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Fundamentals of Fast-Scan Cyclic Voltammetry for Dopamine Detection

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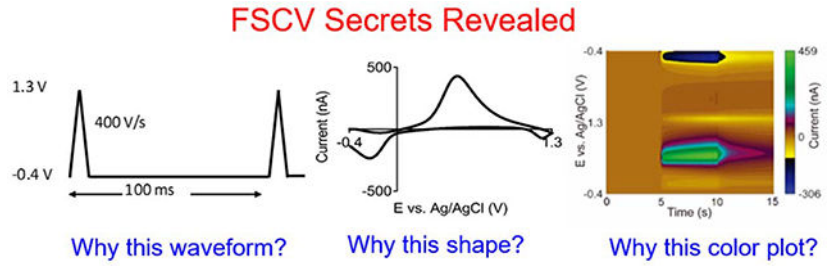
Abstract

Fast-scan cyclic voltammetry (FSCV) is used with carbon-fiber microelectrodes for the real-time detection of neurotransmitters on the subsecond time scale. With FSCV, the potential is ramped up from a holding potential to a switching potential and back, usually at a 400 V/s scan rate and a frequency of 10 Hz. The plot of current vs applied potential, the cyclic voltammogram (CV), has a very different shape for FSCV than for traditional cyclic voltammetry collected at scan rates which are 1000-fold slower. Here, we explore the theory of FSCV, with a focus on dopamine detection. First, we examine the shape of the CVs. Background currents, which are 100-fold higher than Faradaic currents, are subtracted out. Peak separation is primarily due to slow electron transfer kinetics, while the symmetrical peak shape is due to exhaustive electrolysis of all the adsorbed neurotransmitter. Second, we explain the origins of the dopamine waveform, and the factors that limit the holding potential (oxygen reduction), switching potential (water oxidation), scan rate (electrode instability), and repetition rate (adsorption). Third, we discuss data analysis, from data visualization with color plots, to the automated algorithms like principal components regression that distinguish dopamine from pH changes. Finally, newer applications are discussed, including optimization of waveforms for analyte selectivity, carbon nanomaterial electrodes that trap dopamine, and basal level measurements that facilitate neurotransmitter measurements on a longer time scale. FSCV theory is complex, but understanding it enables better development of new techniques to monitor neurotransmitters *in vivo*.

Graphical Abstract



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1. Introduction

In vivo electrochemical measurements require rapid temporal resolution to follow the dynamics of chemical neurotransmission.[1–3] Traditional cyclic voltammetry (CV) has scan rates in the range of 100 mV/s, and is too slow for measuring fast changes in neurotransmission.[4] Differential pulse voltammetry (DPV) provides better sensitivity and discrimination of analytes, but DPV is also performed on a slower time scale.[1,5] Constant potential amperometry allows fast measurements (typically 500 Hz), but holding at a constant potential provides little analyte selectivity.[6] Chronoamperometry steps to specific potentials and can be performed at 1 Hz frequencies, but still lacks selectivity.[7]

Fast-scan cyclic voltammetry (FSCV) is the most popular electrochemical method to measure rapid changes of neurotransmitters in the brain.[1,4,8–11] FSCV scan rates are one thousand times faster than conventional CV, typically around 400 V/s. These scan rates allow FSCV measurements to be made with subsecond temporal resolution.[11] FSCV must be performed at a microelectrode, which has a small time constant for fast capacitive charging.[12] For neurotransmitters, FSCV is almost always performed at carbon electrodes, traditionally carbon-fiber microelectrodes (CFMEs) (Fig. 1).[13] CFMEs are ideal for neurotransmitter detection of cations such as dopamine, because they have surface oxide functional groups that adsorb cations.[13–15] Recent studies have developed other types of carbon, particularly carbon nanomaterials, as microelectrodes.[16–20] At carbon electrodes, the kinetics are adsorption controlled instead of diffusion-controlled.[21] The theory of FSCV detection of dopamine at CFMEs has developed over decades of research and is found in the primary literature,[22,23] but it is not included in depth in any traditional electrochemistry textbooks.

In this review, we cover the theory of FSCV, starting from a framework of how it differs from conventional CV. First, we examine the shape of the FSCV voltammogram for dopamine and describe its characteristic features. Second, we explore the dopamine waveform and the research that led to the particular potentials, scan rate, and frequency employed. Third, we survey data outputs, such as color plots and current vs time plots, as well as data analysis techniques, such as principal components regression, which facilitate automatic identification of dopamine. Finally, the last section explores some of the frontiers of FSCV research, from custom waveform development, to nanomaterial electrodes that trap dopamine, to basal level measurements. Overall, FSCV is a vibrant research technique to study neurotransmitter changes *in vivo* and understanding the theory of the technique allows researchers to continue to develop it to solve challenges of modern neurotransmitter research.

2. FSCV voltammograms: Exploring their shape

2.1. Traditional cyclic voltammetry: precision voltammograms at slow scan rates

To understand FSCV, one must first understand conventional cyclic voltammetry. CV is a common electrochemistry technique that ramps a voltage to a switching potential and back in order to study redox currents at those potentials.[24] The theory for CV is well developed and can be found in any standard electrochemical textbook.[25] A well behaved, reversible,

diffusion-controlled redox couple, such as ruthenium hexamine $Ru(NH_3)_6^{3+/2+}$, will have peaks with a peak separation around 59/n mV, where n is the number of electrons transferred in the reaction.[24] The peak potentials, E_p, are around the formal potential of the redox couple, and the peak currents are proportional to concentration.

Fig. 2 shows an example of conventional cyclic voltammetry for dopamine at a glassy carbon electrode. For dopamine, the potential is scanned from a negative voltage to a positive voltage and dopamine is oxidized on the forward scan and reduced on the backward scan. Kinetics are likely a mix of diffusion- and adsorption-controlled because dopamine adsorbs to surface functional groups on the carbon surface.[14] ΔE_p is 70 mV, larger than the ideal expected for diffusion (~29 mV) or adsorption (0 mV), meaning it is not reversible. Traditional CVs are "duck-shaped." As the potential is swept more positive in an unstirred solution, the diffusion layer grows and there is less mass transport of analyte to the surface so the current decreases.[24] Usually the potential is reversed before the current goes to zero. With conventional scan rates, there is a capacitive charging current, but it is small compared to the Faradaic current and therefore it is not subtracted out. CV is used in many applications, from evaluating catalysts, to understanding corrosion, or measuring the E^0 of different compounds.[24] However, the time scale and sensitivity are not a good match for neurotransmitter measurements. Scanning from 0 to 1 V and back at 100 mV/s takes 20 s, longer than the time scale of neurotransmission. Also, current scales with area of the electrode and scan rate, so microelectrodes have small signals with traditional scan rates.

The time scale of conventional CV is too slow to analyze the time scale of biological changes. Julian Millar started, and Mark Wightman popularized, fast-scan cyclic voltammetry to speed up voltammetry to the biological time scale.[23,26,27] They increased the scan rate to several hundred volts per seconds, so that a single scan is completed in just a few milliseconds. Moving to faster scan rates is actually quite complex. In this section, we investigate the cyclic voltammograms (CVs) produced in FSCV: specifically examining background currents and the peculiar shape of the CVs.

2.2 FSCV Voltammograms: Background subtraction needed

Fast-scan cyclic voltammetry produces large background charging currents, because charging current magnitude is proportional to scan rate.[25] With slower scan rates of traditional cyclic voltammetry, background charging currents are typically smaller than Faradaic currents.[25] Background charging currents arise from the rearrangement of ions around an electrode when the potential is applied and so the charging, like a capacitor charging, is proportional to electrode surface area. FSCV cannot be performed at larger, traditional electrodes such as glassy-carbon electrodes because the background currents would be too large and take too long to stabilize. The background currents at typical CFMEs are in the range of hundreds of nA for FSCV, 10–100 times larger than the Faradaic currents being measured. Thus, it is difficult to distinguish the Faradaic current from the background signal in a voltammogram (Fig. 3A). However, background currents for FSCV are stable and thus the charging current is the same each scan.[27] Therefore, background currents are subtracted out to get a differential signal that is primarily Faradaic current. A background-subtracted CV for dopamine is shown in Fig. 3B.

Background subtraction sets one of the primary limitations of FSCV: it is inherently a differential technique. Therefore, it cannot be used to establish the basal levels of any neurotransmitter but instead is best at measuring fast changes in neurotransmitters. Like any analytical technique, FSCV cannot solve all problems, but it is particularly adept for answering questions about rapid, discrete changes in neurotransmitters.[8] Traditionally, techniques like microdialysis have been used to measure basal levels, providing a complementary measurement on a different time scale,[28] but in section five, we also explore basal levels being monitored with microelectrodes using other electrochemical techniques.

2.3 FSCV voltammograms: Understanding their shape

The background-subtracted voltammogram for dopamine has a characteristic shape (Fig. 3B), but doesn't look anything like the traditional CV with slower scan rates (Fig. 2). First, the peaks are much wider spread apart and the peak separation is a few hundred millivolts. Second, the peaks are symmetrical and come back to zero, losing the "bill" part of the traditional "duck shape". Third, the peak current for the oxidation of dopamine is much larger than the peak current for the reduction of dopamine-o-quinone (DOQ) back to dopamine. This section explains the fundamentals of FSCV for dopamine and how they lead to the characteristic shape of an FSCV voltammogram.

2.3.1. FSCV voltammograms: Peak separation is wider—Our first observation about the FSCV voltammogram is that the peaks are spread far apart and the peak positions are out of place. With slower scan rates, the ΔE_p was not the ideal ~30 mV, but it was still only 70 mV. For FSCV, the peak separation for dopamine is about 750 mV! Similarly, the peak oxidation appears to occur at a much higher potential, around 600 mV, instead of 200 mV as in the slow scan CV. What causes these shifts? There are a variety of possible factors, including an increased cell time constant, current amplifier bandwidth, low pass filter cut-off frequency, and uncompensated ohmic drop, but these factors are mainly concerns for FSCV with scan rates in the kV/s to MV/s range.[29,30] For typical scan rates in the hundreds of V/s, the primary reason for the large ΔE_p is the sluggish electron transfer kinetics for dopamine at carbon electrodes.[14,31] Current density is also a factor, as high current densities at microelectrodes also distort the shape of the voltammogram and cause a higher overpotential.[22] For quasi-reversible systems, the shape of the voltammogram is a function of the rate of electron transfer (k^0), diffusion coefficient, and scan rate (See Bard and Faulkner, 2nd edition, Section 6.55).[25] While k^0 (and E^0 as well) is expected to be the same for traditional and fast-scan rates if the experiment is performed at the same electrode, the increase in scan rate will shift the oxidation peak to the right and make the peak wider. Therefore, in FSCV, the shape of the voltammogram is distorted by scan rate, the rate of electron transfer, and high current density.

With higher scan rates, the peaks shift out to higher voltages for FSCV of dopamine, and ΔE_p becomes larger (Fig. 4A). The larger overpotentials are more problematic for higher scan rates in the thousands of V/s range, as the peaks shift even more, may not be complete in the normal potential scan window, and are more affected by ohmic drop.[29,30] Because the overpotential depends on scan rate, it is not advised to pick potentials for amperometry experiments based on the peak voltage in a FSCV voltammogram. For example, the 0.6 V

potential used as the standard for dopamine amperometry experiments comes from FSCV, but a much lower potential would suffice. Shifting peaks with scan rate becomes a greater problem with analytes that have oxidation potentials near the switching potential, as often the peaks appear on the reverse scan. For example, the peak for adenosine, with an E^o of 1.3 V,[32] shows up on the reverse scan with a 1.45 V switching potential (Fig. 4B).[33] Peaks can also appear on the reverse scan due to electrode activation or cleaning of the surface as the oxidation potential is applied,[34] and some sawhorse waveforms have been developed to pause at the switching potential to allow more oxidation to occur due to the slow electron transfer kinetics.[35–37]

2.3.2. FSCV voltammograms: Peaks are symmetrical for dopamine—One of the other issues with the FSCV voltammogram for dopamine is the peak shape. Traditional CVs are "duck-shaped," because as the diffusion layer grows, there is less mass transport of analyte to the surface so the current decreases, but the potential is usually reversed before the current goes to zero.[24] However, the peaks for dopamine in FSCV voltammograms are very symmetrical, returning quickly to zero current, and do not have the "duck bill." Their appearance is more similar to thin-layer cell voltammograms.[25] In a thin-layer cell, the width of the cell is so small that the analytes are restricted to the electrode surface. All of the analyte is oxidized (exhaustive electrolysis) and the current decreases to zero quickly, as the surface concentration is not replenished by mass transport. [25] Thin-layer cell behavior is also observed when only analyte adsorbed to the electrode is detected, so exhaustive electrolysis occurs and the current drops to zero when all the adsorbed analyte is oxidized.[25] Adsorption-controlled kinetics arise when the rate-limiting step for dopamine detection is due to the kinetics of adsorption.[21] Adsorption is one of the main reasons that FSCV for neurotransmitters is performed nearly exclusively at carbon electrodes. Carbon electrodes have edge-plane sites (defects) and oxide functional groups that attract and adsorb positively-charged neurotransmitters, leading to high sensitivity measurements.[14]

2.3.3. FSCV voltammograms: Peak heights are not the same for oxidation and reduction—Another interesting observation from examining the FSCV voltammogram is that the peak heights for the oxidation and the reduction are not the same for dopamine. Theory from thin-layer cell electrochemistry predicts that the peak heights would be the same if the redox reaction is reversible.[25] Then why is the reduction peak only about half the area of the oxidation peak? The answer again lies in adsorption and specifically the differences in adsorption between dopamine and DOQ. Bath and Wightman[21] found that dopamine adsorbs to carbon-fiber electrodes almost ten-fold stronger than DOQ. Practically, this means that the DOQ is more likely to desorb from the electrode and thus, it is not present on the surface to be reduced back to dopamine. This asymmetry in peak currents is well known and exacerbated *in vivo*, where reduction peaks tend to be smaller.[38]

3. Understanding the dopamine waveform

Most recent reports detecting dopamine with FSCV use a triangular voltage waveform from −0.4 to 1.3 V and back at 400 V/s and a 10 Hz frequency.[39,40] Where did that waveform come from and why is it the standard used today? This section describes the development of

the dopamine FSCV waveform and alternatives that have been used, parameter by parameter.

3.1. Waveform development: Why 10 Hz?

The standard waveform for FSCV is repeated at 10 Hz. But the waveform is typically only about 10 ms long, so it could be repeated at 100 Hz. Why not repeat the measurements faster? The answer to the question is adsorption. A pioneering paper by Wightman's group in 2000[21] showed that the current dramatically decreases if the frequency of FSCV measurement is increased. Indeed, repeating measurements at 100 Hz decreases the signal by over 75 % (Fig. 5A). The time spent at the holding potential for FSCV allows time for dopamine to adsorb to the electrode and preconcentrate. In many ways, FSCV is like a stripping voltammetry experiment, and FSCV has been used similar to stripping voltammetry for metal analysis.[41] After accumulating the analyte on the electrode for a given time, the voltage is ramped up and down quickly to strip (i.e. oxidize/reduce) the adsorbed analyte for detection. The major difference is that the accumulation time is relatively short for FSCV and thus the surface concentration might not reach equilibrium, as it does in stripping voltammetry.[42]

3.2. Waveform development: Why −0.4 V?

The FSCV waveform for dopamine usually starts at −0.4 V. Fig. 5B shows the current response for dopamine with different holding potentials. The FSCV current decreases with increasing holding potential. This decrease is due to adsorption, as a negative potential adsorbs more dopamine, which is positively charged at physiological pH. Thus, the signal decreases dramatically with a positive holding potential. Another consideration for choosing the holding potential is the reduction peak, as it appears around −0.2 V for dopamine at fast scan rates, and it is convenient to scan to −0.4 V to see the whole peak. The final consideration that is not immediately obvious is oxygen reduction. Oxygen can be reduced at voltages below −0.6 V and FSCV waveforms have been developed that briefly scan to negative voltages to detect oxygen.[43–45] Ninety percent of the time in a FSCV experiment is spent at the holding potential and oxygen reduction produces radical byproducts that might cause oxidative stress to cells around the electrode. Thus, while a waveform was developed with a −0.6 V holding potential, which slightly increased sensitivity,[46] it was never widely adopted in order to be conservative about oxygen reduction.

3.3 Waveform development: Why 1.3 V?

For the first two decades of FSCV, the potential was generally scanned to 1.0 V for dopamine.[23] This potential was sufficient to observe the entire oxidation peak and the theory of cyclic voltammetry says that scanning higher should not matter. However, Wightman's group realized in the 2003 that scanning higher than 1.0 V actually increased the current for dopamine.[46] Fig. 5B shows this trend: as switching potential increases from 1.0 V up to 1.5 V (note, the experiment started with 1.0 V and went up), the current increases due to activation of the carbon-fiber microelectrode surface, which breaks carbon bonds and adds edge plane sites.[34] Early studies by Gonon demonstrated activation of a carbon fiber by applying a higher potential.[47] Thus, carbon-fiber microelectrodes have a "memory" and remain activated after having been swept to higher potentials. Note that if you did this

experiment backwards, i.e. you started with a switching potential of 1.5 V and decreased it down to 1.0 V, the currents would be higher for the 1.0 V waveform because the surface was previously activated. At potentials above 1.3 V, the surface of the carbon can be etched away.[34] If you apply the waveform constantly for days, the diameter of the electrode will actually decrease by half![34,48] Electrochemical activation has two advantages: (1) it creates many defect sites that preferentially adsorb dopamine and (2) the electrode surface is constantly being cleaned which alleviates fouling. Higher switching potentials are also advantageous for measurements of compounds with higher formal potentials, such as hydrogen peroxide,[49] octopamine,[50] histamine,[51] and adenosine.[52,53] The upper potential is limited to about 1.5 V because water oxidation occurs above that potential,[54] and 1.3 V is a good compromise for activation of the surface, but avoiding water oxidation and electrode instability with higher potentials.

3.4. Waveform development: Why 400 V/s?

As shown in Fig. 4A, the current for dopamine increases with scan rate. Dopamine current increases linearly with scan rate because it is adsorption controlled (Fig. 5D),[21] and this linearity is observed over several orders of magnitude of scan rate. Background charging current also increases linearly with scan rate. If the kinetics were diffusion-controlled, Faradaic currents would be proportional to the square root of scan rate; thus background would increase at a faster rate than the Faradaic currents, making faster measurements impractical.

Considering the theory, you want to go as fast as possible, as long as your peaks are not shifting too much compared to your scan limits and your background charging current is not overloading your amplifier. One additional consideration for scan rate is stability, and higher scan rates over 1000 V/s tend to result in less electrode stability.[36] In the first two decades of FSCV research, 300 V/s scan rates were used, as scan rate was limited by instrumental considerations, and this set the stage for what a FSCV voltammogram of dopamine “should” look like.[27] While there is nothing particularly magical about 400 V/s, it is a compromise between being high enough to give good, stable currents without distorting the peaks too much and moving them to different voltages.

FSCV performed with the typical dopamine waveform is sensitive to dopamine, with a limit of detection around 15 nM at cylindrical electrodes.[55] FSCV currents are linear with concentration up to 10 μM (Fig. 5E). However, at larger concentrations, a monolayer of adsorbed dopamine is formed on the surface, behavior becomes more diffusion controlled, and the isotherm is no longer linear (Fig. 5F).[21] Physiological concentrations of dopamine are typically below 1 μM so the experiments fall in the linear range.

4. Data analysis for FSCV

4.1 Color plots show all the data

One issue with FSCV experiments is that many cyclic voltammograms are collected, about 36,000 per hour. Thus, examining individual CVs is not practical and there needs to be a way to visualize large amounts of data. Color plots are used in FSCV to examine data over

time.[56] Fig. 6A is a sample dopamine color plot. Voltage is on the y-axis, starting from the holding potential at the bottom, scanning to the switching potential in the middle, and to the holding potential again at the top. The x-axis is time and the color represents different currents. The CV results from taking a vertical swipe through the color plot, examining changes in current at different voltages. To examine the current at one potential (typically the potential of peak oxidation for the analyte) over time, an i vs t trace is generated by taking a cut horizontally (shown here above the color plot, as they are on the same time scale). Color plots can plot minutes of data, and the real limit is the need for background subtraction more frequently. With longer times, there is drift in the background current, which shows up as broad color changes in the color plot. Therefore, while experiments may go on for hours, color plots still typically only plot a few seconds to a few minutes worth of data. The Sombers lab has recently investigated partial-least-squares regression and a double triangle waveform as a way to remove electrode drift and thus better visualize data on the minutes to hours time scale.[57] Drift has also been removed by using a zero-phase high pass filter.[58]

4.2. Current (*i*) vs time (t) traces: tracking dopamine changes over time

A plot of current vs time at the oxidation potential for dopamine (Fig. 6) allows tracking of dopamine changes over time. Sometimes those plots are also changed into concentration vs time plots using a calibration factor to convert current to concentration.[59,60] Current vs time plots are convenient to quickly visualize changes over time, but there are a few considerations that need to be made when interpreting these traces.

4.2.1 *i* vs t traces: Time response is slowed by adsorption—For calibration experiments in a flow injection system, typically buffer is flowed by the electrode, then a valve flipped to flow by dopamine, and then the valve flipped back to flow buffer again (this is the experiment performed for Fig. 6A).[61] The changes in solutions are rapid and if the current were to follow the changes exactly it should look like a square, as it does for ascorbic acid (Fig. 6B). However, for dopamine, the response in a flow injection system is not square, but slightly rounded (Fig. 6A). The cause of this problem, like many others in FSCV, is adsorption. Why does adsorption slow the time response? Simply put, as dopamine starts to flow by the electrode, it takes some time for it to start adsorbing to the electrode, so that it can be detected.[21] The slower the kinetics of adsorption, the slower the response. Interestingly, with less time for adsorption, i.e. with higher scan frequencies, the response is more ideal and more square.[21] With higher frequencies, the amount of surface adsorbed dopamine is lower, but it is faster to reach that equilibrium. Venton and Wightman showed that the effects of adsorption can be removed by deconvolution.[62] While deconvolution is useful for kinetic modeling of uptake, it is rarely done for routine experiments.

4.2.2. i vs t traces: Caution recycling occurring, don't integrate me—One reason that FSCV is so sensitive is that it can detect the same molecule of dopamine again and again. This is due to redox cycling: dopamine that is oxidized to DOQ and then reduced back to dopamine can be detected again on the next scan. This recycling process is not perfect as not every molecule of dopamine is recycled on every scan at CFMEs; as discussed above, some of the DOQ will desorb from the electrode and not be recycled back to

dopamine.[21] One consequence of this redox cycling and amplification is that it is not valid to integrate under the *i* vs t curve to calculate how many molecules of dopamine were present. Peak integration is common in amperometry, where molecules are only oxidized once. and integration of a peak provides information about how many molecules were released from a vesicle.[63] However a similar analysis of FSCV peaks is not valid and might count the same molecule multiple times.

4.3 How to find the dopamine peaks? PCR and machine learning

An issue with taking thousands of voltammograms at a time is that it is difficult to sort through all those voltammograms to find the ones that are actually your neurotransmitter of interest. Thus, data analysis techniques are needed to automate identification of neurotransmitters. The main technique developed for finding peaks is principal components regression (PCR) which is a mathematical technique that reduces CVs to their principal components.[64,65] A training set is needed of the analyte of interest and then the software can choose CVs that look similar to the CV based on the principal components. Most of the training sets involve either electrically-stimulated dopamine release or dopamine transients collected *in vivo*. There is some debate in the field as to whether the training set has to be from a specific electrode or if it can be from a group of typical electrodes, as training sets are particularly difficult to make for chronic electrode implantation experiments.[9,66] Training sets of *in vitro* (i.e. calibration in a flow cell) CVs tend not to work as well because CVs will shift or change slightly *in vivo* due to changing electrode impedance.

PCR has been extensively calibrated for discriminating dopamine from pH shifts.[65] However, PCR assumes that CVs of the analytes are always the same, and some analytes, such as adenosine, have CVs that change over time due to secondary reactions. Other data analysis techniques have also been developed to mine FSCV data. The Venton lab developed methods to pick adenosine peaks based on the patterns of the primary and secondary peaks.[67] Recently, Montague's group developed machine learning approaches that use *in vitro* calibrations to find dopamine and serotonin changes in humans, but these approaches have not been validated in animals.[68,69] More work could be done in the area to apply the latest in machine learning and data science techniques to discriminating different neurotransmitters with FSCV.

4.4 Considerations of using FSCV *in vivo*

Most FSCV theory was developed by doing flow injection analysis experiments, but there are considerations for moving from a beaker into a brain. One consideration is tissue damage. Michael's group has shown that small carbon-fiber microelectrodes cause very little damage to tissue.[70] Most histology performed after FSCV requires that a dye or a high voltage be applied to actually mark the placement of the electrode because it is hard to find without a mark.[71,72] Another consideration for FSCV *in vivo* is whether applying a voltage waveform activates or depolarizes neurons that are next to the electrode. The actual Debye length (i.e. the length of the double layer) is less than 1 nm, so only cells right next to the electrode surface are affected by the voltage. Stable release is observed with the traditional dopamine waveform and recent experiments combining FSCV and electrophysiology show normal firing patterns of neurons near the electrode.[36,73,74]

A third consideration for FSCV *in vivo* is that the CVs and color plots look slightly different *in vivo*. Most of the difference is likely caused by biofouling, i.e. the adsorption of proteins to the electrode, which act as a barrier for adsorption and electron transfer. The sensitivity of electrodes drops nearly 50% from precalibration to postcalibration due to protein adsorption.[60] For some analytes, such as adenosine, it causes the peaks to be appear later and be broader.[53] For others, such as dopamine, the peaks are slightly further apart in the CV due to slower electron transfer, and the ratios of the oxidation to reduction peak currents change due to changes in adsorption. Researchers are addressing the biofouling problems by using different nanomaterial electrodes,[75–77] coating electrodes with polymers,[78–80] or using membranes coverings.[81]

5. Future of FSCV for neurotransmitter measurements

5.1. Custom waveforms for custom analytes

The standard waveforms for FSCV, like all CV, have been triangle shaped. However, the waveform can be customized using other shapes to optimize detection of specific compounds. The earliest example of this is a serotonin waveform developed by Jackson and Wightman that scanned from 0.1 V to 1.0 V, back to −0.1 V and returned to 0.1 V at 1000 V/s.[82] With a positive holding potential and the more rapid scan rate, the electrode suffers less fouling by serotonin oxidation byproducts. Faster scan rates are not always as stable for the electrode, so the Wightman group invented sawhorse waveforms that pause at the upper potential for a few milliseconds in order to clean the electrode and provide good sensitivity.[36] The Venton group adapted these sawhorse waveforms for adenosine detection, as holding at the upper potential allowed more time for adenosine detection and helped discriminate it from ATP.[35] The Sombers group investigated changing the scan rate in the middle of the waveform to improve sensitivity and discriminate compounds, such as met- and leu-enkephalin.[83] In particular, a slow scan rate is used to discriminate compounds with a similar oxidation potential but a fast scan rate is used to keep the time short and outrun fouling. The Ross group has followed up on that strategy to invent waveforms for purine discrimination, such as guanosine and adenosine.[84] Another waveform strategy is to do two pulses, and to use the information from both pulses to verify analytes. For example, the Jang and Lee groups have developed paired pulse methods to discriminate dopamine and adenosine from pH changes.[85,86] Finally, a fast cyclic square wave method has been developed to capture both the advantages of FSCV and square wave, although the data analysis is time consuming.[87] Future research will continue to investigate customized waveforms that enhance both sensitivity and selectivity for neurochemicals.

5.2. Custom electrode materials to change the electrochemical behavior of microelectrodes

This article has explained the behavior of dopamine at CFMEs with FSCV, but recently some new nanomaterial electrodes have been invented that have different electrochemical properties for dopamine. Specifically, carbon nanomaterials such as CNTs that have micron scale roughness can act as thin-layer cells and trap dopamine, causing their current to be independent of FSCV frequency.[88] With these materials, the current is the same for all frequencies and 500 Hz frequencies can be used with higher scan rates.[89] This phenomenon

was first discovered with CNT yarns and fibers,[18,89,90] but has now been expanded to other types of CNT fibers as well as cavity carbon nanopipette electrodes (CNPEs) (Figure 7).[89–91] At a carbon-fiber microelectrode, the response is not very reversible and the reduction peak is typically ~60 % of the oxidation peak because DOQ desorbs from the electrode and then diffuses away.[21] With the nanomaterials or CNPEs, the theory is that dopamine is momentarily trapped in the nanomaterial or cavity, and thus the peak currents for oxidation and reduction are nearly the same because the DOQ can't diffuse away (Fig. 7). Also, some side reactions are observed that are not present for CFMEs, specifically the cyclization of DOQ to leucodopaminechrome, which cause extra peaks in the CVs.[91] These electrodes are exciting because they show that changing the surface structure of a microelectrode can change its properties with FSCV detection. However, CNPEs inherently have slower response times because the solution must equilibrate with the cavity through the small orifice hole. More work is needed to research how these structural changes affect other compounds and to discover other materials that also have these properties.

5.3. Basal level measurements

One disadvantage of FSCV is that it cannot measure basal (or tonic) levels of neurotransmitters but instead only measures fast changes. In recent years, other techniques have been invented to circumvent the background subtraction problem and measure basal levels. All of the techniques must remove the background charging current or trap dopamine long enough in order to look at slower changes. Wightman's group used convolution based measurements to remove the background and measure tonic dopamine levels.[92] Heien's group invented fast-scan adsorption CV (FSCAV), a technique where they clean an electrode surface and then allow dopamine to adsorb to the electrode for 10 s.[93] They remove the background by modeling it and subtract that model to measure the ambient levels that adsorbed to the electrode. Cui's group used an extremely porous structure to increase the surface area and also trap dopamine and then used a slower voltammetric technique like square wave voltammetry to measure the basal levels of dopamine.[94] Fast cyclic square wave voltammetry has also been used to track tonic levels of dopamine.[95] While serotonin has been measured with FSCAV,[96] there is a future need to extend these methods to other analytes.

6. Conclusions

FSCV is a versatile technique for measuring neurotransmitter changes in the brain. At CFMEs, FSCV kinetics are primarily adsorption controlled, and adsorption-controlled kinetics leads to symmetrical peaks shapes in CVs, which are not fully reversible. The electron transfer kinetics are slow; thus, the high scan rates and high current densities cause the peaks to be spread much wider apart than in traditional CV. Because so many CVs are collected, automated data analysis software has been developed to identify neurotransmitters of interest. Current focus is on customizing waveforms for specific analytes and drift removal, designing electrodes to create better surfaces for high sensitivity and lower fouling, and improving methods of background removal to aid in longer term measurements of basal levels. FSCV is a useful technique for researching neurotransmitter signaling and understanding the fundamentals leads to better development of new applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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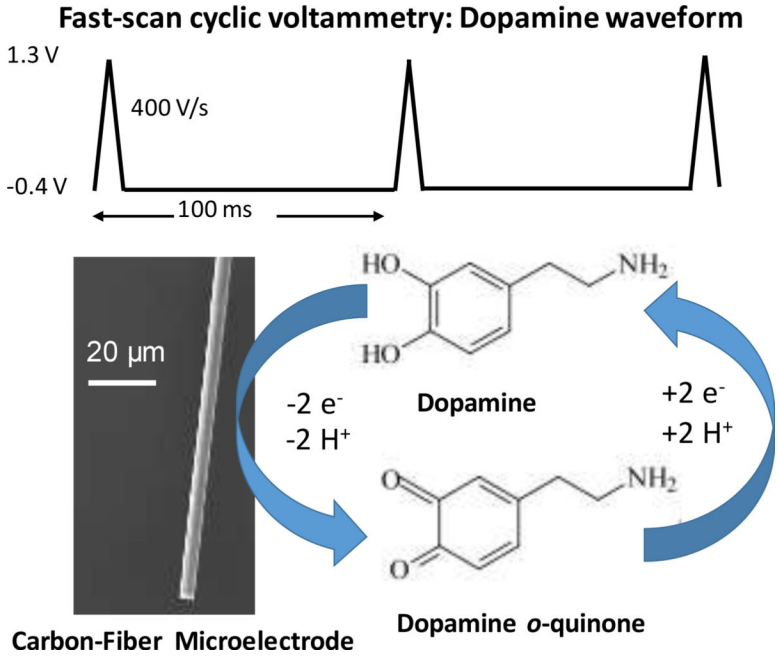

Figure 1:

FSCV waveform for dopamine. Scanning electron micrograph (SEM) of carbon-fiber microelectrode (7 µm in diameter), with the dopamine redox reactions. Dopamine is oxidized to form dopamine-o-quinone, which can be reduced at the electrode back to dopamine.

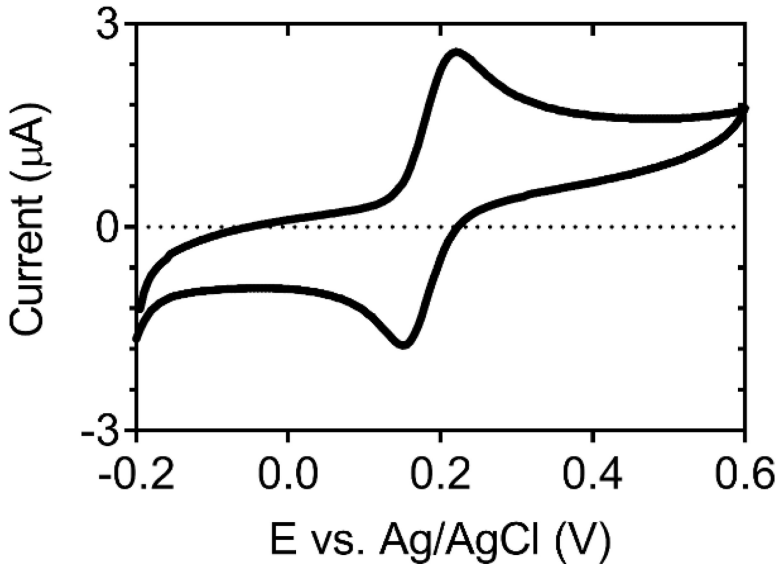

Figure 2.

Cyclic voltammogram of 100 μM dopamine at a glassy carbon electrode (in PBS buffer, pH 7.4). Scan rate is 100 mV/s and scan range is −0.2 V to 0.6 V (All the voltages are measured vs. Ag/AgCl reference electrode in this article). See supplement for Methods.

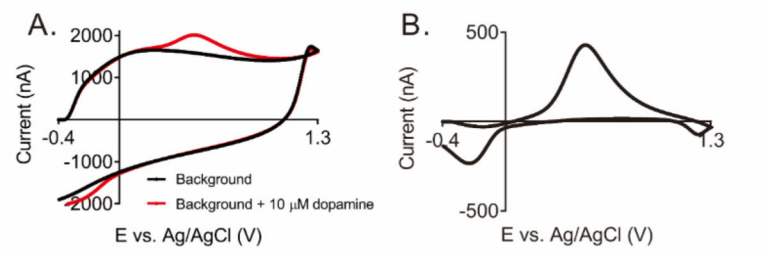

Figure 3.

FSCV detection of dopamine. A. Background current with (red) and without (black) 10 μM dopamine. B. Background-subtracted FSCV voltammogram for 10 μM dopamine. A waveform from −0.4 to 1.3 V with scan rate of 400 V/s at 10 Hz was applied to a 7 μm carbon-fiber microelectrode. See supplement for Methods.

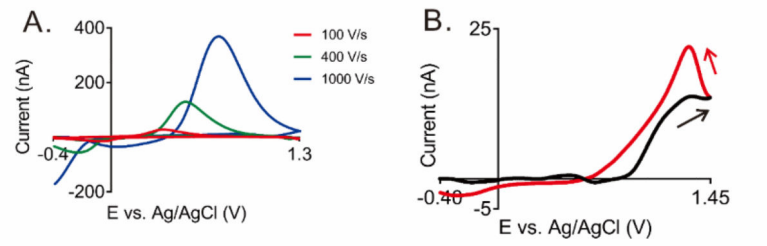

Figure 4.

Scan rate effect of FSCV. A) Higher scan rate shifts out the peak position of dopamine, and peak height increases with scan rate. Waveform from −0.4 to 1.3 V, 400 V/s, 10 Hz was applied to detect 2 μM dopamine (PBS, pH 7.4). B) Adenosine CV. The forward scan is black but the main oxidation peak is observed on the back scan, which is in red. A waveform of −0.4 to 1.45 V at 400 V/s and 10 Hz was applied to detect 1 μM adenosine. See supplement for Methods.

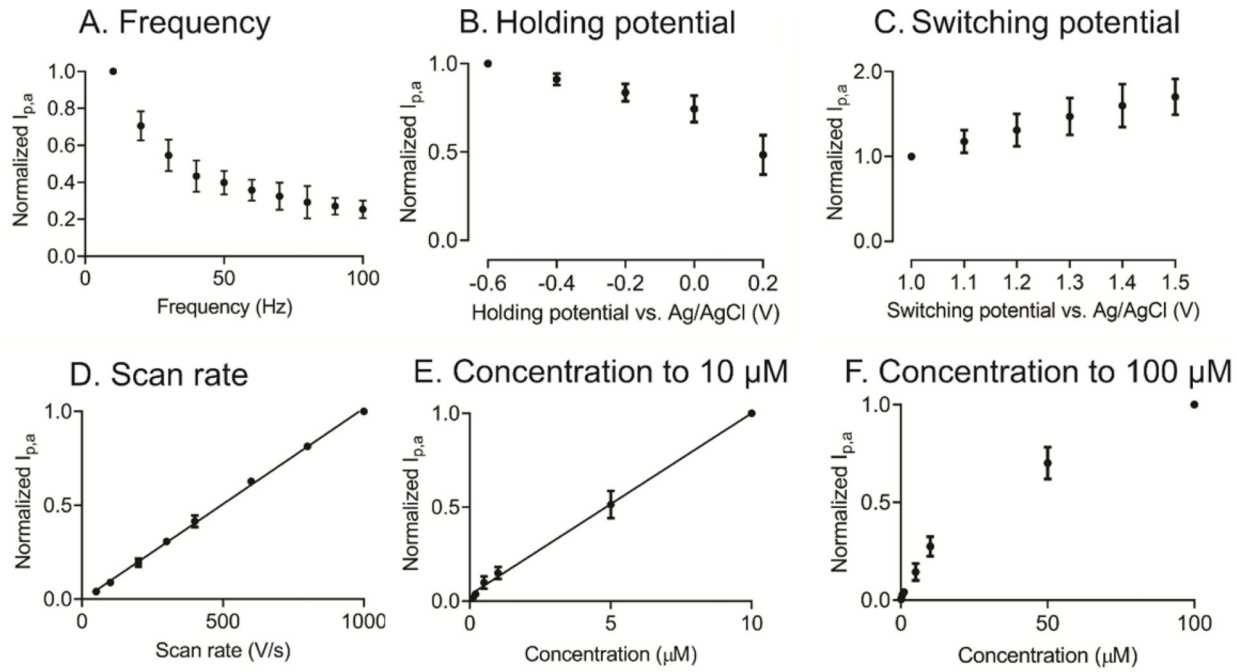

Figure 5.

Factors affecting anodic peak current of dopamine. **A.** Current decreases dramatically at high frequency. **B.** Negative holding potential assists adsorption dopamine. **C.** Higher switching potential enhances current response due to surface activation. Note: potential started at 1.0 V and was increased to 1.5 V. **D.** Current for dopamine is linear with scan rate from 50 to 1000 V/s. **E.** Current for dopamine is linear with concentration up to 10 µM. **F.** Current vs dopamine concentration over an expanded range. Current is not linear at higher concentrations. Other than the changing factor, holding potential of −0.4 V, switching potential of 1.3 V, scan rate of 400 V/s, and frequency of 10 Hz are applied to detect 1 µM dopamine. The anodic peak current is normalized by the largest current, except for switching potential where it is normalized to the 1.0 V current (n = 8 – 10). See supplement for Methods.

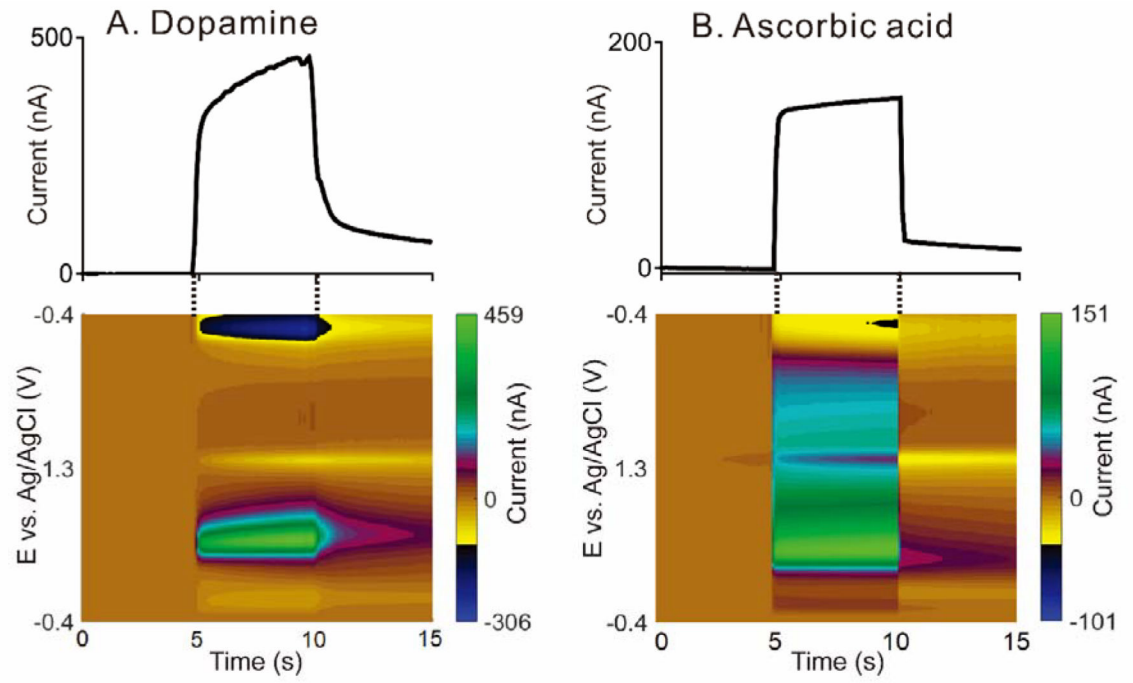

Figure 6.

FSCV color plots and I vs t traces for A. Dopamine (10 μM) and B. Ascorbic acid (20 μM). The x and y axis represent time and voltage applied, and color gradient represents current responses. Dopamine (left) or ascorbic acid (right) was injected to PBS buffer at 5 s, and PBS buffer was injected at 10 s. A waveform with a scan rate of 400 V/s at 10 Hz was applied. See supplement for Methods.

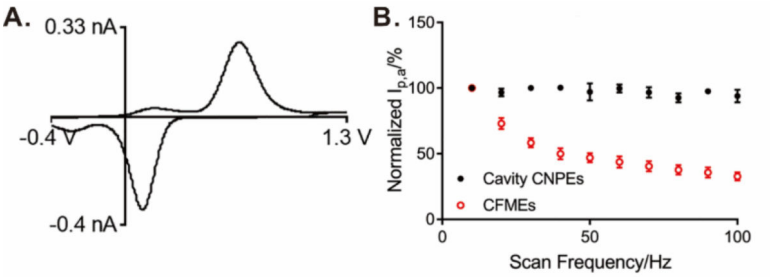

Figure 7.

A. Electrochemical response of 5 μM dopamine in cavity carbon nanopipette electrodes. B. Anodic peak current of dopamine is independent with frequency of FSCV waveform. Adapted with permission from ref. [91]. Copyright 2019 American Chemical Society.