

Video Article

Measuring *In Vivo* Changes in Extracellular Neurotransmitters During Naturally Rewarding Behaviors in Female Syrian Hamsters

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Abstract

The ability to measure neurotransmitter release on a rapid time scale allows patterns of neurotransmission to be linked to specific behaviors or manipulations; a powerful tool in elucidating underlying mechanisms and circuitry. While the technique of microdialysis has been used for decades to measure nearly any analyte of interest in the brain, this technique is limited in temporal resolution. Alternatively, fast scan cyclic voltammetry is both temporally precise and extremely sensitive; however, because this technically difficult method relies on the electroactivity of the analyte of interest, the possibility to detect nonelectroactive substances (e.g., the neurotransmitter glutamate) is eliminated. This paper details the use of a turn-key system that combines fixed-potential amperometry and enzymatic biosensing to measure both electroactive and nonelectroactive neurotransmitters with temporal precision. The pairing of these two powerful techniques allows for the measurement of both tonic and phasic neurotransmission with relative ease, and permits recording of multiple neurotransmitters simultaneously. The aim of this manuscript is to demonstrate the process of measuring dopamine and glutamate neurotransmission *in vivo* using a naturally rewarding behavior (i.e., sexual behavior) in female hamsters, with the ultimate goal of displaying the technical feasibility of this assay for examining other behaviors and experimental paradigms.

Video Link

The video component of this article can be found at <https://www.jove.com/video/56135/>

Introduction

The ability to measure neurotransmitter release in awake behaving animals allows researchers to link specific behaviors with spatial and temporal patterns of neurotransmission—a powerful tool to investigate mechanisms and circuitry underlying both natural and operant behaviors in real-time. Historically, microdialysis has been employed to measure both electrically reactive and nonreactive substances in the extracellular milieu of the brain¹. This technique uses a continuous flow of an aqueous solution of similar ionic composition to the extracellular fluid, through a microdialysis probe composed of a small shaft with a tip made of a semipermeable hollow fiber membrane². After insertion of the probe, neurotransmitters or other analytes of interest can cross the semipermeable membrane by passive diffusion before being collected at intervals for subsequent analysis by high-performance liquid chromatography (HPLC), an analytical chemistry technique commonly utilized to separate, identify, and quantify components in a heterogeneous mixture³.

Although microdialysis is a sensitive technique that can be used to measure virtually any analyte of interest, the temporal resolution is low, with maximum sampling rates on the order of minutes to tens of minutes^{1,2}. The invention of fast scan cyclic voltammetry (FSCV), a technique that relies on the redox potential of electroactive species, can elucidate near instantaneous concentrations of the analyte of interest in the extracellular fluid. In brief (see Robinson *et al.*⁴ for an extensive review), an electrode is applied to raise and lower the voltage in a triangular wave fashion on a fast time scale⁴. When the voltage is in the correct range, the compound of interest is repeatedly oxidized and reduced. This oxidization and reduction results in a movement of electrons that creates a small alternating current. Scan rates take place on the sub-second scale with oxidization and reduction of compounds occurring in microseconds. By subtracting the background current created by the probe from the resulting current, one can generate a voltage vs. current plot unique to each compound. Since the time scale of the voltage oscillations is known, these data can be used to calculate a plot of the current as a function of time. Thus, the relative concentrations of the compound may be determined as long as the number of electrons transferred in each oxidation and reduction reaction is known⁴.

This chemical specificity and high temporal resolution make FSCV a powerful technique for detecting changing chemical concentrations *in vivo*. However, despite these manifold advantages, this technique requires extensive technical expertise and expensive equipment and setup. Further, nonelectroactive neurotransmitters (e.g., glutamate) cannot be measured using this technique. Fortunately, technological advancements in the field of electrochemistry⁵, as well as commercialization of these inventions, has introduced a relatively simple approach to measure non-electroactive neurotransmitters in awake behaving animals without compromising temporal precision—a technique known as enzymatic biosensor

technology. This technique uses enzymatic conversion of the nonelectroactive neurotransmitter of interest into two substrates, one of which is electroactive hydrogen peroxide that is detected as an amperometric oxidation current generated by an applied potential⁵. Commercially available biosensor probes (see **Figure 1**) selectively measure analytes of interest by competitively reducing the contribution of endogenous interferents. In the case of glutamate, the contribution of the common interferent ascorbic acid (AA) is competitively reduced to the measured current by co-localizing AA oxidase onto the active enzymatic surface of the sensor, converting AA to non-electroactive dihydroascorbate and water. In addition, a negatively charged Nafion polymer layer present under the enzyme layer excludes endogenous anionic compounds.

This same biosensor experimental setup can measure electroactive neurotransmitters as in FSCV, but instead it employs a fixed-potential recording⁶. In contrast to the oscillating voltage applied in FSCV, in a fixed-potential recording the voltage is kept at the redox potential for the analyte of interest. Although it is less chemically selective than FSCV as multiple neurotransmitters may have the same redox potential, in brain areas that overwhelmingly skew towards one neurotransmitter, the turn-key nature of this approach outweighs the lack of chemical specificity.

The ability to measure both electroactive and nonelectroactive neurotransmitter release in near real-time and link it to specific behavioral events provides an opportunity to examine converging neurotransmitter release. This manuscript details the use of this system to interrogate both dopamine and glutamate neurotransmission in response to natural reward in awake behaving hamsters. The aim of this paper is to detail the process of measuring this neurotransmitter release during sexual behavior in female hamsters, with the goal of demonstrating its feasibility for examining other behaviors and experimental paradigms.

Hamsters are an ideal model for use in electrochemical recordings

Historically, rat and mice models have been employed in the study of sexual behavior. These rodent species engage in a dynamic copulatory sequence, involving numerous female solicitation behaviors that include hopping, darting, and ear wiggling to entice the male to chase and ultimately mount the female⁷. The mounting by the male (with or without vaginal penetration) lasts only a few seconds, during which the female engages in her sexual behavior posture (termed *lordosis*) also only for a few seconds before resuming active solicitation behaviors. This pattern of behavior, composed of high levels of activity interspersed with brief periods of immobility, is problematic for measuring neurotransmission in behaving animals. First, there can be movement artifacts in the amperometric recordings that are unrelated to neural activity. Second, the locomotion is associated with the release of particular neurotransmitters in certain brain regions. For example, dopamine release has been coupled to locomotor activity in the dorsal and ventral striatum^{8,9}, a finding that formed the basis for microdialysis measurements of dopamine following psychostimulant administration¹⁰. Because the female-typical solicitation behaviors in most rodents involve high levels of locomotor activity, and are represented by the bulk of a 10 minute sexual behavior test, this makes it difficult to ascribe changes in neurotransmission to the explicit components of sexual behavior that collectively last only minutes.

To analyze the neurochemical profile of female sexual behavior, this lab sought out a species in which there is minimal locomotor activity accompanying sexual behavior. The copulatory sequence in Syrian hamsters (*Mesocricetus auratus*) is ideal for neurochemical recordings due to the lack of solicitation behaviors typically seen in rats and mice¹¹. As a consequence, female hamsters will enter and maintain the lordosis posture for upwards of 9 minutes out of a 10 minute testing session¹². With the lack of extraneous locomotor movements by the female, *in vivo* electrochemical recordings that can be associated with components of sexual interactions with the male can be obtained.

Copulatory bouts in hamsters

After the introduction of a male stimulus animal into the testing chamber, the male will initially engage in anogenital investigation (AI) of the female before mounting her (**Figure 2A**). In order for the male to mount, the female must assume a receptive sexual posture known as lordosis, in which she arches her back and deflects her tail so that the mounting male can gain penile access to her vagina. The male will mount the female, clasping her hindquarters with both paws (**Figure 2B**), and begin thrusting in an attempt to gain penile intromission (**Figure 2C**). The male will mount the female (without insertion) as well as intromit a number of times before eventually achieving ejaculation. This sequence of mounts and intromissions leading to ejaculation is termed a "copulatory bout". Males will have several copulatory bouts within a single session.

Protocol

All procedures described here were approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Minnesota, and are in accordance with The Guide for the Care and Use of Laboratory Animals¹³.

1. Animals and Cannulation Surgery

1. Obtain Syrian hamsters from a common animal supplier at approximately 55 days of age.
NOTE: Although the age of animals will vary due to constraints of various experimental paradigms, for sexual behavior it is important to obtain sexually mature animals that are all approximately the same age.
2. House animals in a controlled temperature (22 °C) and lighting (14 h light followed by 10 h dark with lights out at 13:00 h) environment, with food and water available *ad libitum* except during periods of experimental testing.
NOTE: Since hamsters seasonally reproduce, this 14:10 light/dark cycle mimics their natural breeding conditions.
3. Following a 1-week acclimation to the laboratory, perform aseptic bilateral ovariectomies and intracranial stereotaxic cannulations under general anesthesia (e.g., pentobarbital), and administer appropriate institutionally-approved post-operative analgesic and antibiotic treatment as previously described^{14,15}.
NOTE: For the study of sexual behavior, ovariectomies are necessary to remove the major endogenous source of sexual hormones so that the animals can be uniformly induced to sexual receptivity with exogenous hormone in accordance with the experimental timeline.
 1. For both dopaminergic and glutamatergic probe implantation, stereotaxically implant guide cannulae (0.7 mm diameter; **Figure 3**) into the area of interest (e.g., this lab targeted the right nucleus accumbens (NAc)) as described in stepwise detail elsewhere¹⁵.
NOTE: Although the data presented are from single sensor recordings of one glutamate or dopamine probe per animal, this system allows for simultaneous recording of up to 4 sensors in a single animal; thus, 1 - 4 cannulae could be implanted depending on desired experimental design and outcomes.

1. Determine the implant coordinates of the region of interest using a stereotaxic atlas (e.g., *The Golden Hamster Brain* by Morin & Wood), keeping in mind that the sensor extends 1 mm below the cannula into brain tissue.
2. After a cannula is stereotaxically lowered to the desired dorsal-ventral location, affix it to the skull using a cap constructed from dental acrylic, secured with stainless steel bone screws and a plastic head mount (**Figure 3**; see¹⁵ for more details).
3. For carbon fiber testing, insert a reference electrode (350 μm diameter, 7.5 mm total length) into the contralateral hemisphere.
NOTE: A specific location or distance from the cannula is not required; reference electrodes may be placed anywhere in the contralateral hemisphere that is convenient.

2. Biosensor and Carbon Fiber Testing

1. Following 1-week of recovery from surgery, administer the hormone priming regimen to induce sexual receptivity.
 1. Inject 10 μg of estradiol benzoate in 0.1 mL of cottonseed oil subcutaneously (s.c.), approximately 48 h and 24 h prior to the sexual behavior testing, to induce sexual receptivity towards the male¹¹.
 2. Inject 500 μg of progesterone in 0.1 mL of cottonseed oil, s.c., 4 h prior to the introduction of the male to induce sexual receptivity¹¹.
2. Test all animals at the start of the dark phase of their daily cycle, as social behaviors of rodents are subject to alteration under white light and red light testing conditions^{16,17,18}.
3. For enzymatic biosensor glutamate testing, calibrate the sensors *in vitro* (**Figure 4**) before use as described previously^{15,19}.
 1. Make the calibration solutions fresh on the day of testing in 20 mL glass centrifuge tubes. For the analyte solution, dissolve 7.4 mg of L-glutamic acid into 10 mL of ultra-pure H_2O by gently inverting the tube. Make the interferent solution by dissolving 176.1 mg AA in 10 mL of ultra-pure H_2O .
 2. Set up for the calibration by placing a magnetic stirrer on the base of a basic lab ring stand. Place a 20-mL jacketed beaker on top of the magnetic stirrer, and clamp in place using a medium 2-prong clamp.
 1. Add a magnetic stir bar and 20 mL of 100 mM phosphate buffered saline (PBS) into the jacketed beaker. Connect the jacketed beaker to a circulating water bath to heat the buffer solution to 37 $^{\circ}\text{C}$.
NOTE: The enzymatic biosensor calibrations must be performed at body temperature since enzymatic activity is influenced by temperature.
 3. Place the sensor calibration holder on top of the jacketed beaker, and set a 4-channel calibration preamplifier on top, securing in place using a right-angle clamp. Connect the preamplifier to a data conditioning and acquisition device to record calibration data.
 4. Test the sensitivity of each enzymatic biosensor to glutamate (or other analytes of interest for other commercially available biosensor probes e.g., glucose) before the experimental recording by placing the probe into the calibration holder atop a jacketed beaker, submerging the sensing cavity and some portion of the silver chloride (AgCl) reference wire in the buffer solution.
 1. Before connecting each sensor (up to 4) being tested to a port on a 4-channel calibration preamplifier, initiate a new recording on the computer interface utilizing free downloadable acquisition software. Allow the sensors to reach a stable baseline.
 5. Begin adding the analyte injections when the sensors have reached a stable baseline. Use the hole in the calibration holder to introduce a pipette tip to the buffer solution. Use the quick key annotation tool in the recording software to annotate when an injection is made.
 1. Make 10 μM additions of glutamate by pipetting 40 μL of the analyte solution into the buffer solution. Wait for the sensor to stabilize between additions. Add 3 injections of analyte solution before adding a single injection from interferent AA solution (50 μL injection volume for 250 μM change in concentration).
NOTE: The calibration allows for conversion of the changes in the amperometric signal (seen during the enzymatic experimental recording in step 2.12) to changes in glutamate concentration, if desired. See reference¹⁵ for stepwise instruction. The dopaminergic probes used by this lab are calibrated during the manufacturing process by the commercial provider. Glutamate probes must be calibrated at the time of use as their sensitivity can decrease over time as the enzymatic components degrade¹⁹. Because carbon fiber electrodes do not undergo degradation, the calibration performed by the company prior to shipment is sufficient along as there is an increased likelihood of damaging the thin carbon fiber (350 μm diameter) through additional handling.
4. Line the test chamber with bedding taken from the animal's home cage.
NOTE: This increases the female's familiarity with the testing chamber, as well as exposes the male to sexually stimulating olfactory mating cues that were distributed by the female in response to the estradiol treatment.
5. Lightly anesthetize the animals prior to insertion of the probe by either isoflurane or another volatile anesthetic in an induction chamber.
6. Remove the occlusive obturator from the guide cannula, and insert either the carbon fiber electrode or enzymatic probe through the guide shaft.
7. After the probe insertion, place the animal in the testing chamber.
NOTE: This lab uses a 10-gal glass aquarium (24.5 x 48.9 x 29.4 cm), but round chambers of equal area may also be employed.
8. Begin recording the video and time-locked amperometric signal in a commercially available software program, described in detail elsewhere¹⁵.
9. Connect the sensor to the recording system: the potentiostat via an electrically shielded cable and an electrical swivel. Stabilize the sensor connection by screwing a pin attachment onto the head mount. Reinforce the connection using lab film if necessary.
NOTE: A customized connector is needed to attach both the carbon fiber electrode and reference electrode to the potentiostat, while the enzymatic glutamate sensors can be directly connected to the potentiostat.
10. Allow the sensor to equilibrate in the brain before experimental testing.
NOTE: Equilibration times differ depending on the recording sensor (e.g., enzymatic sensors require 2 - 4 h equilibration, while carbon-fiber electrodes only require about 30 min). If both types of sensors are implanted, equilibrate for the longer enzymatic sensor time.

11. After sensor equilibration, introduce a stimulus male into the testing chamber.
 12. Following the first mount with penile insertion (termed intromission) by the stimulus male, continue recording for an additional 10 - 30 min, depending on the experimental goals.
 13. Following behavioral testing, disconnect the female's sensor.
 14. Remove both hamsters from the testing chamber. Remove the bedding and clean the chamber using 70% ethanol.
- NOTE: Due to the brief equilibration required for carbon fiber recordings, steps 2.3 - 2.14 can be repeated to test multiple animals in the same day. In contrast, because the enzymatic sensors require about 4 h of equilibration, test only one animal per day to limit possible inter-subject variation due to differences in the animals' circadian time at testing.

3. Sacrifice and Perfusion

1. Following the conclusion of the testing period, deeply anesthetize the female test subject with a euthanizing agent (e.g., this lab uses an intraperitoneal injection of 0.2 mL phenytoin and pentobarbital solution).
2. Transcardially perfuse the animal with 25 mM PBS, pH 7.6, to remove all circulating blood, followed by a 20 min fixation using 4% paraformaldehyde-PBS.
3. Remove the brain and postfix in ~ 30 mL of the 4% paraformaldehyde solution in a 50-mL conical tube overnight.
 1. Store the brain in a 10% sucrose-PBS solution until ready to serial section.

NOTE: Brains may be stored up to 2-weeks, with weekly sucrose solution changes to avoid potential growth of contaminants.
4. Serial section the brain in 40 μ m slices through the implanted region with either a freezing microtome or cryostat as previously described¹⁹.
5. Mount slices serially on commercially available adhesive-coated slides (or see²⁰ to make). Allow to dry, at least overnight.
6. Stain the slides with cresyl violet dye, clear, and coverslip as previously described²⁰.
7. Image the slides using bright field microscopy to confirm the anatomical placement of the sensor.

4. Behavioral Coding

1. View and annotate the videos using free commercially downloadable software (see **Table of Materials**) in slow motion to precisely code behaviors time-locked to the amperometric signal.
- NOTE: To use the MATLAB code in the analysis (see below), annotate the *start* and *end* of each of the female's and male's behaviors.
1. Annotate *startLordosis* in the frame when the female initiates a dorsoflexion of her back and defects her tail upwards. Annotate *endLordosis* when the female terminates this posture, which often occurs when the female readjusts to another location in the testing chamber.
 2. Annotate *startAI* when the female is in the lordosis posture and the male moves his snout towards the female's anogenital region, where sniffing, licking, or nuzzling of her perineal region may occur. Annotate *endAI* when the male removes his snout from close proximity to the female's anogenital region.
 3. Annotate *startMount* when the male approaches and places his forepaws on the female in a mounting posture, regardless of the orientation of the mount attempt (e.g., side, rump).
 4. Annotate *startIntromission* when successful thrusting gains the mounted male penile access to the female's vagina.

NOTE: An intromission is behaviorally distinguishable from the mounted thrusting that occurs prior to intromission, as the male visibly pulls his upper limbs towards his body, thrusts his pelvis forward, and curls his tail upwards, indicating penetration (see **Figure 4C**).

 1. When applicable, annotate *ejaculation*.

NOTE: At the end of a mating bout and in conjunction with a successful intromission, the male will ejaculate. Although one is unable to visualize the actual emission, male hamsters have a characteristic treading motion with their hind foot during an intromission²¹, thus annotate the ejaculation at the occurrence of the foot movement.
 5. Annotate *endIntromission* and *endMount* simultaneously in the frame when the male has removed his front two paws and thus, has no contact with the female. Due to the frequent inability to visualize his withdrawal, simultaneous code these two behaviors to allow for consistency and reliability across mating bouts.

Note: A mating bout either follows a successful ejaculation or is when the female breaks the lordosis posture following at least one *mount*.

5. Example Data Analysis

Note: The use of fixed-potential carbon fiber and enzymatic recordings for dopamine and glutamate, respectively, allows for the ability to measure both tonic and phasic patterns of electrochemical release. This lab uses a free, commercial acquisition system and software to record and convert recorded signals from analog to digital (bias 0.600 V, sampling rate = 1 Hz).

1. **Description of example data analysis protocol**
 1. Although other programs can be used, quantify the tonic and phasic (transient) changes in the recorded electrochemical signal using MATLAB.
 1. For the presented data, calculate the tonic signal using a 50-point moving average filter. A transient-only normalized representation of the signal was calculated by subtracting the tonic signal from the raw data. When identifying phasic peaks, there are various algorithmic options (e.g., labeling peaks as changes in the signal more than 1 SD above the mean).
 2. Identify the locations of these peaks, i.e., local maxima, from the normalized signal using the MATLAB function *peakfinder* with the minimum peak amplitude set to the root mean square of the signal. The location of peaks detected from the normalized signal was used to extract the amplitude from each peak. The ability to detect peaks allows for the identification of any time-locked behaviors that may be coinciding with, or driving the relative peak in the neurochemical under investigation.

2. Segment the processed data in accordance with the behavioral annotations (see step 3); a Student's *t*-test was used to evaluate differences in the tonic neurotransmitter level, the occurrence of transients, and the amplitude of transients during the pre-mating bout lordosis versus lordosis during mating bouts. To measure changes to tonic patterns of electrochemical release, the data points occurring during the *Lordosis* position directly before a mating bout (*i.e.*, pre-mating bout lordosis) were compared to the data points during a mating bout session using a *t*-test.

2. Preparation of raw data for described data analysis protocol

Note: As stated, although other programs or analyses may be equally viable options, in order to use the MATLAB code described above, the raw data must be prepared in the following way:

1. Export the annotation and voltage files. To do this, under the 'File' tab, select the 'Export' function and select the 'Annotations' tab to save the annotations. Under the 'File' tab, select the 'Export' function and choose the "TSV" tab to save the voltage measurements as a TSV extension file.
2. Open each file with a spreadsheet program and save as 'xls/xlsx' to make the files compatible with MATLAB.
NOTE: For the 'Annotation' file, there are additional annotations that must manually added post-export in a spreadsheet program to dictate the *start* and *end* of both the pre-mating bout lordosis (annotated as *PreMBlordosis*) and the mating bout so that these time points can be directly compared as mentioned in step 5.1.2.
3. Annotate the start of the pre-mating bout lordosis (*startPreMBlordosis*) at the same time as a lordosis behavior begins (*startLordosis*) that also includes a *mount* behavior, and also at the start of a new mating bout if the female remains in the lordosis posture.
4. Annotate the end of the pre-mating bout lordosis (*endPreMBlordosis*) when the first *mount* begins.
5. Annotate the start of a mating bout (*startMB*) at the same time of the *endPreMBlordosis*, as this signifies the start of a mating bout.
6. Annotate the end of a mating bout (*endMB*) at either the end of a *mount* that includes an *ejaculation* (see step 4.1.4.1), or following the female's termination of the lordosis posture (*endLordosis*).

Representative Results

Using the electrochemical and behavioral coding methodology described above, this lab has begun to characterize both tonic and phasic fluctuations in both dopamine and glutamate during *in vivo* recordings of sexual behavior. Due to the temporally-precise manner of this methodology, we can more accurately characterize neurotransmission during sexual behavior; as well as ascribe specific changes in release patterns to corresponding tonic changes during mating bouts, and corresponding transient fluctuations during the female's receipt of individual copulatory behaviors (*e.g.*, intromission, ejaculation) from the mounting male.

Specifically, we used this methodology to focus on measuring the release patterns of dopamine and glutamate in the NAc of separate animals. The NAc is a critical region involved in reward processing²² and has been described as an integral region in the reward aspects of sexual behavior²³.

We observed that during sexual behavior, females demonstrate a tonic increase in dopaminergic levels in the NAc core during mating bouts (**Figure 5**). While these tonic changes are shown for all mating bouts, the methodology described above can measure tonic changes between each mating bout, and thus increase the temporal identification of overall changes to tonic levels of neurotransmitter release throughout the entire session. Further, this described methodology can pinpoint phasic changes and temporally map the behaviors likely associated with these phasic neurotransmitter fluctuations. Specifically, these preliminary results suggest that during mating bouts, dopaminergic transients in the NAc arise during vaginal insertion (intromission) by the male (**Figure 6**). Furthermore, this association with intromission is specific, with negligible dopaminergic responses to other copulatory behaviors, such as the AI.

A similar pattern has been observed in other animals with regard to glutamate release, with rapid transients that correspond to individual intromissions in the dorsal NAc core during a copulatory bout (**Figure 7**). This pattern is region specific, with no discernable signal occurring in the NAc shell or medial caudate of other animals.

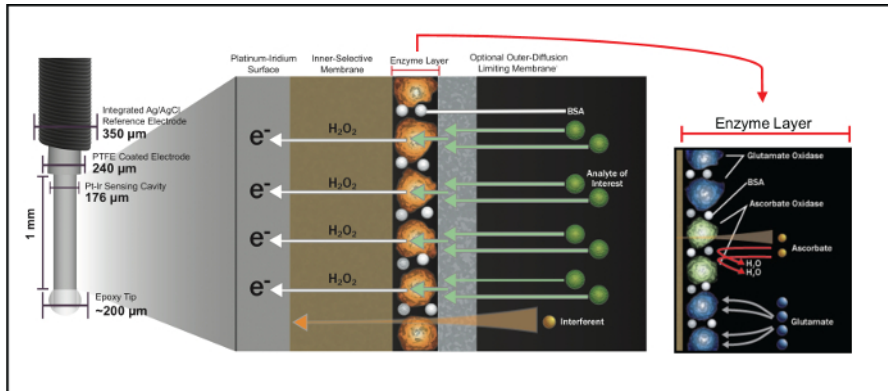


Figure 1: Enzymatic Biosensor Technology.

Standard fabrication of commercially available enzymatic biosensor probes that detect neurotransmitters via enzyme-mediated processing (left image). In the case of glutamatergic sensors, glutamate oxidase is employed in the enzymatic layer (right image) to convert the nonelectroactive neurotransmitter to electroactive hydrogen peroxide (H_2O_2) that is detected by oxidation at the platinum-iridium (Pt-Ir) electrode. Ascorbic acid (AA), a common interferent that is oxidized at the same potential, is excluded via an addition of AA oxidase at the enzymatic layer that converts the interferent to nonelectroactive water. Other negative electroactive interferents present in the brain are excluded via a passive selective membrane. [Please click here to view a larger version of this figure.](#)

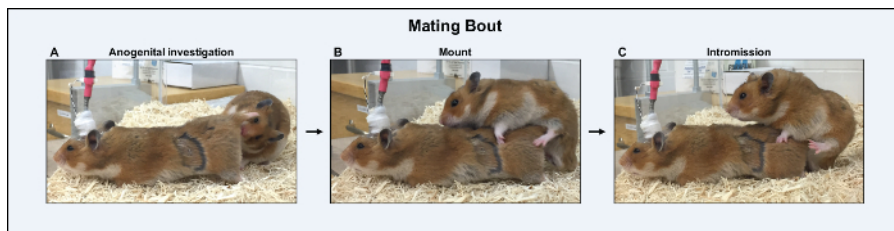


Figure 2: Components of a Hamster Mating Bout.

In hamsters, a mating bout consists of a sequence of copulatory behaviors, during which the female remains in the immobile lordosis posture while the male anogenitally investigates (A), mounts (B), and achieves intromission (C). Note an intromission is behaviorally distinguishable from mounting as the male pulls his upper limbs towards his body, thrusts his pelvis forward, and curls his tail upwards, indicating penetration. [Please click here to view a larger version of this figure.](#)

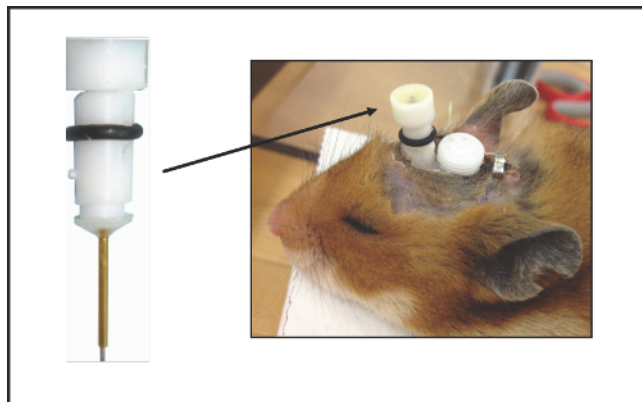


Figure 3: Skull Cap Construction.

Guide cannulae, bone screws, and dental acrylic are used in the construction of a skull cap to allow insertion of carbon fiber or enzymatic probe (see¹⁵ for stepwise details). This figure depicts a single cannula implant over the right NAC for an enzymatic sensor (no implanted reference electrode) and plastic head mount for sensor stability. [Please click here to view a larger version of this figure.](#)

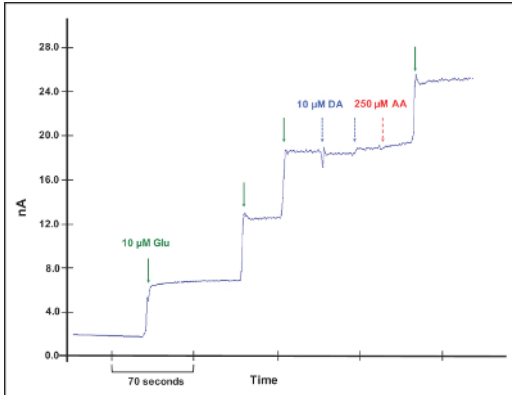


Figure 4: Glutamate Biosensor Calibration.

The sensitivity of each biosensor to glutamate is tested *in vitro* before experimental recording by 10 μM additions of glutamate (green) at 37 °C (since enzymatic activity is influenced by temperature). Each sensor is confirmed to be non-responsive to ascorbic acid (AA; red) or dopamine (DA; blue). This calibration allows for the conversion of changes in the amperometric signal seen during experimental recording to changes in the glutamate concentration. [Please click here to view a larger version of this figure.](#)

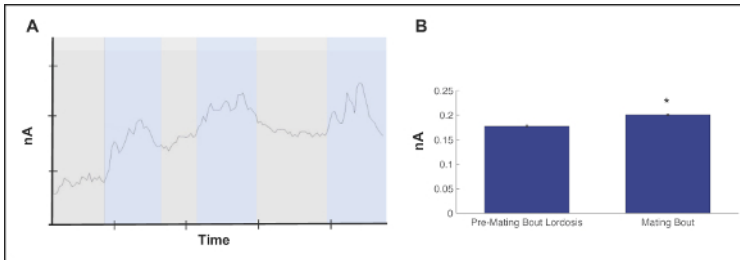


Figure 5: Tonic Increases in Dopamine During a Mating Bout.

A. Stylized example of pre-mating bout lordosis signal vs. mating bout lordosis signal. To measure tonic dopamine release, the amperometric responses to dopamine levels during lordosis before a mating bout (MB) were compared to those during a MB. Gray boxes indicate lordosis before a MB. Blue boxes indicate lordosis during a MB. **B.** Representative quantification from one animal comparing the mean voltage between preMB lordosis and MB lordosis. Error bars indicate standard error of the mean (SEM), and asterisk indicates significant difference (p -value < 0.05). [Please click here to view a larger version of this figure.](#)

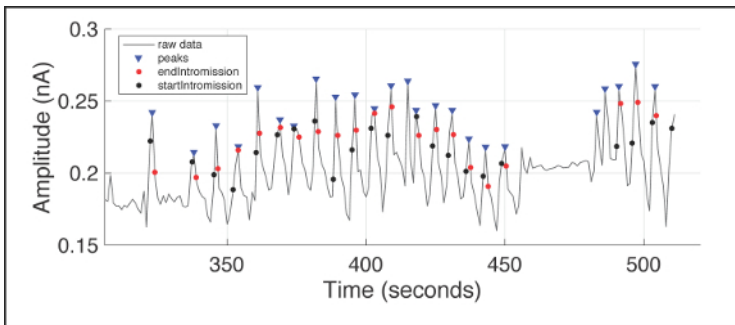


Figure 6: Representative Dopamine Trace.

Phasic release of dopamine is time-locked to intromissions from the male in the dorsal NAc core. [Please click here to view a larger version of this figure.](#)

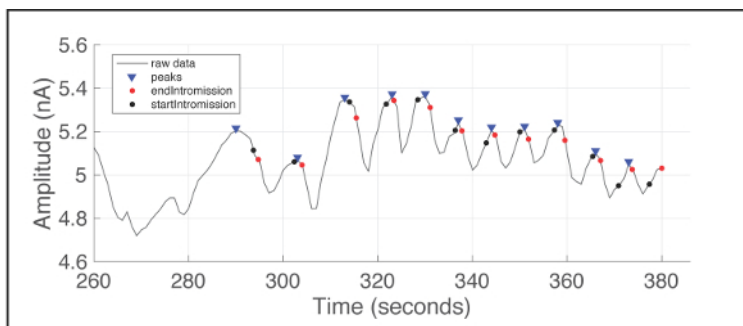


Figure 7: Representative Glutamate Trace.

Glutamate transients correspond to individual intromissions from the male in the dorsal NAc core during an extended sexual experience. [Please click here to view a larger version of this figure.](#)

Discussion

Although relatively straightforward, some issues can arise when employing this technique. First, the stereotaxic placement of the probes must be precise: unlike microdialysis that samples a wider radius of the extracellular milieu surrounding the probe, this technique only allows the measurement of a neurotransmitter that comes into direct contact with the probe. Second, in the case of the carbon fiber recording, due to the small width of fiber, breakage can occur, and the probe must be inserted with deliberate care. In the case of glutamatergic biosensors, enzymatic degradation can occur if the probe is not used within the guaranteed time-frame of 3-weeks. Fortunately, all of these issues are addressable, and can be eliminated with proper care and attention to detail.

A potentially problematic caveat arises in the specificity of fixed-potential carbon fiber recordings. Because the applied potential for dopamine coincides with other monoamines, such as norepinephrine, if the recordings are made in areas that release both neurotransmitters, than this lack of chemical specificity may limit the interpretation of the results. In the NAc this does not pose a major problem, with dopamine being the primary catecholamine transmitter²⁴. Because roughly 98% of the cells in NAc are medium spiny neurons that respond to dopaminergic neurotransmission²⁴, the signal is biased towards detection of dopamine and facilitates the use of this more turn-key approach.

Another benefit of this enzymatic biosensor and fixed-potential carbon fiber recording system is that dual-probe recordings can be performed, so that both electroactive and nonelectroactive neurotransmitters such as dopamine and glutamate can be measured in the same animal concurrently. Although the data presented here come from recordings in separate animals, these techniques can be employed concurrently such that one can evaluate the convergence of neurotransmission in multiple brain regions or in the same region bilaterally. Combination of recordings within the same animal, or comparison of recordings across animals as shown here, are both powerful tools in elucidating mechanisms behind network connections and signaling.

In sum, this paper demonstrates the powerful techniques of enzymatic biosensing and fixed-potential recording to measure multiple electroactive and nonelectroactive neurotransmitters utilizing a single experimental setup. Although the data presented here come from individual neurotransmitter recordings across multiple animals, this system provides the capability to record from up to 4 sensors in one animal concurrently¹⁵. The sample results presented collectively provide insight into rapid neurotransmission in the female hamster resulting from specific patterns of copulatory stimulation from the male. Further, these experiments demonstrate time-locked responses of dopaminergic and glutamatergic transients in the NAc core of the female to intromission from the male, suggesting that this release may be responsible for encoding distinct rewarding properties of sexual behavior. Our goal is to continue utilizing the mating pattern in female Syrian hamsters to develop a more comprehensive picture of the relation of *in vivo* dopamine and glutamate release and the underlying circuitry to individual components of copulatory stimuli from the male. We believe that the capability to characterize the patterns of these neurotransmitters is not only beneficial from a basic science perspective²³, but also in elucidating potential therapeutic approaches for pathological forms of reward behavior such as drug addiction²⁵.

Disclosures

The authors have nothing to disclose.

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