measured current (y-axis) against the applied potential to the electrode (x-axis), dopamine is chemically identified with an observed oxidation peak at +0.6 V and a corresponding reduction peak of dopamine-ortho-quinone is observed at -0.2 V versus a Ag/AgCl reference electrode. The third representation of the data uses a three-dimensional pseudo-color plot (Figure 1B) combining the information from both the current versus time traces and the cyclic voltammogram to form a single plot. In the representative pseudo-color plot, time is plotted in seconds along the x-axis, the applied voltage to the carbon fiber working microelectrode is graphed along the y-axis, and current is represented as false color along the z-axis. Due to the small size of carbon fiber microelectrodes (~ 7 µm in diameter), electrically stimulated dopamine dynamics can be detected in discrete anatomical regions of the striatum (CPu versus NAc core versus NAc shell; Figure 2).

An advantage of using striatal coronal slices is that it eliminates contributions from the dopamine cell bodies, and allows for an investigation of presynaptic dopamine dynamics. Presynaptic control of dopamine release and uptake is not strictly limited to dopamine autoreceptor or transporter functions as others have shown [16, 17]. Heteroreceptors of other neurotransmitter systems also modulate dopamine dynamics [18, 19]. Representative current versus time traces shown in Figure 3A demonstrate that when slices are treated with 1 µM quinpirole (D2/D3 receptor agonist) for 30 minutes, a decrease in electrically-evoked dopamine release is observed. On the other hand, when a substrate for the dopamine transporter, such as methamphetamine, is perfused over the slice for 30 minutes, no difference in dopamine release is observed (Figure 3B). The peak decay is shifted to the right, which is typically associated with alterations in dopamine transporter kinetics (K_m) [3]. Finally, Figure 3C is a representative trace of the current versus time trace once the slice has been bathed in a 100 ng/mL brain-derived neurotrophic factor (BDNF) solution, which has been hypothesized to influence dopamine release dynamics [20, 21]. From this representative trace, it can be seen that BDNF has the ability to enhance electrically-evoked dopamine release. Taken together, these pharmacological treatments emphasize the utility of FSCV to probe dopamine dynamics within the striatum.

The primary limitation of using brain slices to investigate presynaptic dopamine dynamics by FSCV is that the neurocircuitry from an intact brain is lost. With slice FSCV it is impossible to study the effects of neurotransmitters from other brain regions, making it difficult to understand contributions of these systems on the functionality of the region being investigated (e.g. the striatum) or to evaluate non-stimulated dopamine levels. However, recent technical advances in FSCV has allowed for dopamine transient measurements (with and without electrical stimulation) in freely moving rats in response to a pharmacological manipulation, self-administration, or novelty [22 - 24]. Overall, slice FSCV provides valuable information on presynaptic dopamine dynamics, and coupling slice FSCV results to complementary neurochemical techniques such as microdialysis, electrophysiology, and/or freely moving FSCV offers a more comprehensive view of neurotransmitter functioning in the brain.

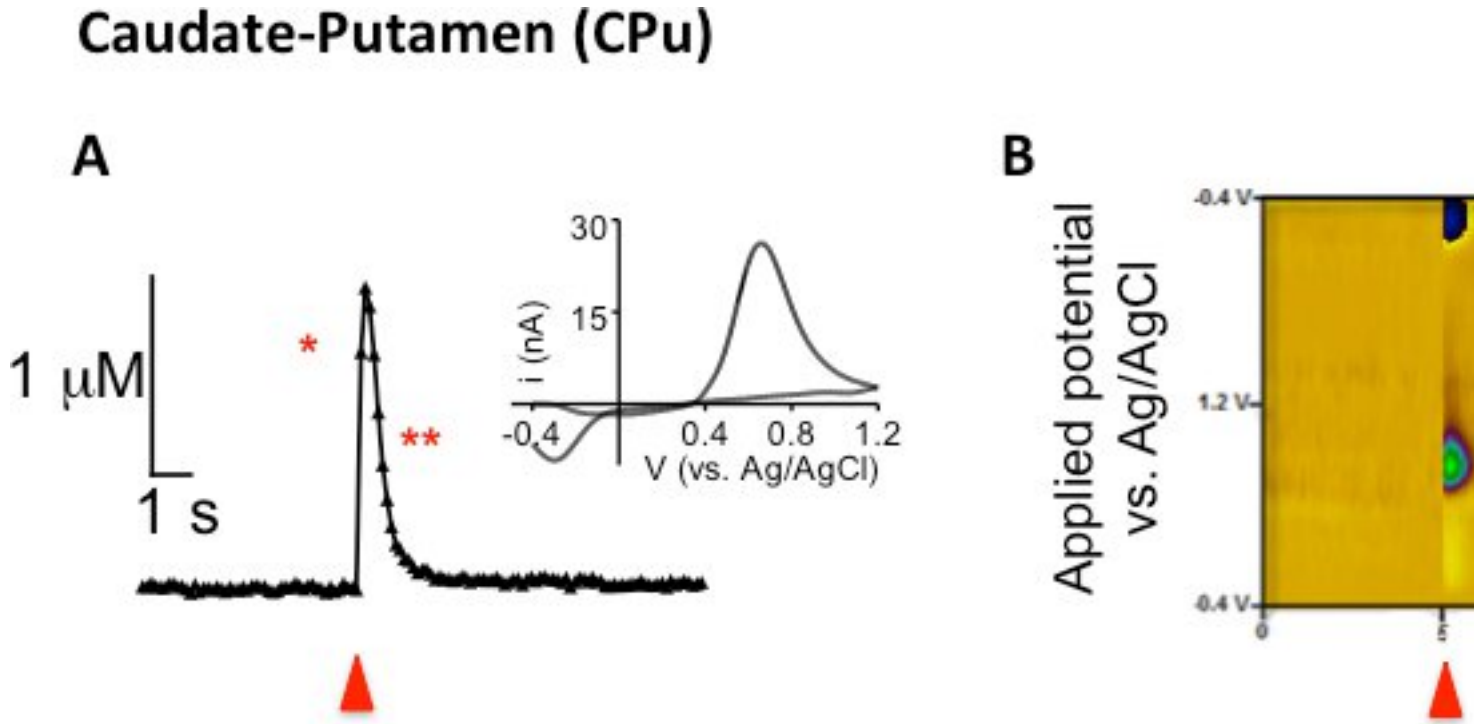

Figure 1. Electrically-evoked dopamine release measured using FSCV following single-pulse stimulation in dorsal CPu slices from C57Bl/6J mice. (A) The concentration versus time trace in which dopamine release was evoked by a single pulse (red arrow). The single asterisk represents the factors that contribute to the rise in concentration, which is predominantly dopamine release, but uptake and diffusion also contribute. The double asterisk represents the peak signal returning to baseline, mainly due to uptake but also diffusion contributes. Inset displays the corresponding cyclic voltammograms. (B) Representative color plots from the dorsal CPu display time (x-axis), applied potential to the carbon fiber microelectrode versus Ag/AgCl reference electrode (y-axis), and current in pseudo-color.

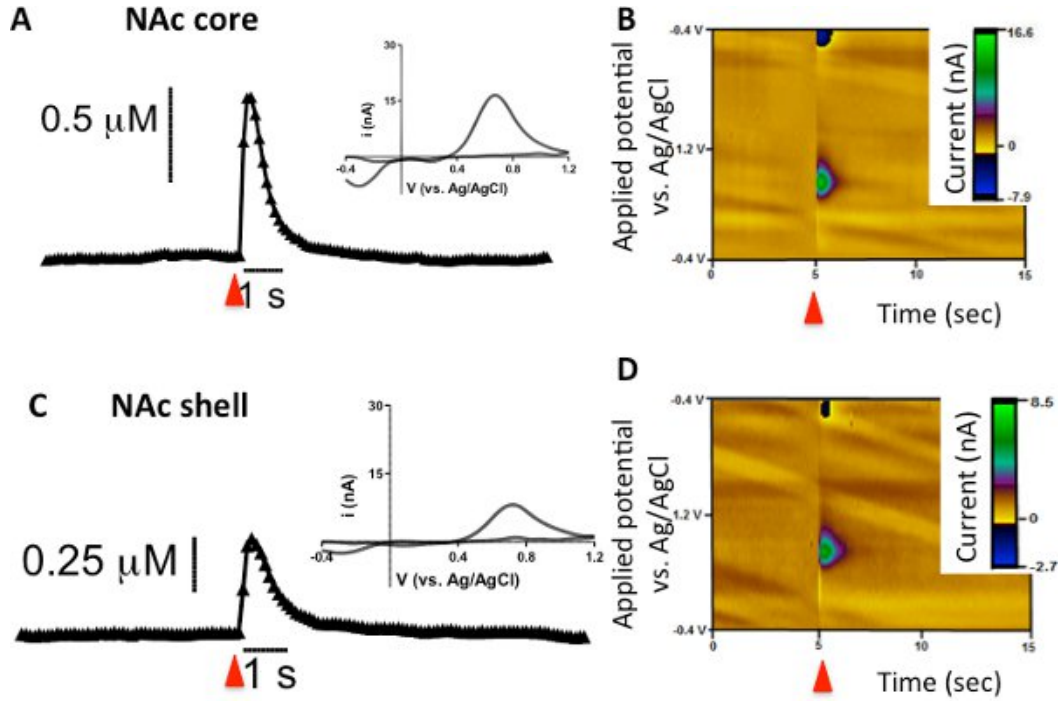

Figure 2. Dopamine release evoked by a single electrical stimulation pulse (indicated by the red arrow) in the NAc core and shell from C57Bl/6J mice. (A and C) The concentration versus time traces and their corresponding cyclic voltammograms (inset) from the NAc core and shell. (B and D) As previously described, representative color plots from the NAc core and shell.

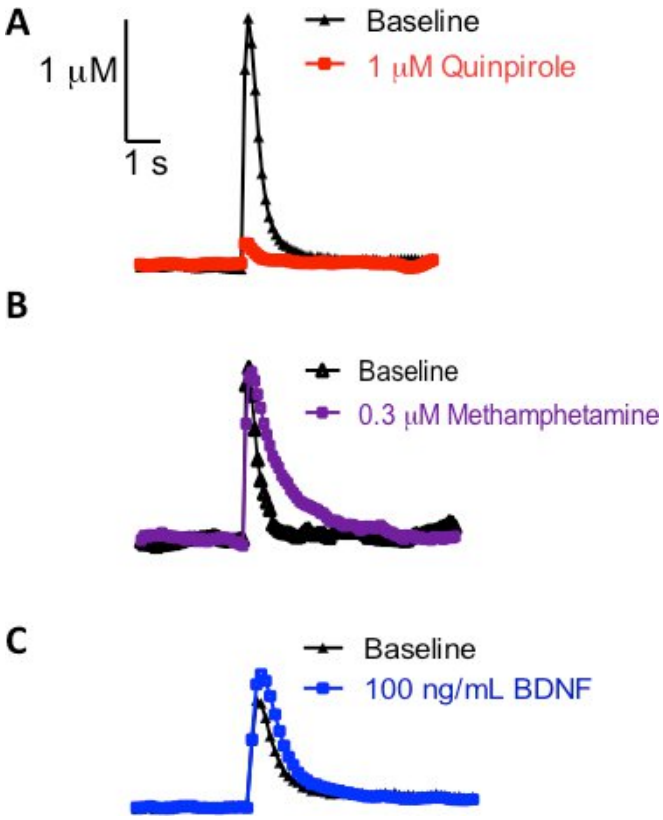

Figure 3. Representative traces after the slice was treated with a pharmacological agent for 30 minutes; in all cases, a single pulse was used to evoke dopamine release in the CPu. (A) Application of the dopamine D2 receptor agonist, quinpirole (red trace) compared to pre-treatment (black trace). (B) Methamphetamine perfusion (purple trace) compared to pre-treatment (black trace). (C) The ability of brain-derived neurotrophic factor to influence dopamine dynamics (blue trace) compared to pre-treatment (black trace).

Discussion

The protocol presented here demonstrates how to prepare and use mouse coronal brain slices for FSCV experiments. Although this method is specific to obtaining and measuring dopamine dynamics, other neurotransmitters such as adenosine, hydrogen peroxide, norepinephrine, and serotonin have been monitored *in vivo* or *in vitro* with FSCV [3, 8 - 11]. FSCV can be used to monitor some of these other neurochemicals by simple modifications of the waveform applied to the working electrode [3, 11]. Since many of these neurochemical species have similar oxidation potentials, the cyclic voltammograms generated provide a unique chemical fingerprint for each oxidizable species, which allows chemical identification. Furthermore, FSCV has been used in various species, from fruit flies to non-human primates, to gain a better understanding of neurotransmission in these model organisms [12 - 15]. One of the primary reasons that FSCV has been used in such a variety of species is due to the small diameter of the carbon fiber microelectrodes, typically less than 7 μm in diameter. As a result, these microelectrodes make it possible to sample tissue from very small environments, as in the case of the fruit fly brain (nL), or to discriminate from discrete sub-anatomical regions like the NAc core versus the shell in larger species [12-14].

In conclusion, the results presented here demonstrate that slice voltammetry is an invaluable electrochemical tool to probe presynaptic dopamine dynamics in the mouse striatum. The representative data focuses on perfusing pharmacological agents over a brain slice from a 'normal or healthy' control and the ability to characterize parameters of dopamine release and uptake. Furthermore, FSCV can be used to evaluate differences in electrically-stimulated release and uptake parameters in genetically modified or treated animals on their own or after pharmacological treatment [15 - 16]. Slice FSCV provides a unique opportunity to investigate dopamine neurotransmission dynamics within discrete anatomical regions that occur on a timescale of milliseconds. Overall, the electrochemical technique of FSCV provides both enhanced spatial and temporal resolution compared to other neurochemical techniques.

Disclosures

No conflicts of interest declared.

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