Psychoanalytical Electrochemistry:

Dopamine and Behavior

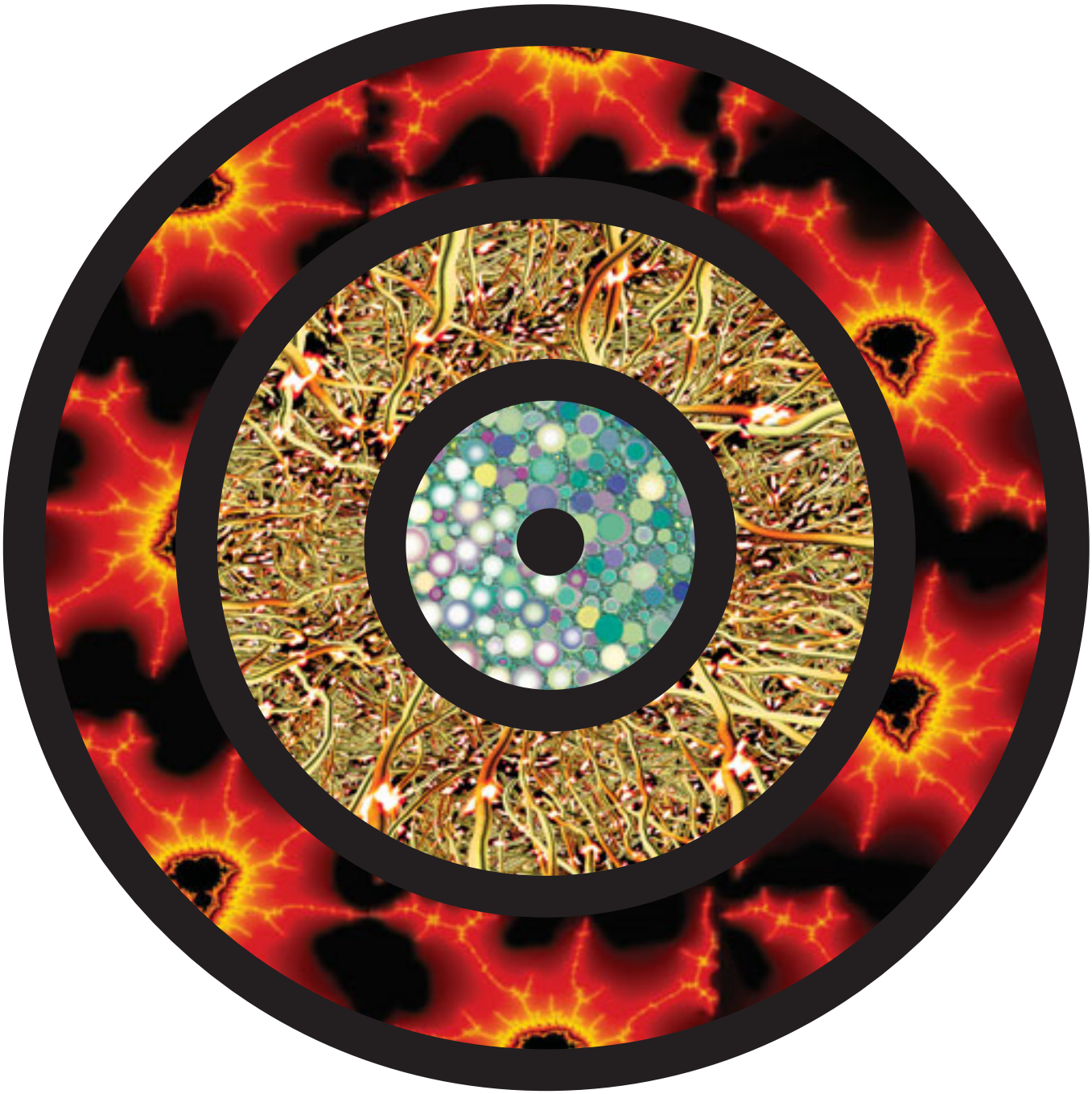
Correlating neurochemical changes in the brain with behavior marks the beginning of an exciting new interdisciplinary field, psychoanalytical chemistry.

B. Jill Venton
R. Mark Wightman

University of North Carolina

In the mammalian brain, neuronal networks process vast amounts of information received from the subject's environment from various senses such as sight, hearing, and touch, which are then combined with signals from throughout the body. Much of the signaling within the brain uses small molecules as chemical messengers (neurotransmitters) between neurons. The processing in brain networks eventually manifests as animal behavior—if the animal is hungry and it sees or smells food, it will go to the food and eat it. By following the temporal fluctuations of the signaling molecules, analytical chemists can play a major role in explaining the processing of sensory information, determining its various control points, and evaluating all of its complexities to help us better understand our own behaviors. We have dubbed this emerging area "psychoanalytical" chemistry and have recently begun to explore the issues of ongoing neurochemical processes with an electrochemical sensor that can detect the neurotransmitter dopamine.

The brain is a challenging environment for chemical sensing because low concentrations of analytes must be detected in the presence of interferences, while disturbing the tissue as little as possible, and because various surface processes inherent to biological systems can affect sensor response.



Thus, to make meaningful measurements, we must understand the properties of the analytical sensor and the general characteristics of the biological system. The perfect sensor would be highly sensitive and selective, with an infinitely fast response time. However, there is always a compromise among these three properties, and biological experiments must be designed with these variables in mind.

Several chemical sensors have been used to measure fluctuations in extracellular concentrations of neurotransmitters. Microdialysis has been the most common method to sample the chemical environment of the brain (*1*, *2*). A 200-μm probe, covered with a dialysis membrane 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 using chromatography. Although efforts are under way to improve the time resolution of microdialysis (*3*), samples are typically collected at 1–10-min intervals, so microdialysis is best suited for measuring changes on a minute timescale.

Adams pioneered the use of electroanalytical sensors to detect chemical changes in the brain in the 1970s, and other researchers addressed electrode sensitivity and selectivity issues in the 1980s (*4*, *5*). Today, microelectrodes allow subsecond measurements during neurotransmission with minimal tissue damage. For example, catecholamine neurotransmitters are easily oxidized

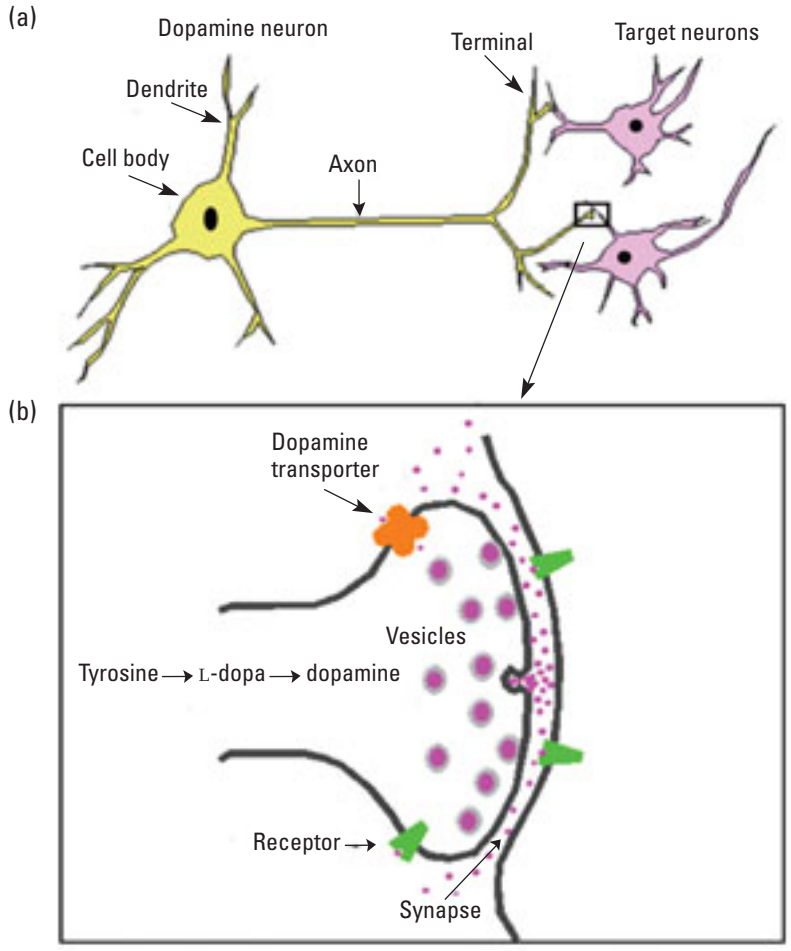


FIGURE 1. Schematic of a dopaminergic neuron.

(a) The dendrites of the dopaminergic neuron receive information from other cells. An action potential then propagates down the axon to the terminals, where neurotransmitters relay information to target cells. (b) Enlarged view of a dopamine terminal. Dopamine (purple circles) is synthesized from tyrosine and packaged into vesicles. In response to an action potential, vesicles release their contents into the synapse. Dopamine can then diffuse out of the synapse, interact with receptors, or be taken up by the dopamine transporter.

and reduced at physiological pH, and their neurotransmission can therefore be observed in real time.

Our lab has investigated dopamine neurotransmission in the brain using carbon-fiber microelectrodes. Dopamine is an important neurotransmitter because it is involved in motor and cognitive functions; deficits in brain dopamine cause Parkinson's disease in humans. Dopamine has also been associated with the reward system, the circuitry in the brain responsible for the motivation to seek out stimuli as well as the emotions of feeling satisfied and satiated in one's environment. It is thought that this system is activated by natural rewards such as food, drink, and sex, as well as by addictive drugs.

We have characterized the regulation of dopamine neurotransmission through the use of electrically evoked dopamine release in slices of brain tissue, anesthetized rats and mice, and freely moving rats (*6*). However, recent developments have extended the technology to measurements of naturally occurring dopamine release in freely moving rats during behavioral situations. We have found that a behavioral stimulus can evoke a transient increase in dopamine, providing the first real-time clues of how a neurotransmitter controls behavior on second and subsecond timescales and revealing how critical rapid, selective, and sensitive measurements are for real-time detection of chemical changes in the brain (*7*, *8*).

Dopaminergic neurons

To understand the challenges associated with measuring dopamine in vivo, it is important to understand the environment in which dopaminergic neurons function. Neurons are the cells that perform the fundamental tasks of information integration and control. They gather, process, and relay specific types of information in the brain, depending on the circuits in which they are involved. Dopaminergic neurons contain two types of projections attached to the cell body, dendrites and an axon (Figure 1). Dopamine can be released at the terminal end of the axon and received by the dendrites of neighboring neurons. Dopaminergic neurons are located in only a few discrete regions of the brain, with the cell bodies found in one area and the axons projecting to another. Most of the cell bodies are in the substantia nigra/ventral tegmental area, and their axons project to various regions, including the caudate-putamen or the nucleus accumbens. These regions are only a couple of millimeters across in their largest dimension, so implanting an electrode into them requires a micromanipulator. The most serious loss of dopaminergic neurotransmission during Parkinson's disease is in the caudate-putamen, and thus this region has been associated with motor control. The projections to the nucleus accumbens play a role in the brain reward circuit. There are only ~10,000 dopaminergic neurons on each side of the brain in this pathway, but each axon can branch and form many terminals.

Dopamine is synthesized in the neuron and then packaged into membrane-bound packets called vesicles (Figure 1). Neurotransmitter release is initiated by an electrical impulse called an action potential. Each neuron has a resting membrane potential caused by ionic concentration gradients across the cell membrane. When an appropriate neurotransmitter binds to receptors on the dendrites or cell body, ion channels open, allowing an influx of Na^+ that changes the membrane potential and initiates an action potential or "firing". It then propagates down the axon to the terminal at a rate of 0.5 m/s (*9*). This firing causes voltage-gated Ca^{2+} channels to open in the terminals. The resultant Ca^{2+} influx triggers the vesicles to fuse with the cell membrane and release their contents, a process termed exocytosis. Because some vesicles are docked adjacent to the

membrane, exocytosis occurs on a millisecond timescale (*10*). The extracellular concentration of neurotransmitter following exocytosis depends on the number of vesicles released and the amount of neurotransmitter in each vesicle, which may be quite small. For example, retinal dopaminergic neurons contain only zeptomole quantities of dopamine (*11*).

After exocytosis occurs, dopamine rapidly diffuses out of the synaptic cleft and interacts with dopaminergic receptors located either on dopaminergic neurons or neurons containing other neurotransmitters (*12*). Its concentration in the extracellular fluid is governed by diffusion and uptake. The dopamine transporter, a membrane-bound protein, controls uptake into neurons by pumping dopamine from the extracellular space. In the caudate-putamen, uptake is very fast, with 4 μM of dopamine clearing every second. Once it is transported back inside the neuron, it is either repackaged into vesicles or metabolized.

Dopaminergic neurons fire action potentials in two patterns, tonic and phasic (*13*). Tonic firing occurs at frequencies of 2–5 Hz, resulting in a steady-state basal level of extracellular dopamine (*14*). Basal levels of dopamine in the caudate-putamen are estimated at ~5 nM by using microdialysis (*1*). However, this may be an underestimate because uptake competes with diffusion of dopamine to the probe, effectively lowering the sampled concentration (*15*). Also, because microdialysis probes are relatively large, calculations of basal levels must take into account the damaged tissue layer that forms around the probe (*16*).

Phasic firing occurs at frequencies of 15–100 Hz, which should result in a transient increase in dopamine concentration to levels that are much higher than the basal levels because the increased firing rate should cause an increase in the rate of release. On the basis of this expectation, we designed behavioral experiments to evoke bursts and found that dopamine transients occur on a timescale consistent with phasic firing (*7*, *8*). The two firing patterns that result in very different extracellular concentrations of dopamine activate different receptors that have both low (1.6 μM) and high (20 nM) affinity states (*17*). Therefore, tonic firing might produce basal levels that activate some populations of receptors while others are only activated by the transient dopamine increases caused by phasic firing.

Electrochemical measurements

Because low concentrations of dopamine are released and rapidly cleared from the extracellular space, the sensor must be fast, sensitive, and selective. Ideally, one would like to be able to measure dopamine concentrations inside the 100-nm synapse. Although this is not currently possible, it is important to minimize the size of the electrode so that synapses can be approached as closely as possible and tissue damage minimized. Typical cylindrical carbon-fiber microelectrodes used in vivo are 5 μm in diameter and 100 μm long.

Measuring dopamine in the brain is complicated by the presence of numerous other electroactive endogenous compounds. Several easily oxidized compounds have been identified in brain fluid samples, including dopamine metabolites such as 3,4-dihydroxyphenylacetic acid and homovanillic acid; the antioxidant ascorbic acid; and other neurotransmitters, such as nitric oxide, norepinephrine, and serotonin (*18*). Because many of these compounds are present in large concentrations, electrochemical selectivity is important in discriminating the dopamine signal. Also, after dopamine neuronal activity occurs, changes in blood flow cause local alkaline pH fluctuations, which can interfere with dopamine detection by altering the background charging current of the electrode in many electroanalytical techniques (*19*). Previous investigations into dopaminergic neurotransmission with electrochemical methods often led to equivocal results because of insufficient selectivity, and the results were described by phrases such as "dopamine-like signals".

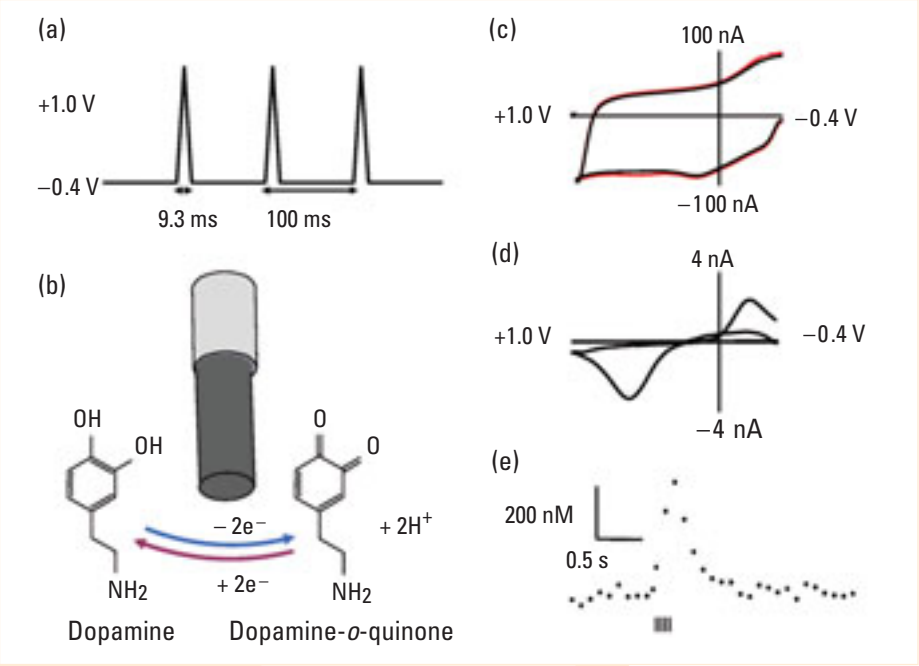


FIGURE 2. FSCV.

(a) The electrode potential is scanned from −0.4 V to +1.0 V and back every 100 ms at 300 V/s. (b) At the cylindrical carbon-fiber microelectrode, dopamine is oxidized to dopamine-*o*-quinone and then reduced back to dopamine. (c) A large background charging current at the electrode (black line) is produced. When dopamine is present (red line), only small changes in the current are detected. (d) The characteristic chemical "fingerprint" cyclic voltammogram for dopamine is obtained by subtracting the black line from the red line in (c). (e) The current at the oxidation potential, converted to concentration using an in vitro calibration value, can be plotted versus time to monitor dopamine concentration changes after a short electrical stimulation (4 pulses delivered at 100 Hz, indicated by hash marks).

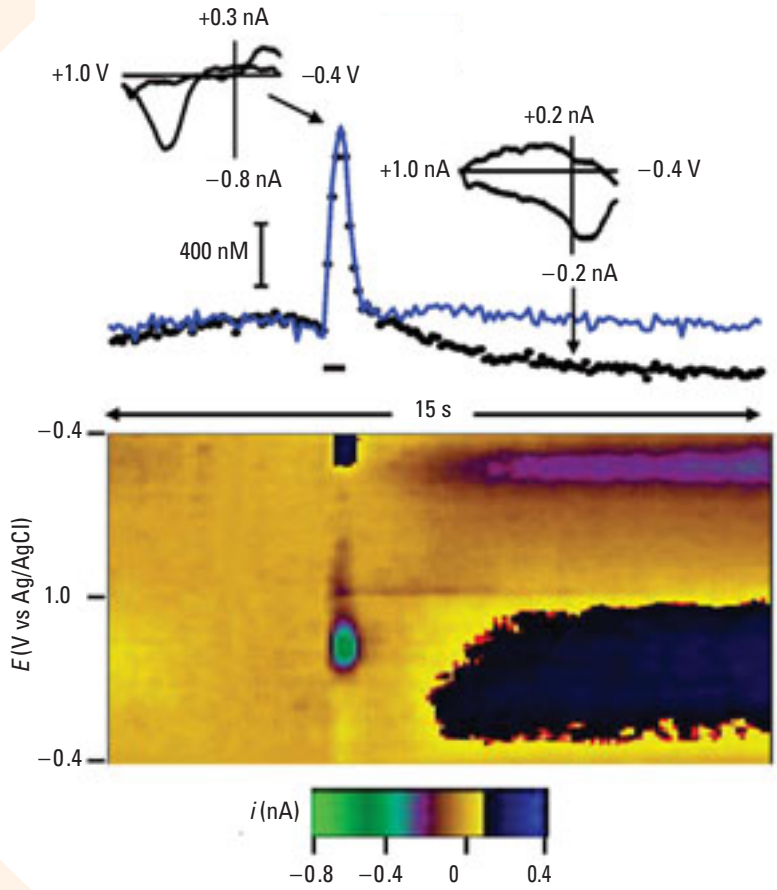


FIGURE 3. A pH change is sensed electrochemically following the dopamine change.

Dopamine release from neuronal terminals was elicited by electrical stimulation of the cell bodies. On the color plot, the green circle centered at ~+0.6 V and the blue circle at ~–0.2 V are caused by dopamine oxidation and reduction, respectively. The broad blue and purple bands that occur later in the plot are caused by an alkaline pH shift. The black dotted trace shows the signal at ~+0.6 V, which has contributions from both dopamine and pH signals. The blue line is the pure dopamine signal with the effects of the pH shift subtracted. The cyclic voltammogram at the peak is consistent with dopamine, whereas the one taken later indicates an alkaline pH change.

Such nonspecific measurements do little to elucidate the functions of dopamine (*13*).

Minimization of electrical noise is critical for measuring low concentrations. Increasing the electrode area can increase the dopamine oxidation current (the signal) relative to the fundamental noise of the amplifiers. Therefore, cylindrical electrodes provide greater sensitivity than smaller disk electrodes for measuring dopamine. Amplifier noise is particularly apparent because the bandpass of the instrument needs to be wider than in conventional electrochemical recordings to follow the rapid dynamics of the dopamine concentration. However, further increases in the electrode area boost both the signal and the noise concurrently because the electrode contributes to the noise through its associated capacitance.

Sensitivity can be further increased by electrochemical pretreatment or by coating the electrode with a polymer film (*20*, *21*). However, the disadvantage of these approaches is a slower time response, which is especially problematic when monitoring dynamic biological systems that require subsecond time resolution. For example, Nafion-coated disk electrodes were used for many years because Nafion enhances sensitivity and selectivity (*21*). However, very fast processes are obscured by the time delay caused by diffusion through the Nafion coating (*22*); therefore, uncoated cylindrical carbon-fiber microelectrodes are now used to detect spontaneous dopamine transients. We always use a fresh electrode to minimize fouling of the surface by the brain environment. To ensure that selectivity and sensitivity are maintained during a recording session, electrodes are calibrated using flow-injection analysis following the experiment to determine the electrode sensitivity after exposure to the brain (*23*).

Fast-scan cyclic voltammetry (FSCV) has emerged as the dominant electrochemical technique for in vivo neurotransmitter sensing because of its rapid sampling, chemical selectivity, and relatively high sensitivity (*24*). In FSCV, concentrations are sampled by repeating scans at regular intervals, and the current arising from analyte oxidation or reduction is monitored. For dopamine detection, the electrode is typically ramped from a resting potential of –0.4 to +1.0 V and back at 300 V/s every 100 ms (Figure 2). These fast scan rates produce a large background current, which can be subtracted. The 10-Hz repetition rate normally used for the cyclic voltammograms allows subsecond time resolution. Although the background-subtracted voltammograms provide a signature of the analyte, the subtraction process removes information concerning basal levels.

False color plots are used to view all of the cyclic voltammograms simultaneously and help discriminate dopamine from interferences (*25*). For example, the color plot in Figure 3 shows data recorded when a train of electrical stimulations was applied to neurons to mimic phasic firing. Time and scanned potential are plotted on the *x*-axis and the *y*-axis, respectively, and current is shown in false color. Dopamine redox processes occur at specific potentials; the oxidation is the green circle at ~+0.6 V, and the reduction is the blue circle at ~–0.2 V. Both are seen immediately after the stimulation. An alkaline pH change occurs after the dopamine increase and is visualized by the long blue and purple bands that are seen over a wider range of potentials. The method responds to pH changes because they cause a small shift in the background current. Above the peak is a trace that shows the signal at +0.6 V, which includes a contribution from both dopamine and an alkaline pH change, and a trace of just the dopamine signal, when the effects of pH have been subtracted. The cyclic

voltammogram for the peak is characteristic of dopamine, whereas the cyclic voltammogram at later times is characteristic of an alkaline pH change (*19*).

Other electrochemical techniques have been used to measure neurotransmitters in the brain. Constant-potential amperometry has almost no selectivity, but it is very sensitive, has a nearly instantaneous electrode response time, and can be used with high sampling frequencies without a decrease in sensitivity (*26*). Although the lack of selectivity makes it unsuitable for measuring naturally occurring transients, it can be used to characterize the rate of electrically stimulated dopamine release and subsequent uptake. Chronoamperometry has less selectivity, and differential pulse voltammetry has a lower time resolution than FSCV, so neither approach offers real advantages for in vivo measurements. Indeed, no single electrochemical technique is suited for all applications. Rather, the various approaches are a series of compromises. Therefore, the goals of the experiment must be well defined to select the appropriate technique.

State-of-the-art measurements

Sensitivity is especially important in detecting naturally occurring dopamine transients and can be affected by the range of the potential scan. Using a scan from –0.4 to +1.0 V versus a saturated calomel electrode, Rebec and co-workers were the first to use FSCV to observe dopamine transients during behavior (*27*). The transient clearly signaled an altered behavioral state because it only occurred when a rat entered a novel environment. Subsequently, we examined whether transients occurred in a male rat when a receptive female rat was introduced into his cage. With this FSCV waveform, detection limits are ~20 nM in vivo, and we were able to observe transients when the female was presented. However, the frequency of transients was even greater after administration of nomifensine, a drug that blocks dopamine uptake and thus can increase extracellular levels (*28*). Therefore, these sensors may have been detecting only the largest bursts of dopamine, and greater sensitivity might have enabled the detection of smaller transients.

Because the detection limit for dopamine oxidation calculated for a diffusion-controlled electrode process during FSCV is in the micromolar range, the observed nanomolar detection limit was unexpected. In fact, dopamine detection is not diffusion-controlled, but rather is dominated by its adsorption to the carbon-fiber electrode. Effectively, the dopamine is preconcentrated between the CV scans. The low detection limits found during calibration persist when the electrode is inserted into the brain, indicating that adsorption sites on the electrode are not blocked by competing proteins in the extracellular fluid. The adsorption process slightly delays the response time of the electrode and leads to a trade-off between time resolution and sensitivity because faster sampling rates result in smaller signals (*29*).

To further increase sensitivity, the scan rate can be increased or the potentials of the scan extended (*20*). For example, 10-fold greater sensitivity is achieved by scanning from –0.6 to +1.4 V and back at 450 V/s (*8*). Several factors contribute to this increase. For adsorbed species, the signal increases linearly with scan rate. Decreasing the resting potential to a more negative value allows more adsorption of the positively charged dopamine to the electrode, apparently because of electrostatic interactions. Increasing the reversal potential causes further oxidation of the carbon surface, a process that adds adsorption sites. When this extended potential range is used, the reduction peak in the cyclic voltammogram is smaller because of changes in adsorption for dopamine-*o*-quinone. The main shortcoming of this extended waveform is that the response time slows by >0.5 s. Therefore, this waveform is primarily useful for experiments in which low nanomolar concentrations must be detected but not for those measuring fast kinetics of release and uptake.

CV in alert animals

Although electrodes are used routinely in anesthetized animals to sense dopamine, extending this technique to alert animals required considerable development. The current transducer had to be miniaturized so it would fit on an animal's head. Appropriate connections for the electronics required a low-noise

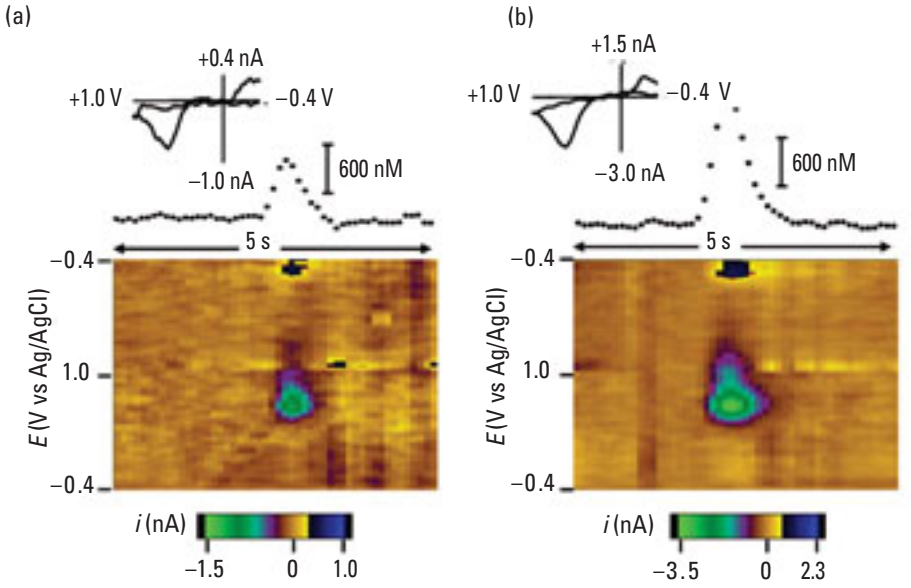


FIGURE 4. Comparison of behaviorally evoked and stimulated dopamine release.

(a) A dopamine transient occurs in response to whisker touching when a stimulus male rat is introduced into the cage of a test male rat. (b) The behaviorally evoked signal is very similar to electrically stimulated release in the same rat.

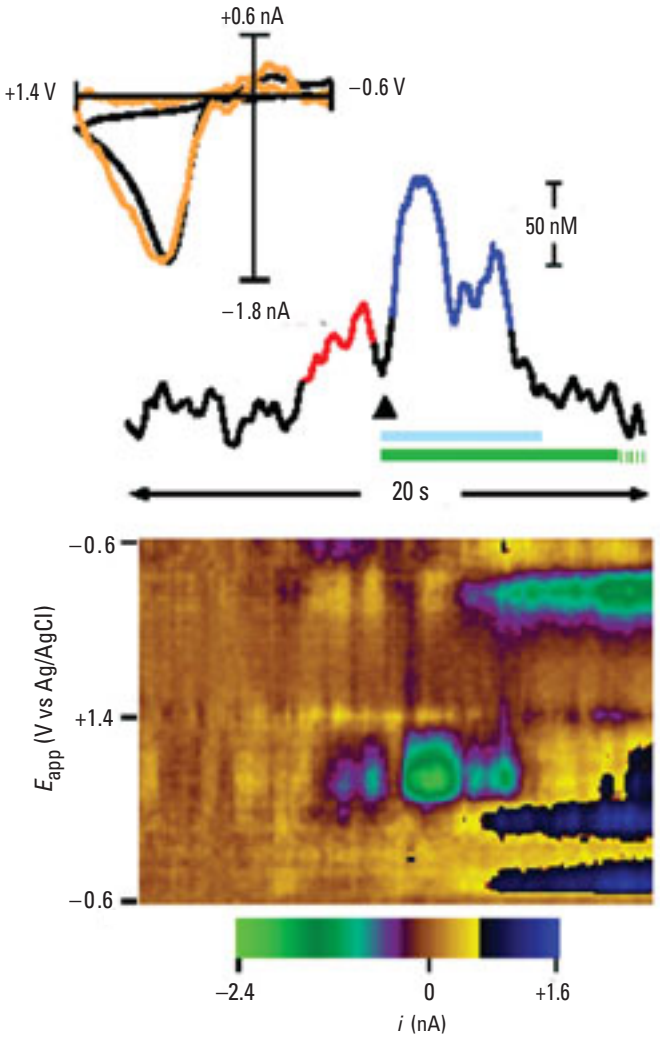


FIGURE 5. Dopamine release during cocaine self-administration.

Rats were trained to press a lever (arrowhead) to receive a small intravenous injection of cocaine. The trace above the color plot shows changes in dopamine. The red peak indicates an increase in dopamine before the lever press. The two blue peaks indicate transients that occurred after the lever press. Underneath the trace, the green bar marks the time the audiovisual cues associated with the lever press were on. The blue bar indicates when the pump was activated to deliver cocaine. The cyclic voltammogram (black) of behaviorally evoked dopamine matches the electrically evoked voltammogram (orange). In the color plot, the broad blue and green bands after dopamine release are caused by pH changes. (Adapted with permission from Ref. 8.)

swivel, so the animal would not tangle the connecting wires. Special holders to implant the carbon-fiber electrode into the brain had to be devised. Electrical noise artifacts caused by animal movements were minimized by implanting the reference electrode directly in the brain and by using slanted walls on the sides of the cage to prevent the rat from knocking its head-mounted instrumentation against the wall. Ethical treatment of animals is imperative and is supervised by a veterinarian and the Institutional Animal Care and Use Committee found on most university campuses. The design of meaningful behavioral experiments is very difficult and requires considerable planning. Our laboratory has benefited from collaboration with Regina Carelli, a psychologist who is an expert in such experiments.

Several other technical advances, uncommon to typical chemistry experiments, were necessary to be able to correlate dopamine transients with behavior. Subsecond synchronization of chemistry and behavior was needed. A video character generator was designed to continuously write the FSCV scan number to the videotaped record of the animal. Synchronization was also achieved in experiments in which the animal self-administers cocaine; each time the animal presses the lever, an electronic pulse is sent to the computer, where it is recorded with the FSCV data.

Another challenge is the processing of the large number of cyclic voltammograms collected to identify the presence of dopamine. For example, a 10-Hz sampling rate during a 2-h behavioral experiment results in >75,000 cyclic voltammograms that must be analyzed. Dopamine identification has been automated by using locally written computer software (*30*). The program calculates correlation coefficients for the experimental cyclic voltammogram and a template cyclic voltammogram of stimulated dopamine release.

Dopamine transients and behavior

To date, we have observed dopamine transients in rats in two different behavioral situations: presentation of other rats and cocaine self-administration. These measurements provide a totally new view of the role of dopamine in behavior. Although researchers have used microdialysis for quite some time to determine changes in basal levels of dopamine during behavior, those measurements were on a minute timescale and differ dramatically from the subsecond changes in concentrations that we observed.

After our first set of trials, in which a female rat was introduced into the cage of a male rat (*28*), we performed a more comprehensive set of experiments to determine if the dopamine transients were of a sexual origin or were generalized responses to the novelty of having another rat in the cage. In these experiments, a male, a nonreceptive female, or a receptive female stimulus rat was randomly presented in the cage of a test male rat (*7*). Figure 4 is an example of a behaviorally evoked dopamine transient after the introduction of a stimulus rat. The transient due to the spontaneous dopamine release has characteristics similar to the electrically stimulated dopamine release in the same rat. All the different stimulus rats evoked transients, but they were more frequent in response to female rats, which indicates that transients occur when an animal is alerted to a change in its environment. These dopamine transients are also similar to those seen when an animal enters a new environment (*27*).

The time resolution of the experiments allowed us to determine what behaviors accompany the dopamine transients on the subsecond timescale. Most of the dopamine transients occurred within a few seconds of the stimulus rat presentation and were associated with orientation behaviors, such as the test rat turning its head toward the stimulus rat. However, other

transients were associated with sniffing or touching the stimulus rat, vocalizations, and sexual behavior. For example, the transient in Figure 4 occurred when the stimulus male rat approached the test male and their whiskers touched. In general, the rats were more active after the dopamine transients; sniffing especially increased. These presentation experiments suggest that dopamine transients are important in causing the animal to switch behaviors and react appropriately to changes in its environment.

In the second behavioral situation, the rat was taught to push a lever to receive a small amount of intravenous cocaine (*8*). Audiovisual cues, such as a change in cage lighting and a tone, were also presented when the rat pressed the lever, and the rat learned to associate these cues with the imminent arrival of the drug. This behavior was reinforced by the cocaine; a well-trained animal will initially press the lever 3 or 4 times to load up on cocaine and then reliably press about once every 5 min. Two phases of dopamine transients were observed around the time the lever was pressed (Figure 5). Initially, the dopamine concentration began to rise ~4 s before the lever press (red peak), deflected sharply as the animal pressed the lever, and then peaked again ~5 s after (blue peak). In Figure 5, there are actually two dopamine transients after the lever press. Typical dopamine concentrations peaked at only 50–100 nM.

There are behavioral links to each of the dopamine peaks during cocaine self-administration. The first peak, before the lever press, corresponded to the animal approaching the lever. To test whether changes in dopamine were sufficient to initiate drug-seeking behavior, short trains of electrical stimulation pulses were administered to the animal to elicit dopamine peaks that mimicked the naturally occurring transients. A few seconds after these experimenter-delivered stimulations, it was more likely that a rat would press the lever to self-administer cocaine, indicating that a burst of dopamine was initiating drug-seeking behavior. The second dopamine peak, after the lever press, is most likely associated with craving. The dopamine transient occurs immediately after the lever press, although the pumping system takes a few seconds to deliver the cocaine. To test whether the dopamine transient was a response to the audiovisual cues that accompanied each lever press, we randomly presented the cues to the rat without it pressing the lever or receiving cocaine. Dopamine transients were observed in response to just these cues, indicating that dopamine was involved in anticipation of the drug effects. Therefore, dopamine is associated with both the initiation of drug-seeking behavior and the anticipation of the drug's effect.

Conclusions

These subsecond measurements of naturally occurring changes in dopamine concentration are the first for any neurotransmitter in the brain of an alert, behaving animal and provide new insights into the way in which behavior is chemically regulated. The rat presentation experiments showed that dopamine transients alert the animal to changes in its environment, and the cocaine studies show that dopamine is involved in both the initiation of drug-seeking behaviors and anticipation of the drug's effects. Psychological interpretations would not be possible without appropriate understanding of the analytical signals obtained. Continued advances in analytical sensors will allow more insights into the dynamics and consequences of neurotransmission in the brain.

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Jill Venton recently received her Ph.D. from the University of North Carolina, Chapel Hill (UNCCH) and is currently a postdoctoral research associate at the University of Michigan. Her research interests include using analytical techniques to study neurotransmission. Mark Wightman is a professor at UNCCH whose research interests are in neurochemistry, including psychoanalytical chemistry, analytical chemistry in small volumes, and electrogenerated chemiluminescence. Address correspondence about this article to Wightman at CB 3290 Venable Hall, UNCCH, Chapel Hill, NC 27599-3290 (rmw@unc.edu).

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