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Detecting Subsecond Dopamine Release with Fast-Scan Cyclic Voltammetry in Vivo

Donita L. Robinson,[1,2,3] B. Jill Venton,[1,2] Michael L.A.V. Heien,[1,2] and R. Mark Wightman[1,2,3*]

Background: **Dopamine is a potent neuromodulator in the brain, influencing a variety of motivated behaviors and involved in several neurologic diseases. Measurements of extracellular dopamine in the brains of experimental animals have traditionally focused on a tonic timescale (minutes to hours). However, dopamine concentrations are now known to fluctuate on a phasic timescale (subseconds to seconds).**

Approach: **Fast-scan cyclic voltammetry provides analytical chemical measurements of phasic dopamine signals in the rat brain.**

Content: **Procedural aspects of the technique are discussed, with regard to appropriate use and in comparison with other methods. Finally, examples of data collected using fast-scan cyclic voltammetry are summarized, including naturally occurring dopamine transients and signals arising from electrical stimulation of dopamine neurons.**

Summary: **Fast-scan cyclic voltammetry offers real-time measurements of changes in extracellular dopamine concentrations in vivo. With its subsecond time resolution, micrometer-dimension spatial resolution, and chemical selectivity, it is the most suitable technique currently available to measure transient concentration changes of dopamine.**

Dopamine is known to be an important neurotransmitter that modulates many aspects of brain circuitry. It is therefore of interest to be able to measure dopamine in awake rats to correlate neurochemistry with behavior. Over the past several decades, two general techniques have evolved to accomplish this: microdialysis and electrochemistry. Although multiple electrochemical techniques exist, those used in freely moving animals are chronoamperometry, differential normal-pulse voltammetry, and fast-scan cyclic voltammetry.

Fast-scan cyclic voltammetry has been used extensively to investigate the rapid events associated with neurotransmission in vivo and in vitro. It is a valuable preclinical tool to evaluate drug mechanisms *(1)* and animal models of disease *(2)* associated with dopaminergic transmission. By exploring the regulation and physiology of neurotransmitter function with fast-scan cyclic voltammetry, novel targets for therapeutic intervention in pathologies such as Parkinson disease, schizophrenia, and drug addiction may be identified.

Relative to other available techniques, fast-scan cyclic voltammetry has several advantages: it measures dopamine concentrations in real time, on a subsecond timescale; it quantifies increases and decreases in dopamine concentrations in the nanomolar to micromolar range; it uses a micrometer-dimension probe that gives fine spatial resolution with minimal tissue damage; and it provides positive identification of dopamine via the cyclic voltammogram. As we will show in this review, these characteristics make fast-scan cyclic voltammetry ideal for measuring the phasic dopamine signals putatively associated with burst firing of dopamine neurons in awake animals.

Extracellular Dopamine

The dynamics of the release and uptake of dopamine into brain extracellular space are currently under intense investigation. It is now well known that dopamine is an extrasynaptic messenger that functions via volume transmission, escaping the synaptic cleft to bind to extrasynaptic receptors and transporters *(3–5)*. Furthermore, there is evidence for both tonic and phasic aspects of extracellular dopamine *(6–8)*, which are best measured by different neurochemical techniques.

Tonic vs Phasic Extracellular Dopamine

Extensive data indicate that a tonic concentration of dopamine exists in target nuclei that may play an enabling role in neurotransmission. In the striatum, these tonic concentrations of dopamine are predicted to be 5–20

[1] Department of Chemistry, [2] Neuroscience Center, and [3] Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27599-3290.
*Author for correspondence. Fax 919-962-2388; e-mail rmw@unc.edu.
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nmol/L by microdialysis *(9)* and differential normal-pulse voltammetry *(10)* and 50–100 nmol/L by theoretical estimations using fast-scan cyclic voltammetry *(11)* and pharmacologic studies *(12)*. If the concentrations are indeed 20 nmol/L or above, those dopamine receptors in high-affinity states would be chronically occupied, which appears to be the case for D_2-like receptors *(13, 14)*. Additional evidence for extracellular dopamine "tone" comes from microdialysis studies in 6-hydroxydopamine-lesioned rats. Basal extracellular dopamine concentrations are maintained until dopaminergic cell loss exceeds 80%, after which both parkinsonian symptoms emerge and extracellular dopamine concentrations drop *(15, 16)*. Tonic extracellular dopamine would be best measured by neurochemical techniques that offer both high sensitivity and a long timescale, such as microdialysis.

In contrast, extracellular dopamine is known to reach high concentrations for brief periods *(17, 18)*, which we describe as phasic activity. These transients are likely to arise from concerted burst firing of dopamine neurons *(19)* that often occur on presentation of salient sensory input *(6, 20)*. Fast-scan cyclic voltammetry and amperometry have been used to measure dopamine release after an electrical stimulation of dopamine neurons that mimics tonic and phasic firing *(11, 21)*. The amount of dopamine released is frequency-dependent; as the stimulation approaches frequencies achieved by burst firing, the dopamine transporter is saturated and high concentrations are achieved. Similar naturally occurring dopamine transients have been measured in the rat, sometimes reaching concentrations >1 μmol/L *(18, 22)*. These higher concentrations are sufficient to activate the low-affinity D_1- and D_2-like receptors *(23)*. Because the events themselves are so brief (<10 s), phasic extracellular dopamine would be best measured by neurochemical techniques that have subsecond timescales.

NEUROCHEMICAL METHODS TO MEASURE EXTRACELLULAR DOPAMINE

High sensitivity, chemical selectivity, and fast temporal resolution are all desirable characteristics when measuring neurotransmitters in vivo. In practice, it is difficult to achieve all of these with one method. In this section, we will briefly discuss the advantages and disadvantages of the methods used for dopamine detection in vivo, with reference to their appropriate applications.

Microdialysis. Microdialysis is the most widely used method to sample the chemical environment of the brain. A dialysis probe that is permeable to small molecules is placed in the brain and perfused with artificial cerebrospinal fluid. As molecules diffuse into the probe, the dialysate is collected and analyzed off-line. Because the dialysate can be analyzed after separations with HPLC, a high degree of chemical selectivity and sensitivity is achieved with this technique. However, a certain volume of dialysate must be collected before analysis can be done; thus, the method has slow temporal resolution and is best suited for looking at concentration changes that happen on the minute-to-hour timescale. Because of these characteristics, microdialysis is routinely used to measure tonic changes in dopamine. [Although measurements of tonic dopamine theoretically include phasic signals integrated across the sample, it has been demonstrated that microdialysis is insensitive to dopamine transients *(24, 25)*.]

Constant-potential amperometry. Constant-potential amperometry is a simple electrochemical technique in which a potential sufficient to oxidize or reduce the molecule of interest is applied to the electrode. The electrode responds extremely quickly to changes in analyte concentration, and high sampling rates can be used; therefore, constant-potential amperometry offers the best temporal resolution among the available techniques. However, it suffers from poor selectivity: any molecule that can be oxidized or reduced at the potential of the electrode is detected, so there is no way to differentiate between molecules. Because of its lack of chemical selectivity, constant-potential amperometry should never be used in the freely moving preparation, where higher chemical selectivity is needed to provide confidence in the identity of the analyte. Nevertheless, it is appropriate for measurement of electrically evoked dopamine release in anesthetized animals or brain slices, and its fast sampling rate makes it ideal to study the kinetics of phasic dopamine signals.

Fast-scan cyclic voltammetry. Fast-scan cyclic voltammetry is a more complex electrochemical technique that provides good chemical selectivity while retaining subsecond temporal resolution. Each measurement consists of a cyclic voltammogram that serves as a chemical identifier to provide chemical selectivity. Thus, possible interferents, such as ascorbic acid, dihydroxyphenylacetic acid (DOPAC),[4] and pH shifts, can be easily distinguished from dopamine via the cyclic voltammogram. Because fast-scan cyclic voltammograms can be repeated every 100 ms, changes in dopamine concentration can be monitored on the subsecond time scale. These characteristics make fast-scan cyclic voltammetry well suited for detecting phasic dopamine changes in the freely moving animal.

Additional electrochemical techniques include high-speed chronoamperometry and differential normal-pulse voltammetry. Both techniques provide more chemical selectivity than constant-potential amperometry but less than fast-scan cyclic voltammetry. High-speed chronoamperometry provides measurements on the second time scale (usually 1–5 s after signal averaging) *(26)*, whereas differential normal-pulse voltammetry provides measurements on the minute time scale *(27, 28)*.

[4] Nonstandard abbreviations: DOPAC, dihydroxyphenylacetic acid; mse, mean squared error; and ICS, intracranial self-stimulation.

Fast-Scan Cyclic Voltammetry

When used with carbon-fiber microelectrodes, fast-scan cyclic voltammetry provides good temporal, chemical, and spatial resolution. In fast-scan cyclic voltammetry, the potential of the electrode is linearly scanned, which causes molecules adjacent to the electrode to be oxidized or reduced. The measured current provides chemical information of the surrounding environment.

The methodology of fast-scan cyclic voltammetry is illustrated in Fig. 1. A triangle waveform is applied intermittently to the electrode (Fig. 1A). The scan rate and potential limits determine the length of each waveform. Under typical fast-scan conditions, the potential is ramped from −0.4 V to +1.0 V, vs a Ag/AgCl reference, and back at 300 V/s. The resulting scan lasts 9.3 ms and is repeated every 100 ms. Variations in these settings affect the sensitivity, selectivity, and time resolution of the measurements.

During fast-scan cyclic voltammetry, a large background current is produced, shown as a cyclic voltammogram in Fig. 1B (solid line; measured current vs applied potential). The background current is chiefly attributable to the charging of the double layer (rearrangement of charged species around the electrode) and is proportional to the scan rate and capacitance of the electrode. Electroactive functional groups present on the electrode surface can also be oxidized or reduced, which adds to the background current of the electrode. The presence of an electroactive species, such as dopamine, may produce only a small increase in the background current (Fig. 1B, dashed line). However, because carbon-fiber microelectrodes have stable backgrounds over several seconds, the background can be digitally subtracted to reveal changes in current. The resulting background-subtracted cyclic voltammogram (Fig. 1C) indicates that, in this case, the change in current is attributable to oxidation of dopamine and reduction of the electroformed quinone back to dopamine. This robust dopamine signal was produced by electrically stimulating dopamine release in an anesthetized rat (biphasic, 60 pulses, 60 Hz, 120 μA).

The background-subtracted cyclic voltammogram provides chemical information on the detected substance; e.g., the location of the oxidation and reduction peaks can be used to chemically identify dopamine. In Fig. 1D, the current at the oxidation peak of dopamine can be plotted over time as the dopamine neurons are electrically stimulated. During the stimulation, the magnitude of current increases as a result of dopamine release, then decreases as a result of uptake. The change in current is confirmed to arise from dopamine by inspection of the cyclic voltammogram. Finally, the current can be converted to dopamine concentration by use of an in vitro calibration.

TEMPORAL AND SPATIAL RESOLUTION

Typical cylindrical carbon-fiber microelectrodes are 5–30 μm in diameter and 25–400 μm in length (Fig. 2). Because of the small size of the probe, minimal tissue damage occurs during insertion into the brain, and tracts are undetectable under a light microscope *(25)*. This is in contrast to larger probes (>200 μm in diameter), such as are used in microdialysis, which cause tissue disruption up to 1.4 mm from the probe site *(29)*.

Fig. 3 shows the response as a carbon-fiber microelectrode is lowered through the nucleus accumbens in 150-μm increments, while dopamine fibers in the medial forebrain bundle were electrically stimulated. Although dopamine fibers to the caudate and nucleus accumbens all travel along the dorsal medial forebrain bundle, the

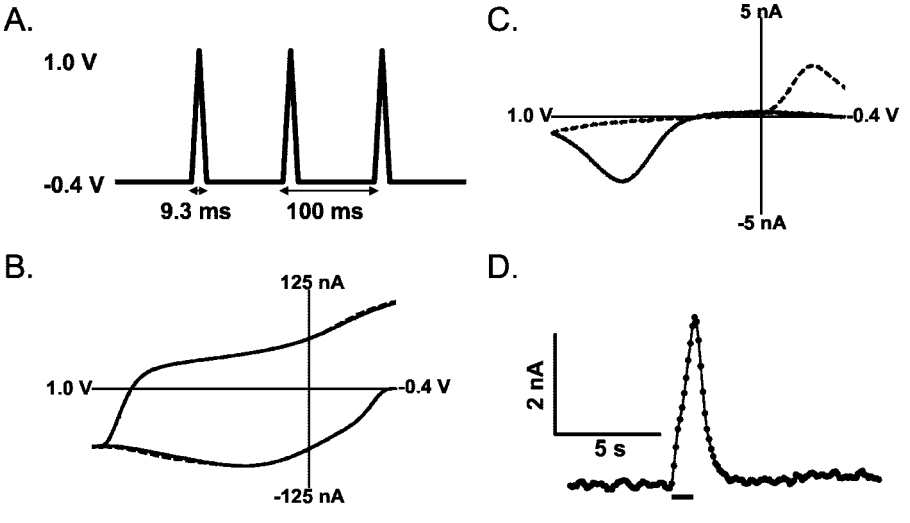

Fig. 1. Fast-scan cyclic voltammetry.

The potential applied to the electrode is ramped from −0.4 V to +1.0 V and back at a scan rate of 300 V/s (*panel A*). This produces a large background current, which is plotted vs applied potential (*panel B*). The *solid line* is the background current, and the *superimposed dashed line* is the current observed in the presence of dopamine. The *dashed line* represents a 3% change in the background current. By subtracting out the background, a cyclic voltammogram for dopamine is generated (*panel C*), which is the chemical identifier. The oxidative wave (potential ramped from −0.4 V to +1 V) is shown as a *solid line*, and the reductive wave (potential ramped from +1 V to −0.4 V) as a *dashed line.* Changes in dopamine current are visualized by plotting the current at the peak oxidation potential of dopamine (+0.6 V vs Ag/AgCl reference) vs time (*panel D*). Each point represents the current from one cyclic voltammogram. The dopamine neurons are stimulated electrically (biphasic, 60 pulses, 60 Hz, 120 μA) as denoted by the *bar*. At the onset of stimulation, the current increases, and then falls after the stimulation ends. The increase in current is confirmed to be attributable to dopamine by examination of the corresponding cyclic voltammograms. The current can be converted to dopamine concentration via in vitro calibration of the carbon-fiber electrode. For this signal, the calibration yielded 300 nmol · L^{-1} · nA^{-1}.

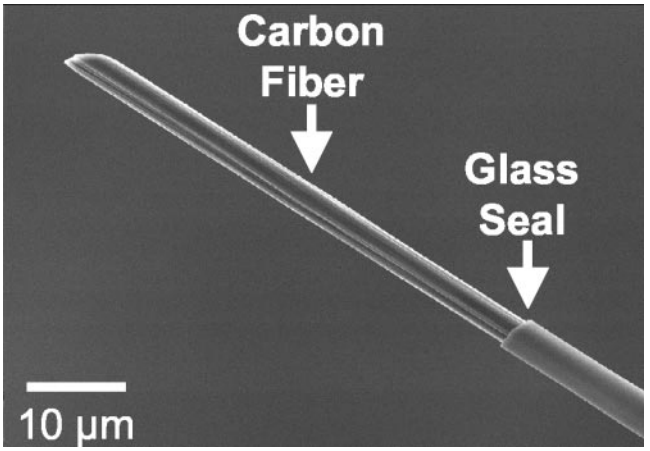

Fig. 2. Scanning electron microscopic image of a cylindrical carbon-fiber microelectrode under ×1200 magnification.

The diameter of the fiber is ~5 µm. The length of the exposed carbon fiber extending from the glass seal is the electroactive area of the electrode.

projections are topographic *(30)*. Therefore, at a given stimulation site, different signals are seen throughout the nucleus accumbens because of the heterogeneity of dopamine release sites. The reciprocal phenomenon was demonstrated by Garris et al. *(31)*, in that release from a single recording site in the nucleus accumbens varied while the stimulating electrode was incrementally lowered through the dopamine fiber pathway. With these methods, an area with high release can be targeted for measurements during an experiment.

It has long been recognized that dopamine adsorbs to carbon-fiber microelectrodes *(32)*. This adsorption process has two main consequences for the voltammetric signal *(33)*. The first is an increase in the signal observed, as dopamine preconcentrates on the electrode surface between scans. The second is a slower time response of the electrode; however, this lag can be removed by deconvolution (mathematically removing distortion) of the signal *(34)*.

Because fast-scan cyclic voltammetry is a differential technique, only changes in analyte concentration can be measured. Although the background current at a carbon-fiber microelectrode is stable over several seconds, it can drift over minutes. As a result, long-term changes in dopamine concentration or basal concentrations cannot be measured, whereas transient changes are easily detected.

SENSITIVITY ISSUES

There are several factors that affect the analyte sensitivity of the electrode with fast-scan cyclic voltammetry. The first is probe size, because the length of the carbon fiber determines how many release sites are being sampled and, thus, sensitivity *(35)*. A second set of factors affecting microelectrode sensitivity are the scanning conditions *(33)*. Because dopamine adsorbs to the electrode surface, the holding potential and time between scans can affect the signal intensity. In addition, the signal will increase

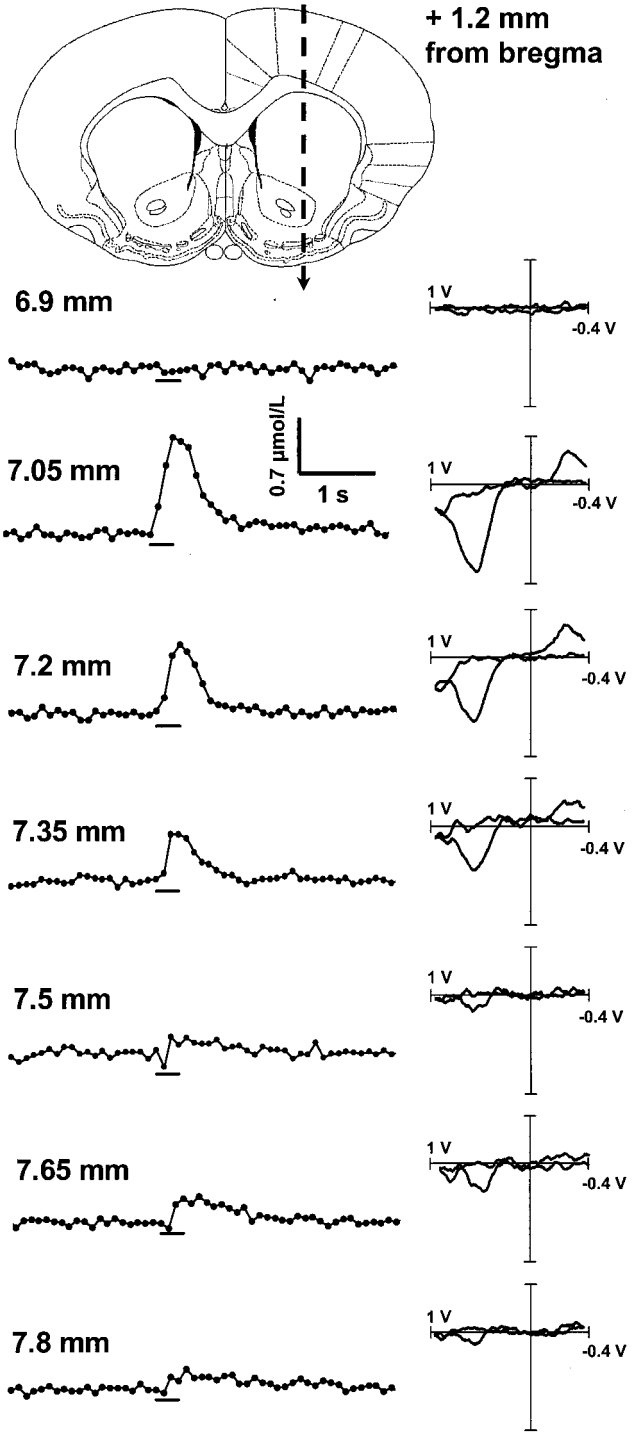

Fig. 3. Heterogeneity of electrically evoked dopamine release in the nucleus accumbens of a freely moving rat.

The carbon-fiber microelectrode is lowered, using a micromanipulator, into the nucleus accumbens at 1.2 mm anterior to bregma *(61)* as indicated by the *downward arrow*. At each ventral placement, the electrochemical signal at the oxidation potential of dopamine (+0.6 V vs Ag/AgCl reference) is converted to concentration, using the in vitro calibration of the electrode after the experiment. The concentration–time scale is valid for each of the traces. The electrical stimulation (bipolar, 24 pulses, 60 Hz, 120 µA) is delivered to the medial forebrain bundle and indicated by the *bar below* each *trace*. The cyclic voltammogram corresponding to the electrical stimulation for each trace is shown on the *right*. The *vertical bar* designates a range of 0.6 to −1.6 nA in each cyclic voltammogram.

linearly with scan rate. Carbon-fiber microelectrodes can be made more sensitive to dopamine by electrochemical pretreatments or extended scan potentials that change its surface chemistry *(36, 37)*. The tradeoff for increased sensitivity with electrochemical treatment is decreased time resolution. Advances in instrumentation and electrode technology have lowered the detection limit of dopamine to 25 nmol/L in awake animals. However, because of background subtraction, this is a differential technique and is best suited to measure transient concentration changes.

To determine the concentrations measured, it is important to calibrate the electrode accurately. Electrodes are typically calibrated in vitro in a flow-injection analysis system, where the electrode response to a known concentration of dopamine can be determined *(38)*. Experiments have been conducted comparing simultaneously collected microdialysis and voltammetry data to determine whether it is most accurate to use a calibration performed before or after the experiment *(39)*. For this study, acetaminophen was used instead of dopamine because that molecule was not subject to the rapid uptake and metabolism seen with exogenous dopamine. The electrode response to the analyte acetaminophen during calibration decreased by two-thirds after the electrode was implanted in tissue for a few hours. The measured microdialysis concentration of acetaminophen in vivo was between the calculated concentrations from cyclic voltammetry using the precalibration and postcalibration factors, indicating that an average of the two might give the most accurate result. However, postcalibration factors are routinely used in our laboratory and are accurate enough to estimate extracellular dopamine concentrations.

Another issue involving sensitivity is acute vs chronic implantation of electrodes. Some researchers implant an electrode during surgery and use it for all subsequent experimental measurements. However, as demonstrated in the calibration experiments, electrodes lose some sensitivity and have a slower time response after implantation in the brain *(40)*. Gliosis can also occur around the electrode after long time periods. Because freely moving animals must recover from surgery for a few days before an experiment can be performed, chronically implanted electrodes are not optimal for use in these experiments. Instead, it is desirable to insert a new electrode for each experiment by use of a micromanipulator *(41)*.

CHEMICAL SELECTIVITY

Fast-scan cyclic voltammetry can be used to detect a variety of electroactive compounds in the brain. One of the advantages of cyclic voltammetry is multiple point identification (each cyclic voltammogram contains ~1000 points), which allows numerous species with unique oxidation and reduction potentials to be detected and differentiated. Fast-scan cyclic voltammetry can be used to detect catecholamines, indolamines, neurotransmitter metabolites, ascorbic acid, oxygen, nitric oxide, and pH changes *(32, 42–44)*. The compounds detected and the ability to resolve two compounds can depend on the applied waveform. For example, the fast scan rates of cyclic voltammetry can cause shifts in the peak potentials attributable to slow kinetics; therefore, the oxidation peaks of some potential interferents for dopamine detection, such as nitric oxide, appear at much more positive potentials *(44)*.

Fig. 4 illustrates the distinct voltammograms for dopamine, norepinephrine, serotonin, ascorbic acid, and DOPAC; the solid line is the oxidative scan, and the dashed line is the reductive scan. Cyclic voltammograms can be compared based on peak height, the relative ratio of oxidative and reductive peaks, and peak location and shape. The shape of a cyclic voltammogram is determined by electron transfer kinetics and how strongly the analyte adsorbs to the carbon fiber. Dopamine and norepinephrine have nearly identical cyclic voltammograms (see below), although the electrode is more sensitive to dopamine. The cyclic voltammogram for serotonin shows multiple oxidation and reduction peaks because its oxidation products are unstable and can break down into additional electroactive compounds. Cyclic voltammetry is particularly useful for serotonin detection compared with other electrochemical methods because the oxidation product, which can foul the electrode, is produced for only a few milliseconds and then is immediately reduced *(45)*. In Fig. 4, the peak oxidation potential for dopamine is marked by a dashed line; it therefore is clear that ascorbic acid and DOPAC have oxidation peaks at a more positive potential than dopamine. Ascorbic acid and DOPAC are also unstable after oxidation, so there is very little reductive peak.

Although cyclic voltammograms are a powerful tool for identifying electroactive compounds, some species, such as norepinephrine and dopamine, have nearly identical cyclic voltammograms and cannot be differentiated by electrochemistry alone. This is because the structures of norepinephrine and dopamine are very similar and the same hydroxyl groups are oxidized on each compound. Therefore, to identify dopamine transients in vivo, electrodes are placed in known dopaminergic regions with low norepinephrine content, and placements are verified histologically. Dopamine can be independently verified by determining the tissue content of the area around the electrode by HPLC after the experiment and by comparison with microdialysis results. Pharmacologic manipulations, such as the administration of a specific receptor antagonist or transporter inhibitor, also allow verification of the signal. Further discussion on guidelines to validate neurotransmitter signals measured with voltammetric methods can be found in Marsden et al. *(46)*.

One of the main interferents for the dopamine signal in vivo is a pH shift. Changes in extracellular pH cause broad changes in the background of the electrode signal as a result of protonation and deprotonation of surface groups, and this affects any technique that requires back-

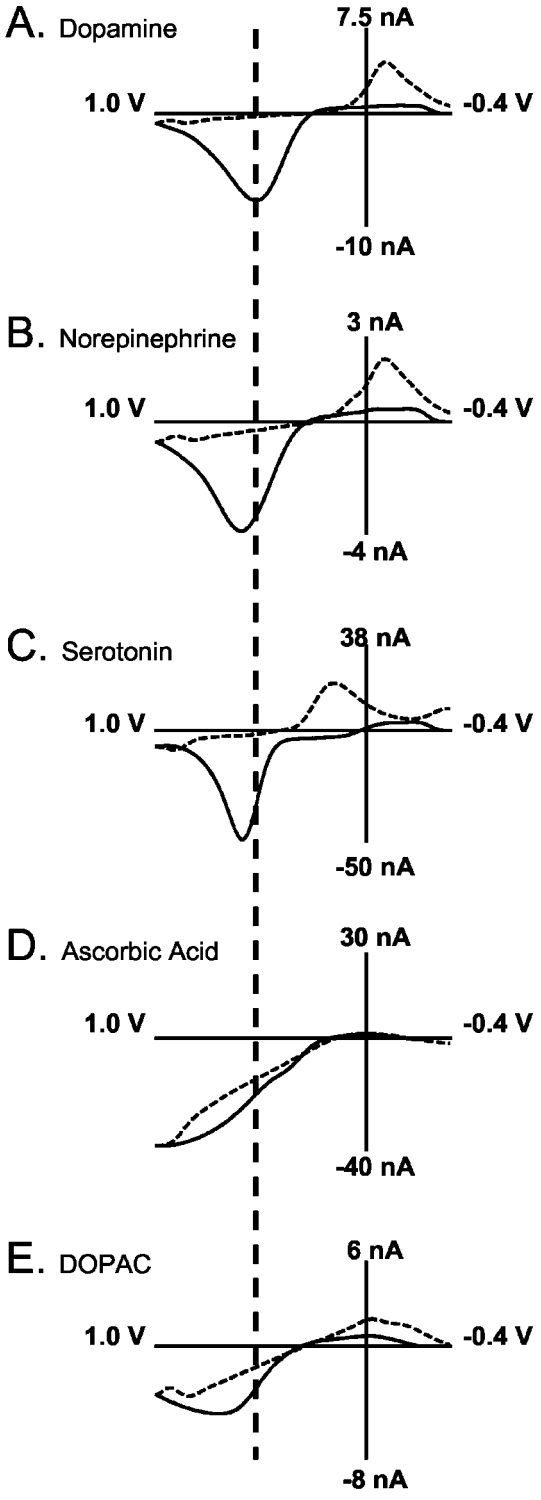

Fig. 4. Cyclic voltammograms of neurotransmitters and interferents.

(*A*), cyclic voltammogram for 2 μmol/L dopamine. The *vertical dashed line* denotes the oxidation peak for dopamine. (*B*), cyclic voltammogram for 2 μmol/L norepinephrine. The oxidation peak is at the same potential as that of dopamine; however, the electrode is less sensitive to norepinephrine. (*C*), cyclic voltammogram for 2 μmol/L serotonin. The cyclic voltammogram for serotonin has a shoulder on the oxidation peak, and two reduction peaks. The electrode is also more sensitive to serotonin than dopamine. (*D*), cyclic voltammogram for 200 μmol/L ascorbic acid. It has a broad oxidation wave. (*E*), cyclic voltammogram for 20 μmol/L DOPAC, which also has a broad oxidation wave attributable to slow electron-transfer kinetics.

ground subtraction (e.g., fast-scan cyclic voltammetry and chronoamperometry) *(43, 47)*. In fast-scan cyclic voltammetry, however, pH changes affect the current at a much broader range of potentials; thus, the effect of pH on the dopamine oxidation signal can be subtracted out *(48)*.

Recently, the occurrence of alkaline pH shifts after evoked dopamine release in vivo has been verified with ion-selective microelectrodes *(49)*. Changes in blood flow cause these local pH shifts by altering the concentration of CO_2, a component of the brain buffering system. Fig. 5 is an example of a behaviorally evoked dopamine transient followed by an alkaline pH shift that occurred when an animal was presented food. The dotted line is the observed signal at +0.6 V, which contains contributions from the both the oxidation of dopamine and an alkaline pH shift. The increase is attributable to dopamine, as verified by the cyclic voltammogram at the peak signal. The cyclic voltammogram from the decreased signal, after the transient, has the characteristic broad changes caused by pH and not the sharp peaks of the dopamine cyclic voltammogram. The effects of the current from the interfering pH signal can be subtracted to give the pure dopamine signal (Fig. 5, dashed line). Attributing the signal at the dopamine oxidation potential only to changes in dopamine would cause a misinterpretation of the results as an increase and then decrease below baseline in dopamine concentration. Examination of the voltammograms allows differentiation of the dual effects of pH and dopamine on the signal.

AUTOMATED DOPAMINE FINDER

During in vivo experiments with a freely moving animal, it can be daunting to look through the data to identify

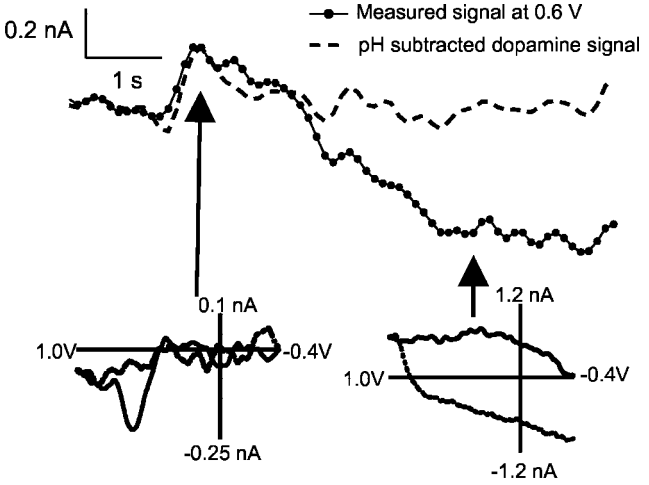

Fig. 5. Behaviorally evoked dopamine and pH signals in the nucleus accumbens at the presentation of food.

●, measured signal at +0.6 V (vs Ag/AgCl reference), which has contributions from both dopamine and an alkaline pH shift. The *dashed line* is the pure dopamine signal, with the effects of pH subtracted out. The *left* cyclic voltammogram is consistent with dopamine, whereas the *right* cyclic voltammogram verifies the change in alkaline pH. The oxidative scan is shown as a *solid line* and the reductive scan as ● in both cyclic voltammograms.

which cyclic voltammograms are dopamine. With cyclic voltammograms typically collected every 100 ms, each with ~1000 points, and experiments lasting up to 2 h, the volume of data can be enormous (up to 76 000 cyclic voltammograms). Therefore, a high-throughput algorithm was developed to find naturally occurring dopamine signals recorded in behaving animals. This method is performed using locally written software in LabVIEW (National Instruments) and analyzes 2000 cyclic voltammograms in <5 s.

In this method, each cyclic voltammogram is background-subtracted using 10 scans taken 1.5 s beforehand. The experimental cyclic voltammogram is compared with a template of dopamine (an electrically stimulated signal from the same rat) and normalized to the oxidation peak. The mean squared error (mse) is then calculated vs the template, given by the equation:

$$\text{mse} = \frac{\sum(x - y)^2}{\text{n}}$$

where x and y are corresponding points on the normalized template and experimental cyclic voltammograms, respectively, and n is the number of points in one cyclic voltammogram. If the mse is small, the cyclic voltammograms are similar; if it is large, they are different. To provide good differentiation between dopamine cyclic voltammograms and the baseline noise, the inverse of the mse is plotted vs time, a threshold is set, and the peaks over the threshold correspond to signals that are dopamine. This process is repeated for each cyclic voltammogram in the data set. An alternative analysis is to calculate the r^2 value between the experimental and template cyclic voltammograms. In this case, we have empirically established a minimum correlation threshold of 0.7.

The automated dopamine analysis for an experiment in a freely moving animal where behaviorally evoked signals are present is shown in Fig. 6. The threshold of inverse mse was set at 20 (the empirically established threshold used in our laboratory); the cyclic voltammogram for a peak >20 matches the dopamine template, whereas the cyclic voltammogram for a peak <20 is not identifiable as dopamine because of the extra oxidation and reduction peaks. Automating the analysis makes the process objective.

Phasic Dopamine Signals in Freely Moving Rats

Fast-scan cyclic voltammetry has been used to monitor electrically evoked and, more recently, naturally occurring dopamine transients. The advantage of electrical stimulation of dopamine release is that sufficient concentrations are achieved to distinguish release and uptake components of the neurochemical signal *(50)*. The recent measurement of similar transients occurring naturally in freely moving rats *(18, 22)* reinforces the validity of electrically evoked transients to study the kinetic mechanisms of phasic dopamine signals. The studies reviewed here are limited to extracellular dopamine detection using fast-scan cyclic voltammetry in freely moving animals; however, note that there is a rich body of research involving use of the method to probe dopamine transmission in anesthetized animals and in brain slices.

ELECTRICALLY STIMULATED DOPAMINE RELEASE

Fast-scan cyclic voltammetry is useful for evaluating the pharmacologic effects of drugs that alter dopaminergic release or uptake. To accomplish this, dopamine release is electrically evoked with a stimulating electrode in the substantia nigra/ventral tegmental area or the medial forebrain bundle. Properly spaced in time, the dopamine release from a mild stimulation train (e.g., bipolar, 24 pulses, 60 Hz, 120 μA) is highly reproducible, with minimal effects on behavior. Therefore, electrically evoked dopamine release and uptake can be monitored before and after administration of a drug *(1, 51–54)*.

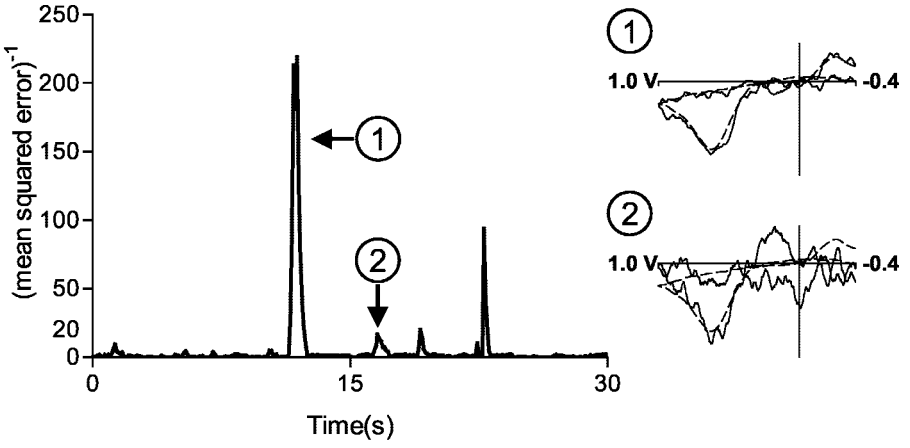

Fig. 6. Automated dopamine finder for large data sets.

Background-subtracted cyclic voltammograms in a freely moving male rat (*solid lines*) were matched to a template of electrically evoked dopamine release in the same rat (*dashed lines*). The rat was investigating a receptive female, and the dopamine signals observed here are spontaneously released. The largest peak at 11.8 s represents a change in dopamine concentration of 250 nmol/L. The first cyclic voltammogram represents the peak at 11.8 s, which has a score >20 (inverse of the mse). This cyclic voltammogram is indicative of dopamine. The second cyclic voltammogram represents the peak at 16.6 s, which has a score <20 and is not indicative of dopamine. Note the extra oxidation wave and the absence of a reduction wave at the potential for dopamine-*o*-quinone, signifying that the signal is not dopamine. A score of 20 represents a mean difference of 22% between the template and experimental cyclic voltammograms. (Concentration/current scales are omitted from the cyclic voltammograms because the target and template voltammograms were normalized to the oxidative peak.)

Examples of the effects of various drugs on electrically stimulated dopamine release in freely moving rats are shown in Fig. 7. Haloperidol (top), a dopamine receptor antagonist, increases dopamine release by blocking D_2 receptor feedback on the dopamine terminals. Nomifensine (middle), a dopamine transporter antagonist, causes an increase in the dopaminergic signal by blocking uptake. Ethanol (bottom), a general depressant that affects multiple ligand-gated ion channels in the brain, decreases electrically evoked dopamine release. Note that the variability in the control signal among the different experiments reflects differences in the precise placements of the carbon fiber and stimulating electrodes, as well as the brain area in which dopamine was recorded. The time course of the pharmacologic effects of drugs on dopamine transmission can be correlated to those on behavior, at a more precise time scale than microdialysis *(53)*. Together, fast-scan cyclic voltammetry and microdialysis provide complementary information about dopamine transmission, providing a more complete picture of extracellular dopamine concentrations after drug administration *(54)*.

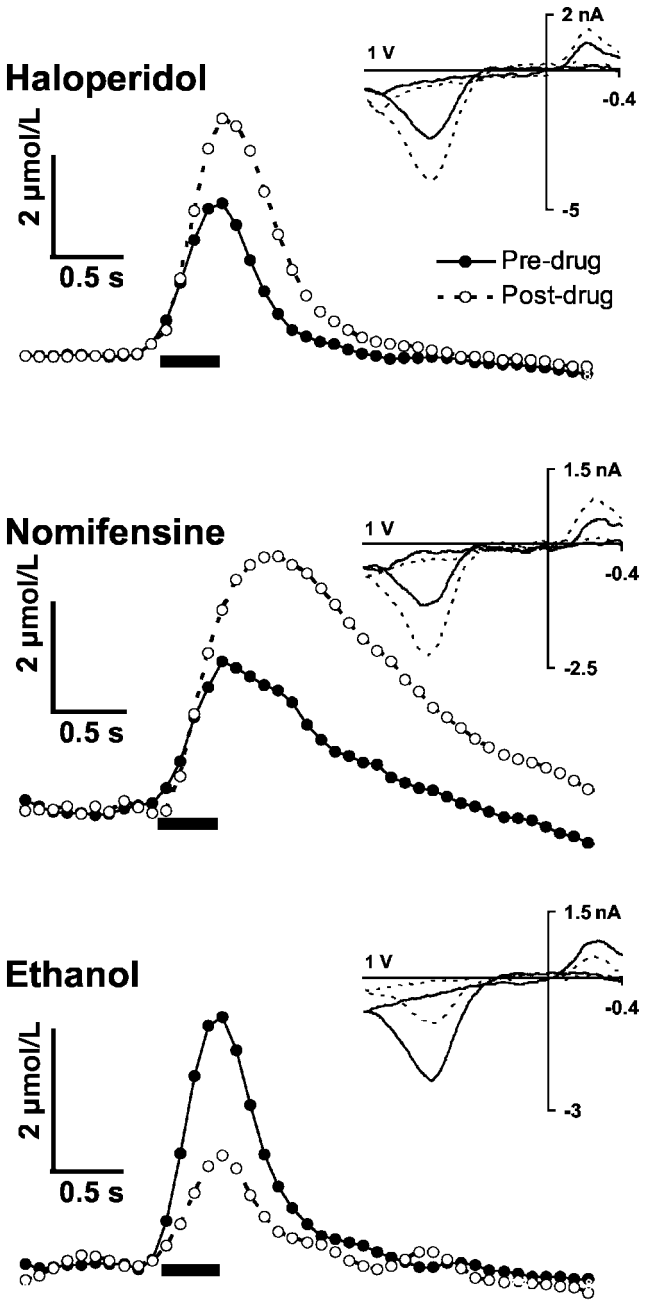

Fig. 7. Effects of haloperidol, nomifensine, and ethanol on electrically evoked dopamine release and subsequent uptake in the freely moving rat.

(*Top*), dopamine release before and 15 min after intraperitoneal injection of haloperidol (0.5 mg/kg). (*Middle*), dopamine release before and 15 min after intraperitoneal injection of nomifensine (7 mg/kg). (*Bottom*), dopamine release before and 10 min after intragastric administration of ethanol (2.5 g/kg, 300 g/L in water). Dopamine concentration was determined by converting the current at the oxidation potential of dopamine (+0.6 V vs Ag/AgCl reference), using the in vitro calibration of the electrode after the experiment. The *inset plots* are the cyclic voltammograms corresponding to the peak of the dopamine response (*solid line*, predrug; *dotted line*, postdrug). The electrical stimulation (bipolar, 24 pulses, 60 Hz, 120–125 μA) is indicated in each plot by the *rectangular bar*.

The electrical stimulation described in Fig. 7 (bipolar, 24 pulses, 60 Hz, 120 μA) is also reinforcing to the rat because it will self-administer such stimulation trains in the substantia nigra and ventral tegmental area in the intracranial self-stimulation (ICS) paradigm. In a particularly interesting series of experiments, the differences in dopamine release produced by experimenter-administered vs self-administered stimulation are described *(55, 56)*. When the experimenter administered a single stimulation, dopamine release was evident in the caudate-putamen and nucleus accumbens core and shell. In addition, dopamine signals occurred as the rat learned to press a bar to deliver the stimulation itself. However, the extracellular dopamine signal disappeared within minutes as ICS behavior became robust. These findings are useful in interpreting the role of dopamine in ICS, suggesting that it is more important in acquisition than maintenance of the behavior. [Note that Kruk et al. *(57)*, also using fast-scan cyclic voltammetry, failed to detect dopamine release even in the acquisition phase of intracranial self-stimulation; however, this may be attributable to the use of chronically implanted electrodes, which are less sensitive than acutely implanted ones *(40)*.]

NATURALLY OCCURRING DOPAMINE TRANSIENTS

The first neurochemical demonstration of a naturally occurring dopamine transient was by Rebec et al. *(17)*. Using fast-scan cyclic voltammetry, they monitored dopamine in the caudate-putamen and nucleus accumbens core and shell of freely moving rats. No signals were detected when the rats were sitting quietly in the test cage. However, when a barrier was lifted and the rats entered a novel environment, dopamine transiently increased in the nucleus accumbens shell. No such signal was apparent in the core or the caudate-putamen. The dopamine transient in the nucleus accumbens shell lasted <10 s, but the concentration was undetermined. These data demonstrate a phasic dopamine response to novelty that is heterogeneous among dopamine target nuclei.

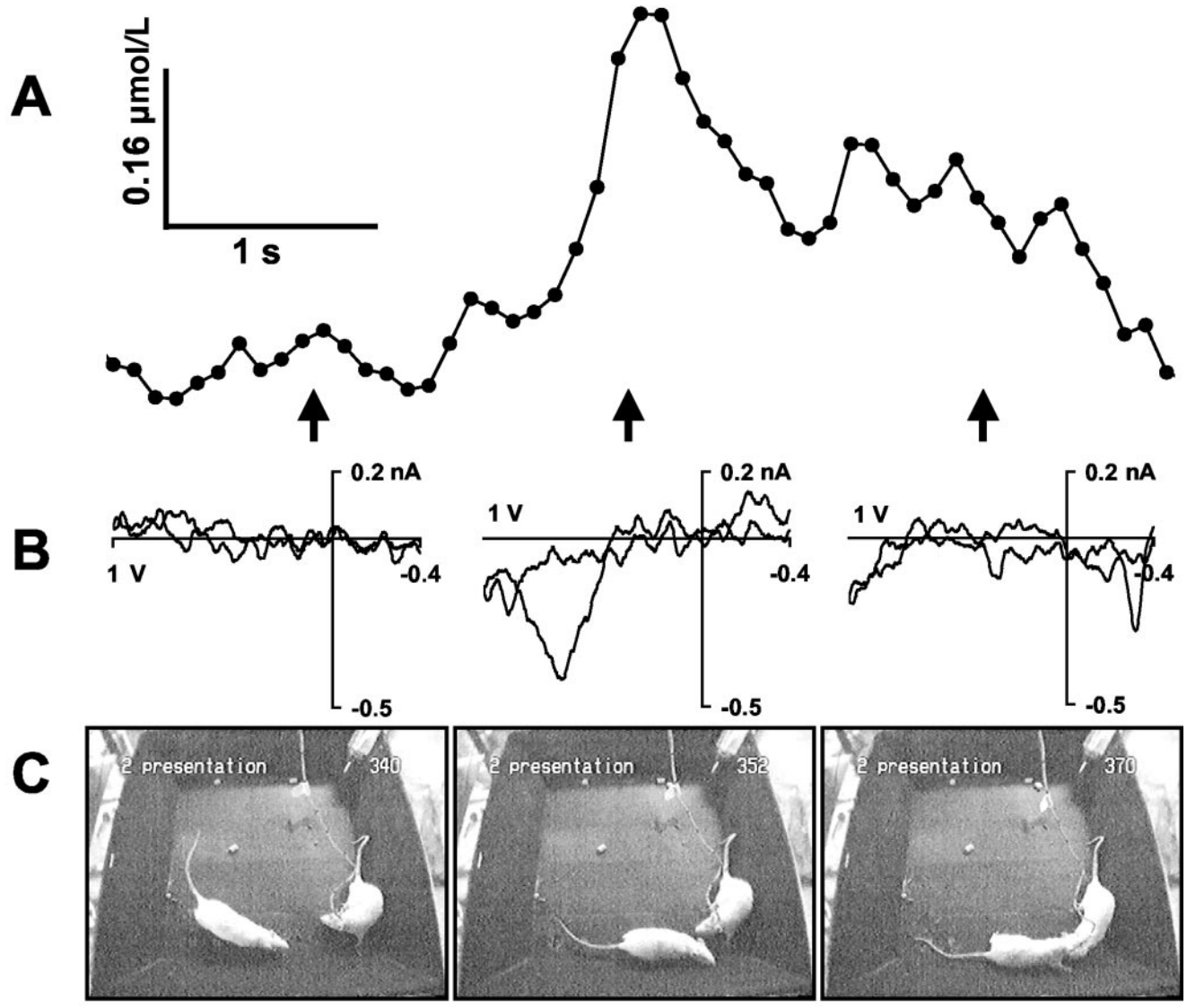

Fig. 8. Naturally occurring dopamine transient in the nucleus accumbens shell of a male rat associated with initial interaction with another male.

(*A*), the electrochemical signal at the oxidation potential of dopamine (+0.6 V vs Ag/AgCl reference) converted to concentration using the in vitro calibration of the electrode after the experiment. (*B*), cyclic voltammograms corresponding to the electrochemical signal at the times indicated by the *arrows*. The sharp increase in current is attributable to oxidation of dopamine (*middle voltammogram*), whereas no changes in dopamine are apparent before (*left voltammogram)* or after (*right voltammogram).* (*C*), video record of the experiment corresponding to the electrochemical signal at the times indicated by the arrows. The dopamine transient coincided with the initial whisker contact of the test male with the stimulus male.

Additional dopamine transients have been measured in the nucleus accumbens core during sexual behavior in male rats *(18)*. Large (200–500 nmol/L), transient (<1 s) dopamine signals were associated with the introduction of a receptive female into the test cage as well as with subsequent appetitive sexual behaviors, such as approaching and sniffing the female. However, it is likely that additional transients were below the detection limits: when the experiment was repeated in two rats after administration of nomifensine, the number of detected transients was greatly increased. Electrically evoked dopamine release in the same rats was consistent throughout the experiment, indicating that the infrequent transients detected during the copulation phase were not attributable to a decreased sensitivity of the electrode or an inability of neurons to release dopamine in a phasic manner. In summary, these data are consistent with numerous microdialysis studies that indicate increased dopamine transmission in the nucleus accumbens during sex behavior *(58)* and extend them by pinpointing exact behaviors and events associated with phasic dopamine activity.

The most robust signals reported by Robinson et al. *(18)* were at the presentation of the receptive female, an event that may include components of novelty as well as social or sexual motivation. This chemical response generalizes to the presentation of nonreceptive females and males *(22)*. In contrast to the signals associated with a

novel environment *(17)*, the dopamine transients associated with conspecific interaction were detected in the caudate-putamen, nucleus accumbens core and shell, and olfactory tubercle *(22)*. Fig. 8 illustrates the phasic dopamine response in the nucleus accumbens core of a male rat to the presentation of another male. In this case, the transient was associated with initial whisker contact with the stimulus male, which was followed by further investigation and sniffing. From the cyclic voltammograms, it is clear that dopamine concentrations increased only at the initial contact with the male and not before or afterward.

Extracellular dopamine transients were also observed in the nucleus accumbens core during cocaine self-administration *(59)*. Whereas previous studies used unexpected or novel stimuli, these rats were well trained to self-administer intravenous cocaine. To increase the voltammetric sensitivity of dopamine, the potentials applied to the electrode were extended to −0.6 to 1.4 V. Dopamine signals were tightly associated with the lever-press response for cocaine as well as with the cues predicting cocaine infusion. Together, these data are consistent with theories of the role of phasic dopamine as an alerting *(6)* or switching *(60)* signal that may be important in reinforcement and learning.

Conclusions

Fast-scan cyclic voltammetry is a useful technique uniquely suited to detect fast changes in dopamine concentrations in vivo. The method can be used to monitor naturally occurring changes or in combination with electrical stimulations to probe drug effects on dopamine release and uptake. Of the techniques available for use in the behaving animal, it provides the best combination of temporal resolution and chemical selectivity. The visualization of dopamine transients in vivo via fast-scan cyclic voltammetry are particularly exciting because they represent the tip of the iceberg in understanding when dopamine transients occur and what role they play in behavior.

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