Video Article

Examination of Rapid Dopamine Dynamics with Fast Scan Cyclic Voltammetry During Intra-oral Tastant Administration in Awake Rats

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Abstract

Rapid, phasic dopamine (DA) release in the mammalian brain plays a critical role in reward processing, reinforcement learning, and motivational control. Fast scan cyclic voltammetry (FSCV) is an electrochemical technique with high spatial and temporal (sub-second) resolution that has been utilized to examine phasic DA release in several types of preparations. *In vitro* experiments in single-cells and brain slices and *in vivo* experiments in anesthetized rodents have been used to identify mechanisms that mediate dopamine release and uptake under normal conditions and in disease models. Over the last 20 years, *in vivo* FSCV experiments in awake, freely moving rodents have also provided insight of dopaminergic mechanisms in reward processing and reward learning. One major advantage of the awake, freely moving preparation is the ability to examine rapid DA fluctuations that are time-locked to specific behavioral events or to reward or cue presentation. However, one limitation of combined behavior and voltammetry experiments is the difficulty of dissociating DA effects that are specific to primary rewarding or aversive stimuli from co-occurring DA fluctuations that mediate reward-directed or other motor behaviors. Here, we describe a combined method using *in vivo* FSCV and intra-oral infusion in an awake rat to directly investigate DA responses to oral tastants. In these experiments, oral tastants are infused directly to the palate of the rat – bypassing reward-directed behavior and voluntary drinking behavior – allowing for direct examination of DA responses to tastant stimuli.

Video Link

The video component of this article can be found at https://www.jove.com/video/52468/

Introduction

Phasic DA release plays an important role in mediating reward-directed behavior [1-3]. However, isolating and studying how a primary reward alters phasic DA release is often complicated by co-occurring behavioral or cognitive processes that are also capable of altering phasic DA release - such as decision making processes or reward-directed motor behavior to acquire the reward. In the current work, we isolate phasic DA responses to tastants through the use of *in vivo* fast-scan cyclic voltammetry (FSCV) combined with tastant delivery through intraoral cannulae. This technique bypasses choice and action and allows us to examine extracellular DA release during direct infusion of a tastant to the palate of a rat.

FSCV is an electrochemical technique with high temporal and spatial resolution, permitting measurements of DA release in a discrete local area (approximately 100 µm) on a sub-second scale (10 Hz resolution). The combination of intra-oral delivery and FSCV provides the advantage of observing rapid, 'real-time' DA responses to a tastant, which cannot be examined using conventional microdialysis methods. Furthermore, phasic DA release in response to either rewards or reward-associated cues occurs at concentrations between 20-100 nM, which is above the 10-20 nM detection threshold for FSCV [4]. The high spatial resolution of FSCV also permits recording from sub-regions of small brain areas, such as the nucleus accumbens core (NAc). Thus, FSCV combined with intraoral infusions of tastants is an ideal model for studying how tastants or other stimuli alter phasic DA release in an awake, behaving animals. Indeed, these techniques have permitted experiments investigating how rewarding and aversive tastants alter extracellular DA release [5].

Over the last decade, FSCV analyses have been successfully combined with intravenous drug delivery [6] and rat self-administration paradigms [7, 8] to identify the role of phasic DA mechanisms in drug addiction models. In addition, combined intraoral delivery with FSCV has been used to examine how tastant cues paired with cocaine availability modulate phasic DA release and behavioral responses that reflect emotional affect [3]. This combined intraoral and FSCV methodology can also be powerfully utilized to examine phasic DA responses to flavorants, such as menthol and oral sweeteners, that are added to cigarettes and dissolvable tobacco products [9-11]. Although many of the flavorants added to tobacco products are appetitive [9-11], it is unknown if these flavorants increase phasic DA release in a manner consistent with a rewarding hedonic valence. Indeed, flavorants added to cigarettes and to dissolvable tobacco products may have direct effects on the DA reward system

and may act through dopaminergic mechanisms to influence the rewarding valence of cigarettes and other tobacco products. Thus, intraoral delivery combined with FSCV can provide new understanding on how flavorants modulate rapid DA release. The use of combined intra-oral and *in vivo* FSCV methodology, and the data obtained from such studies, can also facilitate future studies to determine how flavorants and nicotine interact to alter DA signaling and to potentially modulate nicotine reinforcement. Further, the data gained from such studies can be used to inform regulatory decisions about tobacco product flavorants.

Protocol

All experiments were conducted according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Yale University Institutional Animal Care and Use Committee (IACUC).

1) Pre-surgical Preparations

1. Oral solution preparation
    1. Create solutions of 10% sucrose and 0.005% L-menthol in deionized water (pH 7.4). Store these solutions in closed containers, at RT, and remake every 2 weeks, to prevent degradation.

2. Electrode calibration solutions
    1. Make Tris buffer (pH 7.4) at any time prior to calibration. The solution consists of 12.0 mM Tris-HCl, 140 mM NaCl, 3.25 mM KCl, 1.20 mM CaCl2, 1.25 mM NaH2PO4, 1.20 mM $MgCl_2$, and 2.00 mM Na_2SO_4.
    2. Create a stock solution of 10 mM dopamine (DA; dopamine hydrochloride) in 0.1 M perchloric acid. The perchloric acid helps prevent oxidation of dopamine. Store this stock solution at -20 °C and use for up to 6 months. Make several aliquots of the DA stock solution for storage to use each aliquot only once.
    3. From the 10 mM DA stock, dilute solutions in Tris buffer to concentrations of 1 µM, 500 nM, 250 nM, 125 nM, 62.5 nM, and 31.25 nM.
    4. For pH calibration, prepare solutions of Tris buffer with the pH of 7.2, 7.3, 7.5, and 7.6. This will provide solutions that mimic pH shifts that may occur during the *in vivo* experiment.

3. Electrode fabrication
    1. By vacuum suction, aspirate a T-650 carbon fiber (7 µm diameter) into a borosilicate glass capillary (length 10 mm, outside diameter 0.6 mm, inside diameter 0.4 mm).
    2. Place glass capillary filled with carbon fiber into a vertical electrode puller. For initial parameters, set the heater to 55.0 and turn off the magnet. However, further optimization may be necessary as both heater and magnetic settings vary from lab to lab.
    Note: The electrode should be at least 5.5 cm long (including taper) so that it can easily fit into the micromanipulator and be inserted at least 6.9 mm below dura in the rat brain. If the electrode is too short, recordings from ventral portion of the nucleus accumbens will not be achievable. On the other hand, the total electrode length should not exceed 6.5 cm otherwise it will be too long to fit into the micromanipulator.
    3. Using a light microscope, cut the exposed carbon-fiber so that it extends 75-100 µm beyond the end of the glass. Verify that the electrode has a very tight, barely discernible, seal between the glass electrode and carbon fiber, which will produce minimal noise during subsequent recording. Discard the electrode if there are any noticeable cracks in the glass or if the seal is loose, which is indicative of a poor seal. For more detailed instructions see [12] and [13].

4. Assembling the electrode into the micromanipulator
    1. Obtain a 3 in, 30G wire that is insulated along half the length of the wire and use a small brush to apply a thin layer of silver paint directly to the wire. Immediately after applying silver paint, insert the wire into the hole on the top of the electrode to provide a physical and electrical connection to the carbon fiber. Under a microscope, ensure that the silver wire makes contact with the carbon fiber.
    2. Secure the silver wire and electrode to the micromanipulator using heat shrink.
    3. Place the prepared carbon-fiber electrode into purified isopropyl alcohol (IPA) for at least 10 min to clean the electrode surface and to increase subsequent sensitivity to dopamine. After soaking in IPA, rinse the carbon fiber with tap water to remove the IPA.

5. Reference electrode fabrication
    1. Take a 13 mm silver wire containing an approximately 6 mm Ag/Ag Cl mesh (7 mm silver wire alone, 6 mm mesh, 0.4 mm diameter), cut the silver portion down to approximately half its original length and solder the silver portion of the wire to a gold pin.
    2. Apply epoxy over the solder on the pin.
    3. Dip the Ag/Ag Cl mesh end into a copolymer of 5% polytetrafluroethylene and perfluoro-3,6-dioxa-4-methyl-7octene-sulfonic acid 5 times, with 30 min air drying periods between each dip.
    4. Allow the coated Ag/Ag Cl mesh to air dry O/N.
    5. Cure in oven at 120 ºC for 1 hr.

2) Combined Intraoral Surgery and Intracranial Cannulation Surgery (Approximately 75 min)

1. Oral catheter fabrication
    1. Make the oral catheter, and sterilize by ethylene oxide sterilization, at least 10 days prior to surgery.
    2. Cut PE 100 tubing into 6 cm long segments.
    3. Using a soldering iron, melt one end of the PE tubing and immediately press against a solid surface to cool the PE tubing. The result should create a small, flat disc at one end of the PE tubing and should not be flanged. Use a needle (18 G) or push pin to create a hole in the center of this disc.

4. On the other end of the PE tubing, snugly insert the blunt end of a 18 G needle into the tube.
5. Using a standard paper hole puncher, punch several circular pieces of a polytetrafluroethylene sheet (3.18 mm thick, 6 mm in diameter) to create polytetrafluroethylene washers.
6. Create a hole in the center of the washer. Take the needle attached to the PE tubing and push it completely through the washer. Once the needle is through, slide the washer to the bottom of the PE tubing so that it is flush against the washer.

2. Oral surgery
    1. After sterilizing the surgical tools, cannula (trimmed to 2.5 mm below the pedestal before sterilization), and reference electrode, anesthetize the rat with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg).
        1. After 5 min, apply a noxious stimulus test (foot or tail pinch) to assess the level of anesthesia. If the subject shows any physical reaction to the noxious stimulus test (flinch response, increase in respiratory or heart rate), administer a ketamine boost of 10% to 15% of the original dose and repeat the noxious stimulus protocol above.

    2. After the rat is fully anesthetized and shows no reaction to the noxious stimulus test, use animal hair clippers to shave the rat's fur from the eyes to about 2 mm behind the ears. Then place the rat on a thin water recirculating heating pad to maintain body temperature during surgery. Apply eye lubricant. Administer the non-steroidal anti-inflammatory (NSAID) drug, Carprofen (5 mg/kg), by intraperitoneal injection prior to performing any incisions and prior to inserting intraoral cannulas. Use aseptic technique at all times.
    3. Apply betadine followed by 70% ethanol to clean the skin. Repeat 3 times.
    4. Place the rat on its back, and open the mouth using a sterile cotton swab. Find the first upper molar.
    5. Insert the needle into the tissue between the cheek and the first upper molar until the needle exits just behind and medial to the ears.
    6. Pierce the needle through the skin until the PE tubing exits the skin and is accessible.
    7. Pull up on the cannula (gripping by the cannula itself and not the needle) and secure the polytetrafluroethylene washer into the molars so that the catheter remains in place.
    Note: Cutting the washer into a trapezoid shape and securing it against the molars with the flat side facing the cheek makes this step easier.
    8. Take another polytetrafluroethylene washer and slide it over the exposed needle, down the plastic tubing, and abut against the incision.
    9. To secure the polytetrafluroethylene washer, use sterilized tape to create a triangular "flag" so that the washer does not slide up. Secure the washer against the rat's skin and the sterilized tape.
    10. Cut the exposed catheter to allow 2-4 cm of tubing to be exposed above the rat's head.
    11. Repeat steps 2.2.1 through 2.2.10 for the other side of the mouth.

3. Intracranial surgery for voltammetry
    1. Place the rat in the stereotaxic frame and clean the animal’s scalp using a two stage scrub (a betadine scrub followed by a 70% ethanol scrub, perform with a 3 cycle repetition).
    2. Use sterilized needle nose tweezers and surgical scissors to cut away a 15 x 15 mm section of scalp tissue.
    3. Gently clean the surface of the skull using sterilized cotton tip applicators. Further clean the skull by applying 2 to 3 drops of 3% hydrogen peroxide.
    4. Use a stereotaxic drill to make 3 small (1 mm diameter) holes in the skull for subsequent insertion of screws, 1 hole for the guide cannula, 1 hole for the stimulating electrode, and 1 hole for the reference electrode.
    5. For recordings in the NAc core, position the guide cannula at 1.2 mm anterior and 1.4 mm lateral to Bregma. Lower the cannula apparatus until the plastic base is flush with the skull.
    6. Place the sterilized reference electrode in the hemisphere contralateral to the guide cannula. Lower the reference approximately 2 mm below the dura.
    7. Position the stimulating electrode at 5.2 mm posterior and 1.0 mm lateral to Bregma and lowered 8.0 mm below dura to target the ventral tegmental area (VTA).
    8. Use a sterilized small surgical screwdriver to secure screws to the skull. Then, insert the reference electrode, stimulating electrode, and guide cannula. Finally, secure all components using cranioplastic cement.
    9. For the first 48 hours of post-operative care, administer the non-steroidal anti-inflammatory drug (NSAID) Carprofen by subcutaneous injection (5 mg/kg). Allow at least 1 week for complete surgical recovery prior to performing voltammetry recording.
        1. During post-operative care, flush oral catheters daily with water. Provide food saturated in water for 2 days to aid the animal in chewing and eating. Provide daily monitoring for potential signs of stress of pain (due to mild infection or dehydration) and provide appropriate interventions as necessary (including administration of fluids, topical administration of antiseptics, and or NSAID Carprofen administration at 5 mg/kg).

3) Voltammetric Recordings in Awake, Freely Moving Rat

1. Lowering the electrode and acquiring a DA training set.
    1. On the day of the experiment, check the patency of the oral cannula by infusing 200 µl of water and observing orofacial responses (to verify that the rat is tasting the water).
    2. Attach the FSCV headstage to the rat by inserting the stimulating electrode (on the headstage) into the bipolar stimulating electrode cannula (on the rat). Thus, the headstage (miniaturized current-to-voltage converter) connects directly to the bipolar stimulating electrode.
    3. Remove the dummy cannula from the cannula apparatus and apply two drops of 50% heparin solution into the cannula. Then gently insert a dummy cannula (slightly longer that the dummy cannula that was previously removed) to clear any blood clots or glial scarring that may have occurred, which will break carbon fiber electrodes that will subsequently be inserted during the experiment. Extend the cannula used for clearing clots 3-4 mm below the skull (at least 2 mm above the recording site).
    4. Insert the micromanipulator, with electrode, into the guide cannula and place the omega ring in a "locked" position.

5. Connect the reference electrode wire from the headstage to the appropriate pin. Then connect the working electrode wire from the micromanipulator to the appropriate wire on the headstage.
6. Lower the electrode to approximately 4-4.5 mm below skull.
7. Use a potentiostat to apply a triangular waveform (400 V/s, -0.4 to +1.3 V). Apply the waveform to the electrode at 60 Hz for at least 10 min, then change the waveform application frequency to 10 Hz for subsequent experimental recordings.
   Note: The 60 Hz waveform application step produces a stable carbon fiber surface and will help prevent electrical drift
8. Apply electrical stimulation to the VTA using various frequencies (10 to 60 Hz) pulses (10 to 30 pulses) and currents (50 to 150 µA) all with a pulse width of 2 ms/phase, to evoke different concentrations of DA release and pH shifts. Acquire at least 5 different concentrations of DA release and 5 different pH shifts. Wait (2 - 3 min minimum) between stimulations to allow for vesicular reloading[14].
   Note: It is important to obtain stimulations that produce DA concentrations across the entire range that will be collected during the subsequent experiment since they will be used as a training set for Principal Component Analysis (see section 5.1).
9. Lower the electrode into the NAc core (6.3 mm ventral to dura). Subsequently lower the electrode in 100 µm increments within the NAc core until transient DA release events are observed at frequencies of at least 1 per min.
10. Connect the tubing (inner diameter 1/32", outer diameter 3/32") to a 20 ml syringe, attached to a syringe pump. On the other end of the tube, use a beveled 23 G needle to connect the tubing to the rat's cannula. Ensure that the tubing is filled completely with the desired tastant and verify that no bubbles exist in the tubing.
    Note: Typically, administer a small amount of tastant into the mouth of the rat prior to recording to ensure that the first infusion for recording delivers the full infusion amount and not any residual water or air in the tubing. Moreover, this allows the animal to have some prior experience with the tastant since novel tastants, even appetitive ones, can be aversive initially due to its novelty.
11. For data collection, infuse 200 µl of tastant (6.5 sec using a pump and previously described tubing). Infuse the tastant every 1-3 min. Infuse a total of 25 times per tastant. Each infusion delivers 200 µl of solution.
12. If multiple tastants are being delivered in a single experiment, flush the oral catheter with water after collecting data for one tastant and wait at least 20 min before infusing the new tastant.
13. After the DA recording session, prepare for histological verification by creating a small lesion at the recording site. To preserve the carbon fiber electrode for subsequent calibration, first retract the electrode and remove the micromanipulator. Then use a new micromanipulator with a glass microelectrode and a tungsten wire (extending 100 µm beyond the glass tip) to the same depth (below dura) as the original carbon fiber microelectrode.
14. For histological verification, create a small lesion by applying 3 V at the tungsten wire and increasing the voltage at 1 V increments, every 10 sec, until the 10 V setting is reached.
15. If a standard concentration curve has already been created using multiple carbon fiber electrodes, perform the lesion step above (3.1.14) by applying the potential directly the carbon fiber electrode.
    Note: This lesion method will damage the carbon fiber and prevent subsequent calibration with that specific electrode.
16. Euthanize the animal using a lethal injection pentobarbital (150 mg/kg, i.p.).
17. Dislodge the headcap with the cannulae using pliers by pulling directly upward on the headcap. Do not twist the headcap since this will cause unnecessary damage to the brain and make it challenging to identify the location of the electrode during histology.
    1. Remove the brain and place in 4% formalin for 3 days followed by 30% sucrose for 1 week. Create 30 µm slices using a cryostat, mount on cover slips and examine slices under a light microscope to identify the location of the carbon fiber or tungsten lesion.
       Note: Perfusion is optional for 3.1.17.

4) Electrode Calibration

1. Using the flow cell to create a current-concentration conversion factor.
   1. In order to determine electrode sensitivity, calibrate electrodes after each experiment using a flow "t-cell" apparatus. Lower the electrode into the "t-cell" while Tris buffer, pH solutions, or DA solutions are washed over the electrode surface (at a rate of 2 ml/min). Use a six-position air solenoid actuator to switch back and forth between buffer and pH or DA solution.
   2. For each calibration, collect voltammetric data during 5 sec application of Tris buffer (baseline) followed by 5 sec application of pH or DA solution, followed by 20 sec application of Tris buffer (30 sec total file). Repeat each run 3 times for each calibration standard concentration, counterbalancing between different concentrations of each standard.
   3. For each sample collection, measure the peak DA or pH-evoked current. Average the peak current across each trial to obtain a concentration versus peak current relationship. For more detailed instructions see [12] and [13].

5) Data Analysis

1. Using a training set in principal component analysis (PCA)
   Note: During a voltammetry experiment, output is produced as current, which is the result of dopamine oxidation and reduction, pH shifts, and electrochemical noise. PCA allows separation of these factors so that one can isolate the desired components (DA and pH) and remove additional components that might distort the desired signals (for a more detailed description, see [15, 16]).
   1. Assign a calibration factor to the output current (approximately 5-10 nA/µM) after PCA to allow concentration values of the analytes to be determined.
   2. Use the training set obtained during an experiment to "train" the PCA model to separate out DA, pH, and other factors. PCA takes the training set cyclic voltammograms (collected in section 3.1.8) and determines which essential characteristics of the voltammogram can be used to identify each analyte collected during *in vivo* voltammetry recordings.
   3. To determine the number of principal components to keep, use a Malinowski F-test. Typically, keep only 2 or 3 principal components which normally explain about 95-99% of the variance in the data. For further discussion on implementing the Malinowski F-test, see[17].

Note: Once the number of components are determined, the PCA analysis effectively will project the measured voltammograms from DA and pH training sets onto the principal components that have been retained. The result is a data set that has been filtered so that only DA (or pH) data remains and the residual data that does not resemble DA or pH is removed (See Representative Results for expected output). For a further detailed explanation and step by step instruction for using PCA for voltammetric analysis see Materials sheet and[16, 17].

Representative Results

FSCV combined with intraoral catheter implantation was used to examine how sucrose, an appetitive tastant, modulates phasic DA release in the NAc core. Prior to tastant infusion, electrical stimulation (150 µA, 60 Hz, 24 pulses; indicated by the red bar) of the VTA produces robust increases in phasic DA release in the NAc (**Figure 1**). **Figure 1** shows a color plot with potential on the y-axis, time on the x-axis, and current (represented as false color) on the z-axis. Below the color plot is DA concentration versus time trace. Concentration versus time was determined using the current versus time trace at the oxidation potential of dopamine (indicated by dashed white line) which was converted to concentration, following post-calibration of the electrode. This DA concentration versus time trace was used as part of the training set for PCA.

After the training set was acquired, 25 infusions of sucrose (10%, 200 µl per infusion) were given every 1-3 min. Examples of sucrose modulation of phasic DA release are shown in **Figures 2 and 3**. In **Figure 2**, the 6.5 sec infusion (indicated by the red bar) produced an elevation in current (indicated by the white, downward facing arrow) at the oxidation potential of DA (indicated by the white dashed line). Transforming the data into a concentration versus time plot after PCA verified that the increased current in responses to the tastant is the result of an increase in DA concentration. Another tastant, menthol (0.005%, 200 µl per infusion) was also infused during FSCV recordings in the NAc core. **Figure 3** shows DA concentration versus time example traces for intra-oral sucrose and menthol, revealing increased DA concentration after infusion of either tastant. One should expect to see some trial to trial variability in phasic DA release to tastants (**Figure 2 and 3A**). However, we do not typically see any non-specific trends towards an increase or decrease in phasic DA release over multiple trials. Ranges from 10-100 nM DA can be expected in a typical experiment in a single animal.

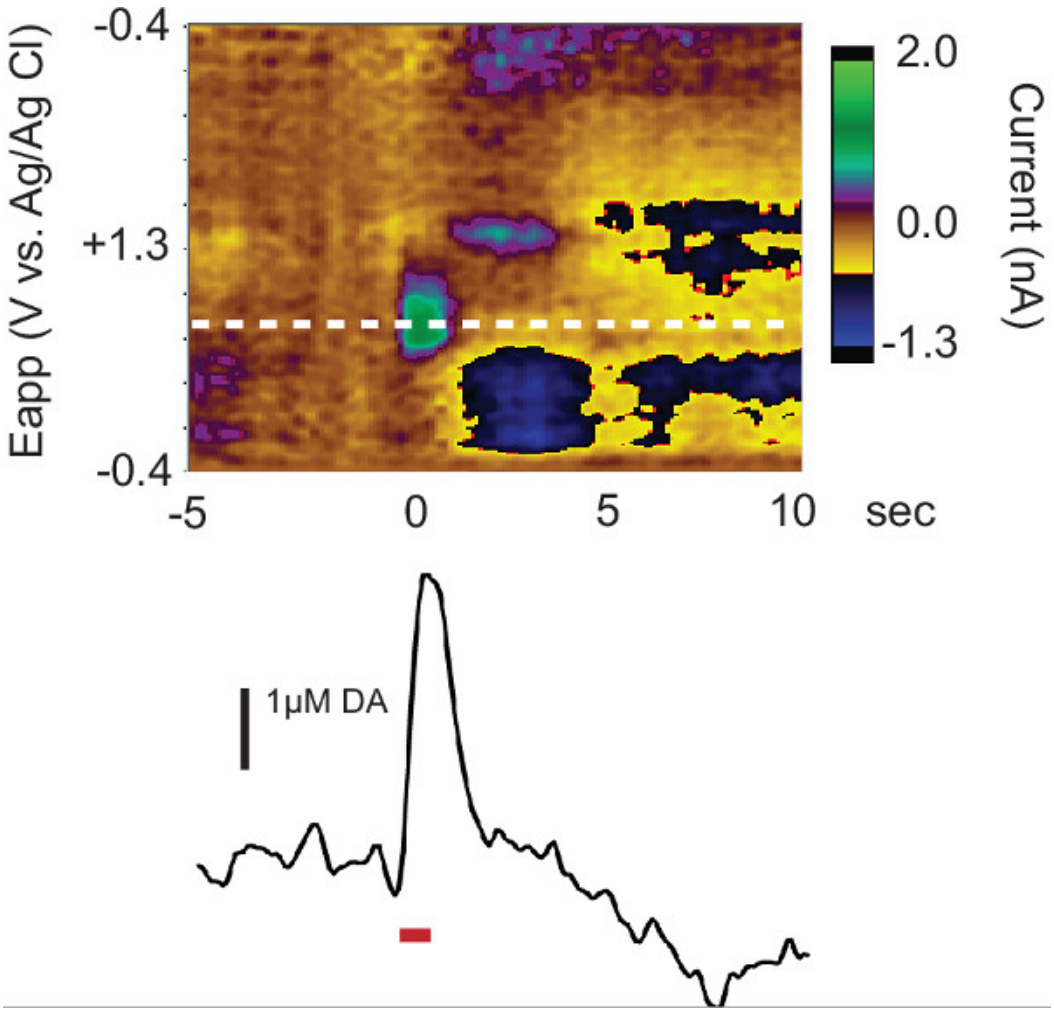

Figure 1. Representative color plot (top) and concentration versus time plot (bottom) of electrically evoked DA release in the NAc core. Red bar indicates stimulation time. Please click here to view a larger version of this figure.

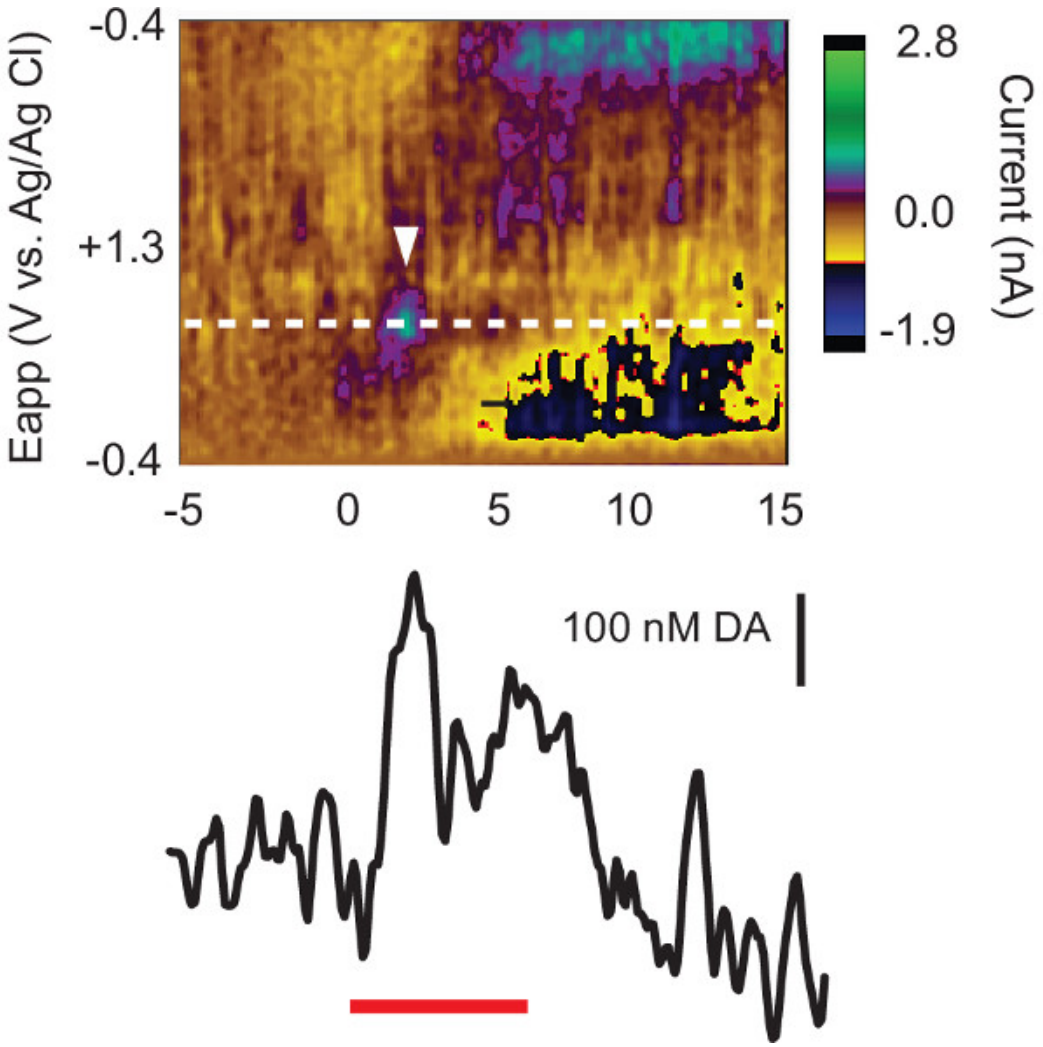

Figure 2. Representative color plot (top) and DA concentration versus time trace (bottom) during a single infusion of sucrose. Red bar indicates 6.5 sec infusion. White downward facing arrow indicates in elevation in current (represented in false color) at the oxidation potential of DA. Please click here to view a larger version of this figure.

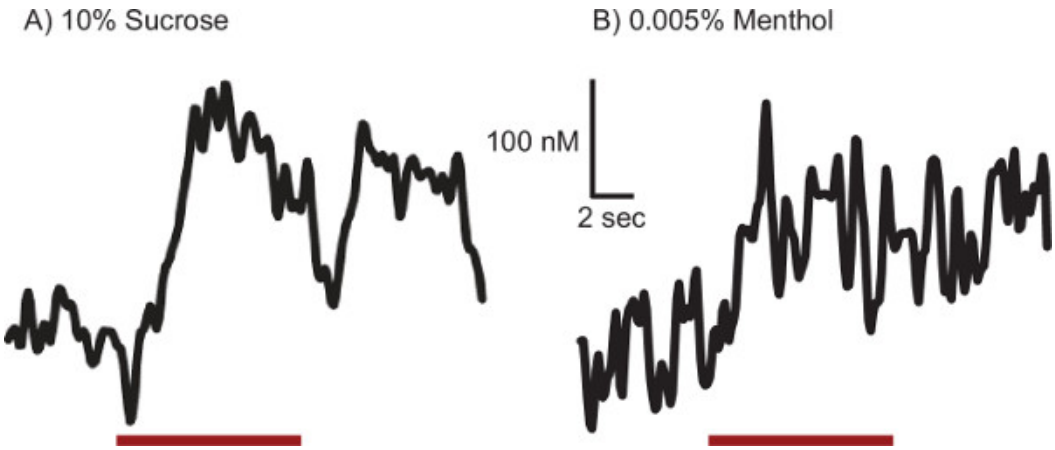

Figure 3. Representative DA concentration versus time traces during (A) 10% sucrose and (B) 0.005% menthol by intraoral administration. Red bar indicates 6.5 sec intraoral infusion. Please click here to view a larger version of this figure.

Discussion

Intraoral tastant delivery combined with FSCV permits analysis of “real-time” DA responses to oral flavorants. There are three critical steps in the protocol that are required for successful DA measurements. First, proper implantation of the oral catheter is critical for delivery of flavorants.

Ensuring that the catheter is inserted behind the first molar and wedged into place prevents the catheter from losing patency and prevents accidental removal by the animal. Flushing the catheter during the first few days after recovery is also important, since the soft food given to the animal during the first 2 or 3 days post-surgery could clog the catheter. Second, the quality of the electrode is critical for increasing signal-to-noise ratio in a freely moving rat. In some instances, electrodes that visually appear to be of high quality and good condition may still give noisy signals. One way to troubleshoot this issue is to take baseline recordings in the dorsal striatum (4 mm below dura). If the electrode produces too much noise, the electrode can be removed and replaced with a new electrode prior to performing the experiment in the NAc core recording site (6-7 mm below dura). A third critical step is finding a suitable recording location in the targeted brain region, where spontaneous DA release events ("transients") are observed at a sufficient frequency (at least 1 per min) with sufficient increases in DA concentration (at least 20 nM to 40 nM). This optimization will ensure that spontaneous phasic DA release events can be detected at specific location, prior to flavorant administration. Lowering the electrode in increments equivalent to the size of the exposed carbon fiber (50-100 µm) maximizes the chances of finding a recording site with detectable transients.

One important caveat of FSCV is the inability to determine absolute analyte concentration at a point in time, since FSCV is a differential technique that measures relative concentration changes (compared to a stable baseline) during each voltammetry recording file. However, the differential technique also allows for quantification of sub-second fluctuations in dopamine concentration in response to both rewarding and aversive tastants [5]. In the context of nicotine addiction, tobacco product additives, such as menthol and oral sweeteners, have been shown to influence smoking behavior [18] and have been recently been banned in cigarettes (except for menthol) by the Family Smoking Prevention and Tobacco Control Act. Thus, intraoral delivery in combination with FSCV provides an important methodological tool that can be used to examine how tobacco product flavorants can alter DA signaling, and potentially the reinforcing properties of nicotine.

Here, we have described the method of combining intraoral tastant infusion with FSCV for the purpose of measuring phasic DA release in response to primary tastant stimuli. It is important to note that while our technique allows the examination of phasic DA release to tastants independent of action or choice, it is also possible that the unexpected nature of the tastant delivery could influence phasic DA release[19]. Thus, a proper control solution, such as water or artificial saliva, can be used in control experiments to compare and examine the effects of an unexpected reward or stimulus on phasic DA release independent of the tastant of interest. However, the technique is not limited to either measurements of DA release or reward. For example, previous work has examined norepinephrine release during both reward and aversion [20]. Since animals do not readily consume aversive tastants intraoral tastant infusion with FSCV is the only technique available for measuring phasic DA release to aversive tastants. However, this technique does not determine whether or not a tastant is aversive or appetitive, rather, simply measures DA release during the consumption of the tastant. In order to determine the hedonic value of a tastant, behavioral tests can be incorporated to observe oro-facial responses during taste reactivity (as described elsewhere [5, 21]).

It should also be noted that FSCV is also suitable for measuring other important neurochemicals, such as serotonin[22] and oxygen [23], but methods for recording these analytes in awake, freely moving animals are not yet available. Furthermore, with the combined voltammetry and intra-oral infusion method, time-locked dopamine responses to tastant delivery can be analyzed in the absence of other behaviors, such as approach behavior or food retrieval, that might also affect DA release. As an additional application, combined intraoral tastant delivery and FSCV may be ideal for measuring DA signaling to rewarding or aversive events during operant or Pavlovian conditioning. Indeed, a recent JoVE publication by Levy and colleagues combines intra-oral tastant delivery with operant behavior to determine whether intra-oral tastants support the acquisition and maintenance of operant self-administration behavior[24]. Thus, such methods can also be combined with FSCV to identify phasic DA response to oral tastants during behavior.

Disclosures

The authors have nothing to disclose.

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