HHS Public Access

Author manuscript

Anal Chem. Author manuscript; available in PMC 2019 January 02.

Published in final edited form as:

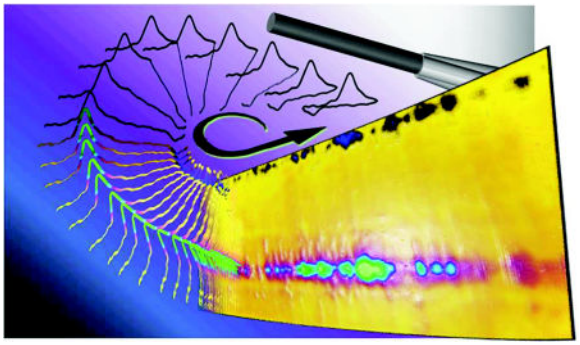
Anal Chem. 2018 January 02; 90(1): 490–504. doi:10.1021/acs.analchem.7b04732.

Fast Scan Cyclic Voltammetry: Chemical Sensing in the Brain and Beyond

James G. Roberts and **Leslie A. Sombers***

North Carolina State University, Department of Chemistry, Raleigh, NC 27695, United States

Graphical abstract



Introduction

In this review we describe the electroanalytical method known as fast-scan cyclic voltammetry (FSCV), how it has advanced over the years, and in what way(s) it is impacting other sciences. To begin, a brief history will be discussed. The various means to enhance chemical selectivity by manipulating the applied potential will be covered. Some limitations of this electroanalytical method will be highlighted to inform and provide clarity to the scientific community. The intent is to seek out effective solutions to the difficult problems associated with the technique, so that the field will continue to flourish. Rounding out the review, the utility and adaptability of this powerful bioanalytical technique will be addressed, as new frontiers of research are established for FSCV outside the scope of the neuroscience community.

FSCV: A Brief History and Overview

The History

Cyclic voltammetry is simply the combination of two segments of linear sweep voltammetry. This electroanalytical strategy can provide substantial insight into chemical species present at the electrode surface, as the experimenter can control a reversible reaction in either direction. For this reason, CV is widely used to determine the electric and thermodynamic properties of molecules and also to probe reaction kinetics across various

*Corresponding Author. lasomber@ncsu.edu, ORCID (0000-0002-0978-9795).

fields of chemistry including inorganic chemistry, organic chemistry, and biochemistry.[1] FSCV is a variant of this technique that has been particularly useful for monitoring molecules in neuroscience applications.

During the late 1950's,[2] Arvid Carlsson discovered that dopamine acts as a neurotransmitter in the brain. This lead to an explosion of research focused around quantifying this molecule, and other biogenic amines, as a means to understand the complexities of neurochemical disease states. Many of the early detection paradigms required physical sampling of tissue, which can be quite an invasive process. The first occurrence of voltammetry in brain tissue was by Leland "the father of biosensors" Clark, using a glassy carbon sensor in 1965.[3] Around the same time, Ralph Adams began studying the oxidation of catecholamines and other compounds associated with neuronal communication at the 'solid' carbon electrode.[4,5] Being captivated with neurobiology and unaware of Clark's earlier work, Adams converged his chemical expertise with his interests in neuroscience to voltammetrically record what was assumed to be catecholamine in the lateral ventricle of an anesthetized rat.[6] These recordings were achieved with 'solid electrodes', which were constructed from graphite mixed with mineral oil, packed into Teflon tubes, and then cut to expose a 0.5 mm diameter, disc-shaped electrode.[7] These electrodes were lowered into the desired brain region and cemented into place for recording over multiple days (Figure 1). This intrepid and exciting venture demonstrated that neurochemicals could be quantified *in situ*, without physically sampling or removing tissue. This could provide information that was not accessible with alternative approaches, as the neural circuitry remained intact. The creative and remarkable efforts of the Adams group during the early 1970's lead the charge for the use of electrochemical methods to quantify endogenous catecholamines, ascorbate, and other redox-active molecules in live brain tissue.

Later, Mark Wightman collaborated with Julian Millar and others to develop the electroanalytical method now known as FSCV,[8] which primarily uses carbon-fiber microelectrodes.[9] The popularization and broad impact of FSCV in the field of neuroscience has arisen due to the ease of sensor fabrication, the simplicity of the instrumentation, the ability to miniaturize and multiplex the components, and the ability to follow neurochemical dynamics in real time, which enables measurements to be time locked to discrete biological phenomena and behavioral events.

The Overview

Voltammetry relies on the simple principle that scanning the potential of the sensing surface through the region at which a molecule is redox active generates current flow at specific energy levels, and a waveform is specifically chosen to encompass this region. The plot of current versus the applied potential, the voltammogram, has features that can be used to identify and quantify an analyte.[10] The wavelimits define the driving force for the electrochemical reaction(s) at the electrode surface, and they can be selected to allow (or disallow) electron transfer for specific analytes. The scan rate defines the flux of analyte to the electrode surface or, more specifically, the thickness of the diffusion layer. The frequency of waveform application limits the time available for an analyte to accumulate in the vicinity of the electrode surface, and directly determines the temporal resolution of the

measurement. The waveform supplies the energy to transform the chemical environment around the electrode, and manipulation of this crucial component allows for tailored monitoring of specific classes of analytes.

Triangular cyclic waveforms have been the most utilized in voltammetry, primarily due to the straightforward nature of scanning through a compound's redox potential to drive a controlled reaction, and then reversing the chemistry on the return scan. The cyclic waveform can be repeated to obtain kinetic information about the concentration of species in solution, products created through electrolysis, coupled chemical reactions, and more. For these reasons, the triangular waveform was the starting point for the quantification of catecholamines and their metabolites in brain tissue. Some of the first applied waveforms ranged from −0.2 to +1.2 V at a scan rate of 150 mV/s, and another from 0.0 to +1.0 V, for intraventricular measurements and measurements in the caudate nucleus, respectively.[6,11] These waveforms were successfully used to quantify neurochemicals, but the relatively slow scan rates required an excess of 13 seconds to collect a single voltammogram. This time course is not commensurate with that of neurochemical signaling. The waveform parameters were likely designed to strike an intentional balance between the inherent background charging current(s) and the expected faradaic current(s) for the analytes in tissue. 'Background charging current' is a bulk term used to describe current responses that are characteristic of the electrochemical cell, being a sum of double-layer capacitance, current derived from redox-active groups on the electrode surface, and pseudocapacitance.[12] Under certain conditions, the background current can be quite large, particularly with respect to faradaic current generated by analytes of low concentration. To improve temporal resolution and enhance sensitivity, the scan rate can be increased. In doing so, peak redox current is amplified, as it is proportional to the square root of the applied scan rate for diffusion-controlled electrochemical reactions. However, the charging current is *directly* proportional to scan rate, and at some point it conceals the faradaic current.[12] Furthermore, the intensity and shape of the charging current can vary with continual application of the waveform, as this treatment alters the chemistry on the carbon surface.[13]

To work around these (considerable) issues with the charging current, alternative electrochemical strategies were pursued for electroanalytical measurements in neuroscience. These included chronoamperometry,[14] normal pulse voltammetry,[15] differential pulse voltammetry,[16] and even differential double pulse voltammetry.[17] All of these methods successfully minimize the contribution of charging current to highlight the faradaic response, but they lack the chemical information that is provided in the reverse scan, which is beneficial for mechanistic analysis and chemical resolution. Square wave voltammetry,[18] cyclic staircase voltammetry,[19] and branch addition techniques[20] were later developed. These alternatives to cyclic voltammetry provide chemical information with a reverse scan, and work by sampling the current in various ways - either by sampling after the potential step when the charging current has substantially decayed, or by differentially quantifying current before and after a step in the potential. Further refinement and research into these methods transpired, but ultimately digital cyclic voltammetry dominated the field of electroanalytical neuroscience.

The advent of computer-controlled instrumentation for cyclic voltammetry enabled an effortless command of scan rates and the ability to rapidly switch potentials. Modern construction of these devices made them cost effective, scalable to a portable size, and even wireless.[21] Unfortunately, digitization does not allow for a smooth voltage ramp, such as that created using analog voltammetry. Modern potentiostats generate a linear sweep as a series of very small potential steps of defined height and duration, which is analogous to staircase voltammetry. Fortunately, actions can be taken to correct for this digitization.[22] By using sufficiently small potential steps (< 0.26 mV),[23] oversampling the current response, or filtering to smooth the applied waveform, digital cyclic voltammetry can produce voltammograms comparable those collected using traditional analog instrumentation.[24] With the advent of digitization, an effective approach to removing the contribution of charging current was finally devised by Julian Millar and colleagues in 1985 for high scan-rate voltammetry, and this is considered the advent of present day background-subtracted FSCV.[8] It was realized that the background charging current stabilizes after a period of repeated electrochemical cycling, and that this current could be subtracted to highlight faradaic current resulting from rapid changes in chemical species at the electrode surface.[25] Adoption of this tactic permitted faster scan rates, millisecond temporal resolution, and the qualitative analysis capabilities that are inherent to FSCV for the analysis of rapid neurochemical fluctuations today.

The advent of carbon-fiber microelectrodes was another key driver of the rise of FSCV. Carbon-based electrode materials are largely preferred over transition metal electrodes for work in tissue, because they are less susceptible to the adsorption of oxidation products and fouling.[26] The carbon-paste electrodes originally used by Adams were eventually phased out in favor of smaller profile, glass insulated carbon-fiber microelectrodes, first introduced by Jean-Francois Pujol.[9] These sensors essentially revolutionized *in vivo* electrochemical measurements, as they result in less tissue damage and allow the application of faster scan rates (due to decreased time constants). The small surface area minimizes the effects of ohmic drop, which can distort potentials. Over the years, many strategies have been explored to advance carbon-fiber microelectrode design, with the intent of increasing chemical selectivity or improving sensitivity while mitigating the effects of biofouling. The cylindrical geometry is most common, and other popular designs include a polished planer disc[27] and a flame etched cone.[28] A cylinder electrode is best for evaluating the extracellular environment in tissue, as the fiber can sample from all sides.[29] The flame-etched conical geometry offers increased surface roughness and enhanced mass transport when compared to the standard cylinder.[30] Finally, the planer disc geometry is most useful for measuring exocytosis from discrete recording sites, such as at the surface of individual cells in culture.[31]

Most carbon fibers are a product of the pyrolysis of either a polyacrylonitrile or mesophase pitch source to form conductive turbostratic structures.[32] The surface of the carbon fiber or, more specifically, the edge plane of the graphitic lattice is functionalized with various oxygen-containing groups.[33] The presence of this oxidized layer contributes to the adsorption of many analytes and can enhance electron transfer rates.[34–36] The electrode surface can be conditioned by applying specific potentials to manipulate this chemistry, where application of a sufficiently positive potential (>1.0 V) facilitates oxidation of the

carbon surface. This treatment also serves to etch a layer of carbon in what is hypothesized to be a Kolbe-like electrolysis (anodic oxidation of carboxylic acids or carboxylates followed by a decarboxylation step that generates carbon dioxide), to reveal a fresh sensing surface with each scan.[26,37] As this technique is intended for use in living tissue, fouling of the electrode surface is an expected consequence of the non-specific adsorption of proteins and the products of electrolysis. The regenerative nature of the surface allows these sensors to operate in tissue for extended periods of time. Additionally, the surface of the electrode can be chemically modified with various polymers and membranes to impart added chemical selectivity and enhance sensitivity. These coatings include Nafion,[38] PEDOT,[39] and polypyrrole.[40] However, it is important to note that sensor performance is then dependent on the integrity and stability of the membrane on the dynamic carbon surface. These coatings also generally decrease the temporal response of the sensor, because mass transport is restricted.

FSCV was developed as a means to study chemical communication between cells on a sub-second time scale. As such, this technology has been applied to many biological studies, in a variety of preparations. FSCV can be used at single cells in culture to evaluate fundamental mechanisms that govern individual exocytosis events. Such measurements were first demonstrated using a carbon-fiber disc electrode to amperometrically oxidize catecholamines that were released from bovine adrenal chromaffin cells.[41] Later, FSCV was used to identify the co-release of multiple chemical constituents in exocytotic events at chromaffin cells.[42] The *ex vivo* tissue slice is also commonly used in voltammetric studies. It is a more complex preparation, in that multiple cell types exist in their natural environment with local connections intact; however, afferent and efferent connections from other brain regions are generally lost, unless slicing is modified to retain a specific signaling pathway. Slices can be prepared for a variety of tissue types, including brain,[43] spinal cord,[44] and adrenal glands.[45] This preparation is preferred when investigating the role of specific proteins at work in nerve terminals, such as those that govern the release and reuptake of chemical messengers from vesicles.[46,47] Electrodes can be easily positioned, and pharmacological agents can be locally delivered to probe the system of interest. Anesthetized animals are useful when intact projections and pathways are required, but the added complexity of sensory inputs is not. However, there are some caveats to anesthesia, as neuronal function can be affected.[48,49] Electrochemical measurements in awake and behaving animals are perhaps the most complicated, as the animal is moving and at times unpredictable. However, this preparation is unique in that it can be used to study neurotransmitter release events that underlie learned associations with specific environmental stimuli.[50] Indeed, scientists can use this preparation to correlate specific neurochemical fluctuations with discrete aspects of an animal's behavior.[51] Since the inception of FSCV, increasing numbers of investigators have adopted this technology and its fidelity to answer their own specific research questions. This conceptually simple, but intensely challenging method of quantification is continually being updated and advanced to encompass new chemical targets or to enable coupling with new technologies to provide exciting and specific chemical information.

Waveform Development

FSCV is most commonly used for catecholamine (dopamine) detection, and the typical waveform ranges from −0.4 to +1.3 V using a scan rate of 400 V/s and an application frequency of 10 Hz.[52] The position of the peaks in the background-subtracted voltammogram serve as a qualitative identifier, and the amplitude of the signal indicates the change in analyte concentration at the electrode surface. As voltammograms are consecutively collected, they are presented in a fashion to easily observe rapid chemical fluctuations and evaluate electrical noise. This is done by concatenating the voltammograms and plotting voltage on the ordinate, acquisition time on the abscissa, and current is represented as false color.[53] This manner of plotting voltammetric information is called a color plot, and these are advantageous because they enable simultaneous evaluation of thousands of voltammograms.

Voltammetric waveforms can be applied with different potential limits and various scan rates to control the surface concentration of a particular chemical species in solution, or to alter the surface of the electrode in ways to attenuate interferents and amplify analytes. The following sections review and evaluate some key adaptations of the applied waveform to benefit the quantification of specific analytes. These adaptations to FSCV were developed out of a demand for more chemically specific and selective measurements, enabling the expansion of this technique into exciting new research areas.

Triangle Waveform

The standard triangular waveform is used by the majority of FSCV researchers, as it is the most straightforward to apply and interpret, and it provides substantial chemical information.[54] For neuroscience applications, the triangular waveform is typically utilized for the quantification of catecholamines, purines, hydrogen peroxide (H_2O_2), and shifts in pH.[55–57] The general limitations for waveform design require that the potential limits do not exceed the potential at which oxygen is reduced (negative extent) and that at which water is oxidized (positive extent), as these reactions will obscure detection of the neurochemical species of interest (unless studying oxygen itself). It is also important to note that optimal waveform parameters vary based on experimental requirements.

Catecholamines are synthesized from tyrosine in neurons that contain the enzyme tyrosine hydroxylase, with dopamine being the first catecholamine in the biosynthesis pathway.[58] Norepinephrine is created from dopamine through dopamine-beta-hydroxylase, and then converted to epinephrine by phenylethanolamine-N-methyltransferase. Each neurotransmitter serves distinct signaling functions and undergoes multiple divergent metabolic pathways. When quantifying any catecholamine, a cyclic waveform from −0.4 V to +1.3 V is most often employed at 400 V/s, with a brief ~90 msec period between scans during which the potential is held at −0.4 V (Figure 2A). The negative charge applied to the electrode between scans serves to pre-concentrate the analyte on the electrode surface, before electrolysis. Extending the duration of this holding period results in amplified peak currents, but at the expense of temporal resolution.[59] In this manner, FSCV for catecholamines is somewhat analogous to anodic stripping voltammetry, on a shorter time scale. A preconcentration step is immediately followed by electrolysis of the adsorbed

analyte, which subsequently desorbs.[12] The quasi-reversible nature of the catecholamine redox couple requires a waveform large enough to observe both anodic and cathodic peak currents. The voltammogram appears less reversible as the scan rate is increased, until peak separation exceeds the limits of the potential window, and peaks are no longer quantifiable. The waveform potential limits have a significant effect on the redox processes for catecholamines, as the use of a higher positive potential limit populates the electrode surface with more oxygen-containing functionalities that enhance catecholamine adsorption and electron transfer kinetics.[13,34] If multiple catecholamine species must be distinguished, it has been shown that norepinephrine and epinephrine can be differentiated by simply altering the applied waveform.[42] At higher potentials, epinephrine undergoes an electrochemical cyclization to form adrenochrome. This secondary reaction generates an additional peak in the voltammogram, providing an effective means to ensure selective quantification, even in the presence of norepinephrine and dopamine.

The purines are a class of heterocyclic biomolecules that range in function from DNA bases to psychostimulants (caffeine). Adenosine, one such purine, has been studied using FSCV, largely by Venton and colleagues.[60] This neurotransmitter is a product of adenosine triphosphate (ATP) degradation, and is implicated in numerous biological processes, ranging from sleep to arrhythmia. The triangular waveform used for its detection extends the positive potential limit to +1.45 V, similar to the waveform used in experiments to differentiate epinephrine from norepinephrine. It has been proposed that a higher potential limit may be necessary to drive the first oxidation step at +1.4 V, which forms a product similar to isoguanine. This product then subsequently undergoes an additional oxidation at +1.0 V, which appears in the subsequent voltammogram. These reactions lead to a characteristic voltammogram for adenosine with two oxidation peaks that can be used to discriminate against catecholamines and other species. The optimal triangular waveform for adenosine detection has not changed drastically, but a sawhorse-shaped waveform has been introduced to improve selectivity over potential interferents, which will be discussed in a later section.

The detection of hydrogen peroxide (H_2O_2) is of particular interest in the field of neuroscience, because H_2O_2 is a biologically relevant signaling molecule,[61] a reactive oxygen species and precursor/product of free radical generation in biological processes including cellular respiration,[62] and it can also serve as a quantitative gauge of cellular activity.[63] The optimal waveform for H_2O_2 detection ranges from −0.4 V to +1.4 V at 400 V/s, and this has been used to detect endogenous fluctuations of this small molecule in live brain tissue.[64] The electrochemical oxidation of H_2O_2 is contingent on a sufficiently oxidized carbon surface, and thus requires a higher potential limit than that required for quantification of catecholamines.[65,66] However, because the triangular waveform also readily supports detection of catecholamines, dopamine and H_2O_2 can be simultaneously monitored in brain tissue.[67]

The oxidized surface of a carbon electrode is quite sensitive to the concentration of protons in solution, allowing FSCV to be used as a measure of pH.[68] A local shift in pH alters the redox properties of the oxygen-containing functional groups on the carbon surface, generating a shift in the background current. As such, the background-subtracted voltammogram reflects a change in the double-layer charging current, and also currents due

to quinone redox activity on the carbon surface.[57] It is important to note that all of the waveforms discussed in this review are sensitive to local changes in pH, and this is amplified when larger waveforms are employed.[66] This is a direct consequence of the population of surface oxides that develops on the electrode surface at extended potential limits.[13]

The triangular waveform is also well suited for monitoring the oxidation of ascorbic acid in live tissue. This antioxidant is prevalent throughout the body, and its detection with FSCV is quite interesting. When the standard triangular waveform that is routinely used for the detection of catecholamines is used for the detection of ascorbic acid, the resultant voltammograms are irreversible, not well resolved, and the electrode is not as sensitive to ascorbic acid as it is to the catecholamines. At a physiological pH, ascorbic acid is negatively charged, and should have no driving force to pre-concentrate at the negatively charged electrode surface. Interestingly, when the positive potential limit of the applied waveform is increased, the voltammogram becomes more reversible, more defined, and sensitivity is substantially improved.[66] This result beautifully demonstrates that a key component of FSCV is the continual cycling and regeneration of an oxidized carbon surface with every scan. Indeed, the condition of the overoxidized, electrochemically pre-treated surface is important in the detection of nearly all neurochemicals studied to date.

From Two Sweeps to Three: the ‘N-Shaped’ Waveform

Not all voltammetric waveforms resemble an isosceles triangle. Adding a third linear sweep results in an ‘N-shaped’ waveform. This can be beneficial to enhance chemical information by controlling the composition of specific analytes at the electrode surface. For example, the typical waveform used for catecholamine detection can be transformed, so that the starting potential is +0.33 V, instead of a negative potential.[69] This N-shaped waveform generates a surface with a positive charge during the accumulation period between scans. The resulting voltammogram exhibits less redox current for the oxidation of catecholamines, as the surface coverage of these molecules (positively charged at physiological pH) is diminished. Presumably, the location of the redox peaks remains comparable to voltammograms collected using a triangular waveform, but the relative intensity of the peaks reflects the fact that a larger portion of the current is derived from diffusion-controlled electrochemical processes, as compared to the typical adsorption-controlled voltammetric detection of catecholamines. The ability to alter the concentration of adsorbed species and mitigate undesired redox currents can be advantageous when increased chemical selectivity or increased coverage of negatively charged analytes is desired.

Oxygen and serotonin are analytes that are often (but not always) targeted for detection with the use of an N-shaped waveform. Real-time oxygen measurements in the brain can provide important information on cerebral blood flow and neural activity.[70,71] On the other hand, serotonin is a neurotransmitter derived from tryptophan that is often targeted for the treatment of psychological disorders including stress, depression, and anxiety.[72] Interestingly, serotonin also plays a key role in the regulation of cerebral blood flow.[73] At physiological pH, serotonin is positively charged, but molecular oxygen carries no net charge and would not readily adsorb to a polarized electrode surface. As such, these analytes require distinct conditions for optimal quantification using voltammetry, which a simple

triangle does not afford. Application of a negative potential reduces oxygen to H_2O_2. Thus, the optimized waveform for oxygen detection starts at 0 V with a positive sweep to +0.8 V, then down to −1.4 V and back to 0 V at a scan rate of 400 V/s (Figure 2B).[74] Using this approach, oxygen has been quantified in the ventral bed nucleus of the stria terminalis,[75] medial forebrain bundle,[76] motor cortex,[77] and nucleus accumbens core.[77] The electrochemical detection of serotonin is problematic because the products of oxidation readily polymerize and adsorb to the electrode, effectively fouling the surface and slowing electrode response times.[78] For a brief time, there was a waveform colloquially referred as the 'flying W' used for its detection, with a fourth linear sweep section.[79] To date, the most effective waveform begins at +0.2 V and ramps to +1.0 V before falling to −0.1 V, and then subsequently returns to +0.2 V, all at a scan rate of 1000 V/s (Figure 2C).[80] The addition of a Nafion coating has been used to counter fouling, and it was later found that 5-hydoxyindole acetic acid (5-HIAA) adsorption is the biggest contributor to this problem.[81] This N-shaped waveform has proven to be stable and sensitive to serotonin detection while minimizing the detrimental effects of electrolysis. With this approach, serotonin has been selectively quantified in the dorsal raphe nucleus[82] and the substantia nigra reticulata.[81] Serotonin can also be readily distinguished from catecholamines with this waveform to permit the simultaneous detection of both neurotransmitters.[83,84]

In summary, the key to the enhanced performance of the N-shaped waveform resides in the ability to manipulate the holding potential, and also scan across the redox potentials for a given analyte. Altering the charge of the electrode surface between scans can shift mass transport in a direction that is desirable for the experiment, while retaining the use of an extended potential window to maintain an oxidized electrode surface.

Multiple-Scan-Rate Voltammetry

As described, the parameters of the voltammetric waveform can be altered to impart chemical selectivity by attenuating the response to potential interferents and amplifying desired oxidation currents. This can be accomplished in a single scan with variations in the amplitude, scan rate, and number of linear sweep sections incorporated into the applied waveform. Importantly, the wavelimits of individual sweep segments can be exploited to impart enhanced chemical selectivity. The inclusion of multiple scan rates in a given voltammetric sweep is not a topic covered in electrochemical textbooks, but nevertheless the foundation of this clever approach is rooted in the fundamentals of voltammetry.

Recently, several cyclic waveforms have been designed to include a segment during which the potential is held constant while the passage of current is recorded – in essence, this is a period of 'zero scan rate'.[85,86] In the realm of cyclic voltammetry, these 'sawhorse waveforms' (Figure 2D) are a novel development; however, this approach is somewhat reminiscent of the detection strategy underlying stripping voltammetry. The goal in stripping voltammetry is to use a negative holding potential to adsorb positively-charged analyte to the electrode surface, followed by linear sweep voltammetry to subsequently desorb and detect the analyte.[12] There is also an additional cleaning step that extends the potential beyond the limit of the linear sweep section to fully desorb any remaining analyte or products on the electrode surface. Recently, sawhorse waveforms for FSCV have incorporated a brief

amperometric section between the linear sweeps of a triangular waveform at the point of the most positive applied potential.[85,87] The desired effect is to remove a layer of adhered material from the electrode surface, in order to overcome electrode fouling, maintain reproducible measurements, and potentially gain more chemical selectivity.

One interesting example of this has been used in the detection of adenosine. It has been shown that adenosine, adenosine triphosphate, and H_2O_2 all generate a strong oxidation peak near +1.3 V when detected with a triangular waveform applied to a carbon-fiber microelectrode, and this singular peak complicates discrete quantification of these species. To achieve selectivity, the waveform can be altered to contain a segment of zero scan rate.[87] This waveform begins at −0.4 V and the potential is swept at 400 V/sec to +1.35 V, where it is held for 1 ms before falling back to −0.4 V (Figure 2D). The hold time at the apex serves to oxidize the exposed graphitic edge plane[37] and eventually strip the carbon surface[26] to generate a new surface for subsequent recordings. This, in turn, improves the electrochemistry by allowing for better peak separation, and clarifying or enhancing features in the voltammograms that are used to qualitatively identify each analyte.

A more complex voltammetric waveform that incorporates a segment of zero scan rate has recently enabled direct detection of met-enkephalin using FSCV. The tragic opioid epidemic that has gripped the nation has triggered increased research into the function of opioid receptors and the modulatory effects of the endogenous opioid peptides themselves. However, these peptides are notoriously difficult to measure directly, and efforts at quantification have been primarily indirect.[88] Met-enkephalin is a five amino acid, endogenous opioid peptide that contains two electroactive residues (tyrosine and methionine). When using a triangular waveform with FSCV, these amino acids oxidize at potentials similar to the peak oxidation potentials for catecholamines and H_2O_2, respectively.[89] Additionally, the products of tyrosine electrolysis adsorb strongly to the electrode surface, analogous to the fouling evident in the voltammetric detection of serotonin. To combat these issues and enable reproducible voltammetric measurements, a multiple-scan-rate approach was designed to attenuate the contribution of catecholamines, amplify the intensity of the signal due to oxidation of tyrosine and methionine, and clear the surface of adsorbed products of electrolysis (Figure 2E).[89] A mild 100 V/s scan rate is used in the first portion of the forward scan (to +0.6 V). This is done to mitigate the faradaic current generated in the oxidation of catecholamines, and to shift the position of the quasireversible catecholamine oxidation peak to a lower potential (as compared to standard detection using a 400 V/s scan rate). The second linear sweep section begins at the point of acclivity (typically +0.6V). The potential is swept to +1.2 V, a potential sufficient for oxidation of methionine, at a faster scan rate of at least 400 V/s to intensify the current generated in the oxidation of tyrosine, and to shift the tyrosine oxidation peak well right of that generated in catecholamine oxidation. A brief segment of zero scan rate follows, during which the potential is held at +1.2 V. As described above, this serves to strip a layer of adsorbed species from the electrode surface, refreshing it for repeated measures. The result is a reproducible voltammogram with distinct features for the quantitative evaluation of met-enkephalin dynamics in real time.

There are far too numerous combinations of applied waveforms to cover in the scope of this review, but manipulation of the applied waveform is the most direct and cost effective manner to achieve chemical selectivity and sensitivity. The ability to finesse the applied potential in order to control the chemical reaction at the electrode surface for analytical purposes is almost an art, and it clearly demonstrates the power of FSCV.

FSCV: Under Construction…

The goal of this section is to highlight some limitations of FSCV, with the objective to clarify and add transparency to a diverse and complicated area of research. The intent is to seek solutions to these pressing issues, and certainly not to tarnish many years of important research. As part of the FSCV community, we have an obligation to describe the drawbacks in addition to the strengths, so that new users have the knowledge necessary to make informed measurement decisions. Hopefully this is seen as a call for action, so that solutions to these problems will emerge and FSCV will become even more robust, powerful, and chemically diverse.

Cutting the Cables

One of the most crucial issues to address when combining FSCV and behavioral analysis is how the animal is interfaced with the instrumentation. When performing *in vivo* experiments, sensors, guide cannulas, stimulating electrodes, optical fibers, and more can be cemented to the skull of the animal, so that electrical cables and fluid lines can be directly connected to these implanted objects. These wires are intrusive and are likely distracting to the animal. The behavioral assays that are typically coupled with FSCV generally involve a single animal housed inside a box or cylinder with an open top, with the cables centrally suspended from above. With the animal confined to a small space, the cables remain out of reach and are not typically damaged. However, in some instances, these cables can get coiled up or even damaged by inquisitive, active, or aggressive test subjects. In addition, the wires can limit range of motion and preclude experiments that could investigate interaction between multiple subjects tethered in the same proximity. Wireless experiments would enable voltammetric investigation of research subjects that interact and roam freely, as well as more complex behaviors that range from navigation of mazes to mating. Currently, there are only a few wireless FSCV systems that can potentially be adapted to these sorts of studies to provide chemical information on a time scale that hasn’t been attainable to date. These devices rely on miniaturized electronics and radio frequency communication such that a low-power, wireless potentiostat can be attached to the skull of the behaving animal. The Wireless Instantaneous Neurotransmitter Concentration System (WINCS)[90] was developed by researchers at Illinois State University working in collaboration with the Mayo Clinic, the University of Memphis, and Case Western Reserve University. This device was designed for rats (another now exists for human studies),[91] and is powered with an external battery pack. Pinnacle Technology (Lawrence, KS) has also created a commercially available device for wireless FSCV in rats.[92] The external battery packs fit into a vest that secures the battery to the dorsal thoracic area of the rodent. The grand challenge with these devices lies in reducing the weight of the components, which include the circuit board, case, and especially the battery. The mass and the bulk of these systems are substantial, and could thus affect the

subject's performance. To combat this, protocols have been developed that reduce the quantity of data collected. A study by McCarty et. al. reduced the FSCV sampling rate from 10 to 1 Hz, decreasing the quantity of data collected by an order of magnitude, without negatively impacting sensor responsivity or selectivity.[93] Another study used a means of analog background subtraction and a two-step cyclic analog-to-digital convertor to reduce overall data density, as well as a duty-cycled transmitter to reduce power consumption.[94] These studies are expected to substantially decrease power requirements, enabling the use of smaller, lighter batteries. Furthermore, a decreased data burden will facilitate scale up to simultaneous recordings at multiple electrodes. A collaborative research team at Cornell University has recently developed a system that weighs only 4.3 g, including the power supply (Figure 3).[94] Collectively, these advances are enabling exciting new FSCV experiments, and advancing the field by introducing the possibility of experiments in which animals can interact, or navigate complex environments. The leap to wireless devices can also impact other fields of research that would benefit from improved flexibility, such as chemical monitoring applications in hard-to-reach places, or in hazardous environments. As this wireless technology continues to develop, features to enable electrical or optical stimulation and drug delivery will likely be added, significantly increasing the utility and flexibility. Indeed, at least two research groups are actively developing systems for closed-loop neurotransmitter regulation.[95,96] These devices use both wireless FSCV and electrical stimulation to monitor and influence neurochemical levels.

Sensor Fragility

With most instrumental approaches to analysis, the detector is the real site of discovery. This is true whether the detector is optical, electrochemical, field effect, a semiconductor, a charged coupled device, or even a thermistor. All of these sensing modalities are susceptible to noise, saturation, and fouling, but the most detrimental factor is the flawed human user. Carbon-fiber microelectrodes are fragile, inherently variable, and challenging to manufacture in bulk as each stage in the fabrication process results in significant loss of yield. The carbon-fiber microelectrodes that have traditionally been coupled with FSCV measurements are glass insulated, making them particularly vulnerable to big thumbs. This electrode design has transformed measurements of dopamine in neuroscience, but the patience and resolve of countless researchers have been tested over the years (Figure 4A). The fragility of these sensors is due to the thin layer of insulating borosilicate glass that is more resistant to bending than is the carbon fiber. Excessive distortion fractures the glass, resulting in insulation failure. This exposes more carbon surface area than desired, or shorts the carbon fiber, so that the metal wire used to address the electrode assumes the role of working electrode. To prevent this, the carbon-fiber sensor can be insulated with a polyimide-coated, fused-silica capillary and effectively sealed with epoxy.[97] When coated with polyimide, the fused silica is flexible, significantly improving the mechanical stability of the sensor (Figure 4B). However, it is important to note that torsion applied to the carbon fiber itself is not translated well to the fused-silica insulation (substantially larger diameter), causing the carbon fiber to shear at the interface.

Metal electrodes are less susceptible to breakage, as metals are more malleable. Furthermore, transition metal electrodes are considered to be favorable, even catalytic, for

electron transfer (relative to carbon electrodes). Thus, many believe that metallic sensors would provide a promising alternative to carbon for studies in biological samples. Indeed, there are whole markets devoted to the design of platinum/tungsten/gold-based sensors and stimulating electrodes that are very effective for electrophysiology studies. For instance, the Utah[98] and Michigan[99] arrays consist of many electrodes to electrophysiologically record from large areas of tissue. Unfortunately, these metal electrodes are susceptible to corrosion, passivation, and a high degree of fouling by protein adsorption that adversely affect neurochemical measurements.[100] These issues are largely attributed to the favorable surface that these substrates present for electron transfer, and they necessitate the use of multiple chemically exclusive coatings to achieve reproducible neurochemical measurements. Carbon-based sensors have also been constructed on silicon wafers using microfabrication methods to create sensor arrays.[101] These planer carbon electrodes are often formed from a patterned photoresist that is pyrolyzed to generate glassy carbon electrodes. The benefit of this design is that a large number of electrodes can be manufactured in a nearly automated fashion, decreasing electrode-to-electrode variability (as compared to handmade carbon-fiber microelectrodes). Unfortunately, the bulky silicon substrate (125–200 μm thick wafer)[101,102] can induce substantial tissue damage when implanted for *in vivo* measurements. A critical need remains for an effective, user-friendly design to enable stable, reproducible, and robust measurements. Addressing these issues will facilitate broad application of FSCV in diverse chemical monitoring applications, especially if these sensors are made commercially available.

Sensor Drift

When recording continuous data, electrochemical sensors almost always show some degree of baseline instability known as sensor drift, the nemesis of every electroanalytical chemist. This inconsistent signal often necessitates correction by re-calibration, cleaning, or even replacement of the sensor. Drift is a low-frequency change in the sensor response over time that may result from factors including temperature fluctuations, changes in the chemical environment, or chemical changes at the sensing surface itself. Broadly speaking, drift results from a shift in system impedance. The impacts of drift are variable, and they ultimately reduce quantitative confidence, particularly as acquisition time increases. With background-subtracted FSCV, sensor drift can even result from changes inherent to the carbon surface itself, as continual waveform cycling to potentials greater than +1.0 V alters the surface chemistry on the edge plane of a graphitic carbon sheet (Figure 5).[37] Indeed, scanning to a potential of +1.3 V has been shown to etch the carbon surface at a rate of 0.24 nm/min (~70 femtometer/voltammogram),[26] presumably by way of carbon dioxide generation at the electrode surface.[103]

It is precisely the dynamic nature of the carbon surface that makes predicting or correcting for sensor drift challenging, even when the electrochemistry is straightforward and performed in a solution of known composition. In living tissue, sensor drift is further confounded by slow changes in the nature of the recording environment, and the nonspecific adsorption of proteins and other material (including some products of electrolysis) to the sensor surface. Only a few studies have evaluated a means to contest sensor drift in FSCV measurements. In a recent work, a high pass filter was designed to address this issue, but the

only justification for the removal of information was to smooth the signal.[104] Another study cleverly combined analog background subtraction with principle component analysis to partially remove artifacts in the data resulting from sensor drift.[105] This approach enabled longer FSCV measurements (up to 30 min), but significant variability in the nature of the drift limits the utility of this approach. In both of these studies, there was no consideration as to how shifts in impedance alter the applied voltage, and thus the electrochemical response, at a fundamental level. Furthermore, the straightforward removal of baseline drift does not account for shifts in sensitivity and formal potential that result from shifts in system impedance. Figure 5A shows what sensor drift can look like in an *in vitro,* benchtop voltammetric recording over the course of 15 minutes. At the time marked with a red arrow, a bolus of H_2O_2 was introduced to the electrode surface in a flowing buffer solution. This chemical event is somewhat masked by the instability of the electrode. Closer examination of the data in Panel B reveals the characteristic peak for the oxidation of H_2O_2 at +1.3 V, along with interference from drift. With appropriate correction for the shifting baseline, the true representation of this concentration fluctuation can be observed (Figure 5C). To begin to address these issues, a recent study systematically evaluated how shifts in system impedance alter the position of peak redox current generated when using FSCV.[106] This important study demonstrates that the background voltammograms that are normally subtracted and discarded in FSCV contain a wealth of information that can be leveraged to enhance quantification. There is a critical need for a robust and user-friendly method of drift compensation in order to enhance accuracy in both the identification and quantification of chemical species using FSCV, particularly in longitudinal studies.

Sensor Calibration

To accurately predict the concentration of unknown analytes, the sensor response must be calibrated to known standards. Carbon-fiber microelectrodes are handmade and, as such, there are inherent variations in the surface area and condition of the sensors. Additionally, the carbon fiber is far from a highly ordered graphite, and the disorder in the carbon substrate contributes to inconsistencies in sensor response. Sensor drift and variations in the local environment also contribute to uncertainty in analyte quantification. In FSCV, peak current from analyte voltammograms can be plotted versus multiple concentrations of the target molecule, such that the slope of the regression line through the data indicates the sensitivity of the sensor.[107] This is a straightforward and relatively simple calibration procedure; however, it does not account for the presence of multiple analytes. Multivariate analysis uses every dimension of the data to describe sensor response, and then reduces the dimensionality to a few components that express the maximum variability. In FSCV, the primary method used for calibration and predictive modeling of multiple analytes is principle component regression (PCR), which is essentially a combination of principal component analysis and least-squares regression.[10] However, the central problem with existing FSCV calibration procedures is that they do not account for the effects of the environment on sensor performance, because the recording environment is often the brain of a living animal. Ideally, calibration of any analytical tool would take place in a setting that accurately mimics the composition of the recording environment.[107] Unfortunately, the brain is neither homogenous nor static, which complicates the problem. Additionally, in some

experiments the microelectrodes are cemented to the skull, preventing calibration even with the traditional benchtop approach.

These issues clearly demonstrate the need for an improved calibration strategy. The drawbacks associated with any method of calibration for FSCV measurements always stem from the principle that the *in vivo* environment is complex, and the concentrations (and even some of the components) of the system are unknown. At the time of this review, two methods are most often used to quantify dopamine fluctuations evident in FSCV data collected *in vivo*.[108] The first uses dopamine responses of various intensities collected *in vivo* as the training set for PCR, and an electrode-specific calibration factor (when the electrode is successfully removed) is used to predict the concentrations of the analytes in the training set.[97] When the electrode is not available for post-calibration, an average calibration factor (generated from other electrodes) is used. This method relies on electrically stimulated dopamine signals to quantify naturally evoked dopamine fluctuations that occur at other time points during the experiment. A second method is preferred when a separate stimulating electrode is not implanted. This approach uses the delivery of an unexpected sucrose pellet to elicit a natural dopamine release event. Then, the position (potential) of peak current is used to search a calibration data base for a data set with comparable redox features that is then used to analyze the data.[109] These methods suffer similar problems, as they rely on a calibration factor for quantification that wasn't obtained while in the recording environment. One recent study took steps toward solving this problem by relating the overall charge inherent in the background voltammogram to electrode sensitivity.[66] A variety of species that are commonly targeted when using FSCV in live brain tissue were investigated. The background voltammograms contain abundant information about the condition of the electrode and the surrounding environment at the time of each voltammetric scan. Thus, careful evaluation of the background current at any time point in an FSCV experiment allows for prediction of sensitivity to various species. This approach has been shown to be at least as accurate as estimates of sensitivity established by removing the electrode and calibrating on the benchtop after experimentation.[66] Furthermore, this approach allows the electrode to be sacrificed at the end of the experiment by the passage of high current. This serves to lesion the tissue and accurately marks the recording site, which is important for neurochemical studies. Although no perfect solution to these problems exists, the ideal method of calibration for quantification of FSCV data should be free of user bias, allow the sensor to be sacrificed, and account for so-called 'matrix effects' that stem from the unknown recording environment. A more standardized method of calibration and quantification would facilitate comparison of data across research groups.

Chemical Selectivity

Chemical selectivity is a primary concern when using any electrochemical approach to molecular monitoring. The principal advantage of cyclic voltammetry over other electrochemical methods for bioanalytical quantification is the wealth of information encoded in the voltammogram. Cyclic voltammograms can serve to identify multiple analytes, as long as the targeted species exhibit redox activity at distinct potentials. However, FSCV cannot always distinguish molecules of a given chemical class. For instance, dopamine, norepinephrine, and epinephrine are all catecholamines that generate similar

voltammetric features. Thus, caution must be exercised if these molecules coexist in the same environment, such as in adrenal tissue and some regions of the brain. Furthermore, the precursors and metabolites for these catecholamines include L-3,4-dihydroxyphenylalanine (L-DOPA), L-tyrosine, 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA), among others.[110] These have similar structures and are suspected to generate similar voltammograms when detected using a simple triangular waveform. Notably, most of these molecules are not present in high concentrations in the brain, but they highlight the importance of focusing on selectivity when interpreting voltammetric data. One redeeming advantage of FSCV is the differential nature of the background-subtracted technique. This means that only *changes* in the concentration of electroactive analytes contribute to the background-subtracted voltammograms, and species that do not rapidly change in concentration are removed with the background. The anatomical distribution of the targeted analytes varies across brain regions, so effective studies can be designed with prior knowledge of the concentration distribution of structurally similar interferents. Additionally, pharmacology can be used to manipulate the biological system in a predictable manner, and thus increase confidence in analyte identification.

Improvements to sensor selectivity can be achieved by altering the electrode surface using chemically selective coatings, or through incorporation of advanced carbon materials. Nafion is a perfluorinated polymer that is perhaps the most popular coating used with sensors for FSCV measurements. The structure of Nafion is quite similar to that of Teflon, with the addition of negatively charged sulfonate side chains.[111] When applied to a surface, Nafion forms a porous cation-exchange membrane that hinders transport of negatively charged species to the electrode surface. Thus, Nafion coatings are especially useful for the detection of positively-charged molecules, such as the monoamines.[83] Enhanced sensitivity has also been demonstrated in the detection of some uncharged species, such as H_2O_2, in comparison to detection using a bare electrode.[65] The use of Nafion has also been extended to the reference electrode, and this has been shown to improve stability.[112] Unfortunately, coating a cylindrical microelectrode with Nafion is not trivial, and attempts to do this often generate a film that is neither uniform nor highly reproducible.[40] Copolymerization with the conductive polymer, poly(3,4-ethylenedioxythiophene) (PEDOT), establishes a more uniform coating, while maintaining the beneficial charge-exclusion properties of the Nafion coating.[39] Additional research has focused on functionalization of the carbon-fiber surface with carbon nanotubes to enhance sensitivity.[113] One study significantly enhanced electrochemical detection by replacing the traditional carbon fiber with a yarn spun from individual carbon nanotubes.[56] This boosted faradaic currents and substantially improved peak resolution for multiple analytes, which translates to an increase in chemical selectivity. Overall, these engineered coatings and advanced materials are particularly promising when combined with mathematical methods and clever waveforms to enhance selectivity.

Data Analysis

One of the biggest hurdles to overcome when using FSCV for analysis of chemical dynamics recorded *in vivo* is related to the nature of the data itself. In most instances, the targeted chemical events are numerous and short in duration, such that many hundreds or

even thousands of events can occur over the course of a typical recording session. These can occur in a manner that is seemingly unpredictable, requiring the analyst to painstakingly identify the events manually. This can introduce inconsistencies, reduce confidence in conclusions drawn, and confound the comparison of data sets across laboratories. As such, there is a critical need for an automated and comprehensive data analysis process to facilitate accurate quantification of chemical fluctuations without human bias. FSCV uses background subtraction to quantify chemical fluctuations, but a standardized protocol for selecting the most appropriate time point for background subtraction has not been established. The Venton group recently published the first steps toward an automated data analysis algorithm for FSCV.[114] This approach assesses FSCV data for chemical information by first choosing a time to describe the background. After background subtraction, the algorithm identifies fluctuations in the concentration of the chosen analyte from the background-subtracted data. Next, the paradigm chooses another time for the background subtraction, and re-analyzes the chemical information. This iterative process continues to update until the signal-to-noise ratio is optimized. This seminal work is a critical step toward an automated data analysis paradigm that has the potential to address the concerns of user bias, reduce data analysis time, and facilitate comparison of data across laboratories by standardizing many aspects of data analysis. Such fundamental advances to FSCV are sorely needed in order to advance FSCV into new research areas.

Multivariate statistical methods have proven to be very useful for quantitative determination of individual chemical contributors to complex voltammetric data, and as described above, PCR is the most commonly adopted approach.[108] PCR uses information collected across the entire potential window in a training set comprised of voltammograms of known analytes (and ideally, known concentrations) to determine principal components (PCs), or basis eigenvectors, that describe the variance in the training data.[34,37] The first ranked component captures the maximum variance inherent to the training set; and subsequent components describe the remaining variance, in a manner such that all PCs are orthogonal to another.[37,52] Then, concentration dynamics for the targeted species can be predicted in unknown data by projecting the data onto the PCs to extrapolate the contribution of each analyte contained in the training set. This approach can be very effective; however, it struggles when two analytical signals have similar sources of variance (voltammetric features).[34,35] Furthermore, if the redox features for an analyte shift across voltages over time, such as in response to changes in system impedance, the model can erroneously quantify the analyte.[115]

Other methods of statistical analysis can be used to potentially combat these issues. For instance, partial least squares (PLS) regression analysis is an alternate approach that removes some of the restrictions inherent to PCR. The two methods are similar in that they both utilize predictor components to describe the observed data, but they differ in how these components are constructed. Principal component analysis is considered to be an unsupervised dimensionality reduction technique, because it creates PCs to describe variance in the predictor variables without considering the response.[52,55] By contrast, PLS regression analysis is a supervised dimensionality reduction technique that projects both the predictor and response variables onto a new vector space to find components that maximize the covariance of the projected structures.[55,56] This ‘supervision’ generally allows PLS

regression models to describe the training data more efficiently with fewer components.[40,42,56] PLS is well suited for data sets with more predictors than observations, and it is more equipped than PCR to oppose multicollinearity. Still, other methods for multivariate data analysis have been less explored and could potentially be valuable to describe the complexities of FSCV. For instance, data analysis could move away from restrictive orthogonal modeling and explore independent component analysis (ICA),[116] or non-linear dimensionality reduction to redefine the space with diffusion maps.[117]

Basal vs Transient

Many of the strengths and weaknesses inherent to FSCV stem from the use of background subtraction. Any chemical information encoded in the background is removed to enable the observation of small-amplitude chemical fluctuations. Unfortunately, this is at the expense of information related to basal levels of neurotransmitters.[118] A few strategies have been evaluated to tease out information about the basal concentration of catecholamines using FSCV. Transient dopamine fluctuations are rapid, low-intensity signals attributed to the burst firing of dopaminergic neurons.[119] One study investigated the role of these transients on basal dopamine concentrations by simply averaging the data contained within color plots that were recorded over the span of several minutes.[120] It was postulated that the accumulation of phasic release events establishes the extracellular level of catecholamine. Continual averaging of phasic chemical information acts to reduce noise and smooth the data, and doing so revealed an average dopamine concentration of ~20 nM. This value was found to be an order of magnitude higher than that reported with microdialysis (~2 nM).[121] Another method, termed fast-scan controlled-adsorption voltammetry (FSCAV), has been developed to estimate basal neurotransmitter concentrations using the FSCV platform.[122] This promising concept uses the principles of stripping voltammetry to evaluate monoamine concentration in the bulk solution. It works by extending the negative ‘hold’ period between voltammetric scans to create a ‘pre-concentration phase’, during which positively-charged monoamines accumulate sufficiently to saturate the electrode surface. Then, FSCV is performed to quantify surface coverage. This method has recently been adapted and used to estimate basal concentrations of serotonin in the hippocampus to be ~65 nM.[123] This again falls about an order of magnitude lower than estimates made using microdialysis (~1.6 nM).[124] Unfortunately, calibration for FSCAV suffers from the same confounding issues that plague accurate quantification when using standard FSCV, because the heterogeneous nature of living tissue needs to be addressed when calibrating with any method. These statements by no means discount voltammetric data, because microdialysis is certainly plagued by its own issues related to accurate quantification.[125]

New Frontiers

The utility of FSCV continues to expand as new lines of research adopt this powerful analytical technique. This approach to real-time molecular monitoring is undoubtedly the most versatile, chemically descriptive, and robust electrochemical detection method used in neuroscience. Its many advantages include millisecond temporal resolution, a simple two-electrode system, a lack of physical sampling requirements, incredibly small size, and low cost. These advantages (and others) promise to provide information that hasn’t been readily

available to date in a wide range of fields that can substantially benefit from continuous chemical measurements.

Measurements at Single Cells in Culture

As described herein, FSCV has been used to quantify synaptic overflow in various biological preparations, but this technique is less often applied to the study of vesicular release from a single cell. Amperometry has been the primary method for studying the dynamics of vesicular release at cells in culture, largely due to the rapid nature of vesicle exocytosis events.[30,126,127] Amperometric measurements are often collected at a frequency of 10 KHz, enabling quantification of individual exocytosis events. By contrast, FSCV measurements are typically collected at a rate of 10 Hz. This sampling rate does not provide sufficient temporal resolution to fully resolve the dynamics of individual exocytosis events, which encompass fusion pore opening and extrusion of vesicular content. A vesicle can release a portion of its content in a “kiss and run” mode of action in less than 100 ms,[128] and only amperometry is fully able to evaluate neurochemical events on this timescale. On the other hand, amperometry offers little chemical selectivity and can’t easily distinguish between catecholamines and the variety of other electroactive molecules that can be co-released.[129,130] At least one study has evaluated the use of FSCV in distinguishing release events from single cells. Norepinephrine and epinephrine were discriminated as they were co-released from bovine chromaffin cells using FSCV with extended wavelimits.[42] Additional voltammetric studies with this system would allow the composition of individual vesicles to be evaluated.

Soil, Water, and Beyond

The utility and attributes of FSCV have seen this technique begin to move into areas including soil science and environmental research. Stripping voltammetry has been used for many years to determine trace amounts of metals in solution, and FSCV has recently been presented as an alternative for quantification of trace levels of copper with sub-second temporal resolution.[131] FSCV has also been applied to a study in soil science.[132] Siderophores are specific organisms in the soil that secrete endogenous chelating agents to sequester metals in the environment. The binding efficiency of metal sequestration was investigated with FSCV. This study is important, because increased understanding of this process will enable these organisms to be exploited in efforts at soil remediation. FSCV has recently been used to monitor reaction intermediates, where oxygen-centered radicals produced in the oxidation of H_2O_2 were trapped and subsequently identified with spectroscopy.[133] FSCV research has extended to larger mammals, including primates[134,135] and humans.[136] And recently, this method has been adapted to non-mammals to evaluate neurochemical dynamics in the zebrafish telencephalon,[137] the nerve cord in Drosophila,[138,139] and the striatum of songbirds.[140] These are only a few examples of the broadening impact of FSCV. Such studies highlight the power of this technique, and further demonstrate the need for improved, user-friendly technologies for FSCV data collection and analysis.

Electrophysiology

FSCV has been coupled with electrophysiological recordings for decades,[141] as this powerful combination can be used to shed light on both neurochemical release events and the response of the local cells receiving the chemical message. For instance, a recent study used a multimodal sensor to demonstrate that dopamine released during an intracranial self-stimulation task modulates distinct populations of neurons in the nucleus accumbens.[142] The sensor combined FSCV for detection of dopamine release with electrophysiology performed at the same sensor (between voltammetric sweeps) to monitor the firing of local striatal cells potentially responding to the chemical event. These technologies were combined with iontophoretic ejection of specific drugs to identify dopamine receptor type(s) on the local neurons. The results of this technically challenging and important study demonstrate well-defined dopamine release events in response to cues related to lever availability, as well as in response to the lever press itself (lever press electrically stimulates the ascending dopamine pathway). Interestingly, dopamine released in response to the presentation of the cue was correlated with a cellular response that was selectively mediated by D2-like receptors, whereas dopamine released with the lever press was correlated with cellular activity that was mediated by both D1-like and D2-like receptors. Another recent investigation simultaneously quantified oxygen and dopamine using FSCV, while incorporating electrophysiology at the same probe to evaluate cellular activity during spreading depolarization.[77] Overall, these studies are important because they link neurochemical release events to the cellular response to these events.

FSCV and electrophysiology have also been combined to investigate whether application of a fast voltammetric waveform can, in and of itself, alter the activity of local neurons. To investigate this important question, electrophysiology was used to record neuronal activity at the carbon-fiber microelectrode in an ~90 ms period between voltammetric scans.[143] This study demonstrated that the recorded activity of the neurons was unaffected by application of the voltammetric waveform, but the ‘pseudo-simultaneous’ experimental design did not truly assess cell firing *during* application of the FSCV waveform. To definitively resolve this, a recent experiment in brain slices used patch-clamp recordings in the vicinity of the voltammetric probe to demonstrate that FSCV did not alter the firing of neighboring cells.[144]

FSCV Coupled with Enzyme-Modified Microbiosensors

Since the Clark electrode was first introduced to enable electrochemical detection of glucose,[145] an abundance of research has focused around first-generation biosensors for monitoring non-electroactive species. The vast majority of these technologies use amperometric detection coupled with enzyme-modified electrodes for molecular monitoring. Enzymes are inherently selective to a substrate, and biosensors are designed to take advantage of this property. However, amperometric measurements are inherently non-selective, and biosensors coupled with this electroanalytical technique typically incorporate chemically selective coatings to exclude as many interferents as possible. Biosensor function is dependent on membrane performance, and these exclusion layers are not absolute. Thus, enzyme-modified electrodes are often coupled with an enzyme-free recording site adjacent to the active electrode, to serve as a point of reference. The electrochemical response

recorded at the enzyme-less recording site is subtracted from that recorded at the enzyme-modified site, to provide a selective measure of the analyte targeted by the enzyme. This subtraction paradigm is applicable *in vitro*, but it is less-than-ideal for measurements in the spatially and chemically heterogeneous environment of living tissue. To avoid these complications, a glucose biosensor was recently developed that coupled FSCV with glucose oxidase-modified carbon-fiber microelectrodes for the detection of rapid glucose fluctuations in brain tissue. The chemically-selective nature of FSCV does not require the use of multiple coatings for chemical exclusion that limit response times, enabling glucose measurements on a previously inaccessible timescale.[146] Furthermore, a major strength of this approach lies in the ability to simultaneously detect multiple species at a single recording site.[67] This is in direct contrast to traditional biosensors, which are specifically designed to exclude all molecules other than the analyte of interest. Extending beyond glucose, an acetylcholine biosensor has been developed using the carbon-fiber microelectrode platform coupled with FSCV, to provide another tool for real-time measurements of neurochemicals that are not inherently redox active.[147]

Concluding Remarks

The field of bioanalytical research continues to expand into exciting new dimensions. The development and progression of FSCV has seen the technology move beyond the originally targeted catecholamines to investigate many additional neurotransmitters, and it has even crossed into other sciences to advance new molecular monitoring applications. This is an exciting time for electrochemistry, as FSCV can be readily combined with powerful new chemogenetic and optogenetic tools to answer previously inaccessible research questions. For instance, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) offer the ability to express engineered G protein-coupled receptors that are activated by synthetic non-endogenous molecules.[148] These designer proteins are used to selectively activate specific cell types, providing unprecedented information.[149] Optogenetics provides another set of valuable tools that can be combined with FSCV. With this approach, neurons are modified to express light-sensitive ion channels, allowing for depolarization of selective neuronal subtypes with light. Current FSCV protocols and methodologies must be made more robust, automated, and user-friendly, so that neuroscience researchers and electrochemists alike can effectively combine FSCV with other exciting technologies inherent to their own fields, without having to clamber a steep learning curve in order to do so. This progression will lead to broad application of FSCV for rapid molecular monitoring in multiple markets, and enable transformative new measurements to address global research challenges.

Acknowledgments

Research in the Sombers laboratory on these topics has been funded by grants from the National Institutes of Health, the National Science Foundation, and NCSU Department of Chemistry. In addition, we gratefully acknowledge our coworkers, past and present, for many of the studies cited in this review.

Biographies

James G. Roberts achieved his B.S. from Lenoir-Rhyne University in 2008 and his Ph.D. from North Carolina State University in 2013 for his research with Leslie Sombers on advancing electroanalytical measurements. His postdoctoral work has focused on advancing fast-scan cyclic voltammetry by improving the means of data analysis, developing innovative sensors, and expanding the scope of the technique.

Leslie A. Sombers received her B.S. in 1996 and her M.A. in 1998 from the College of William and Mary. In 2004, she received her Ph.D. from the Pennsylvania State University, under the direction of Andrew G. Ewing. Her postdoctoral work was completed at the University of North Carolina-Chapel Hill, with R. Mark Wightman. In 2008, she took a position as Assistant Professor of Chemistry at North Carolina State University, where she is now Associate Professor. She is currently a member of the Board of Directors for the Society for Electroanalytical Chemistry (SEAC), and also a member of the Board of Directors for the International Society for Monitoring Molecules in Neuroscience. Her research interests center around the development of new electroanalytical tools for monitoring molecules in neuroscience.

References

1. Bard, AJ., Faulkner, LR. Electrochemical Methods: Fundamentals and Applications. 2. John Wiley; New York: 2001. p. 833
2. Carlsson A. Pharmacological reviews. 1959; 11:490–493. [PubMed: 13667431]
3. Clark LC Jr, Lyons C. The Alabama journal of medical sciences. 1965; 2:353–359. [PubMed: 4955092]
4. Hawley MD, Tatawawadi SV, Piekarski S, Adams RN. Journal of the American Chemical Society. 1966; 89:447–450.
5. Adams, RN. Electrochemistry at Solid Electrodes. Dekker, M., editor. New York: 1969. p. xiiip. 402
6. Kissinger PT, Hart JB, Adams RN. Brain Research. 1973; 55:209–213. [PubMed: 4145914]
7. Marcoux LS, Prater KB, Prater BG, Adams RN. Analytical Chemistry. 1965; 37:1446–1447.
8. Millar J, Stamford JA, Kruk ZL, Wightman RM. Eur J Pharmacol. 1985; 109:341–348. [PubMed: 3872803]
9. Gonon F, Cespuglio R, Ponchon JL, Buda M, Jouvet M, Adams RN, Pujol JF. Cr Acad Sci D Nat. 1978; 286:1203–1206.
10. Keithley RB, Wightman RM. ACS Chemical Neuroscience. 2011; 2:514–525. [PubMed: 21966586]
11. Adams RN. Analytical Chemistry. 1976; 48:1126–1138.
12. Bard, AJ., F, LR. Electrochemical Methods: Fundamentals and Applications. 2. John Wiley; New York: 2001.
13. Roberts JG, Moody BP, McCarty GS, Sombers LA. Langmuir. 2010; 26:9116–9122. [PubMed: 20166750]
14. Conti JC, Strope E, Adams RN, Marsden CA. Life Sci. 1978; 23:2705–2715. [PubMed: 739848]
15. Ponchon JL, Cespuglio R, Gonon F, Jouvet M, Pujol JF. Analytical Chemistry. 1979; 51:1483–1486. [PubMed: 484865]
16. Lane RF, Hubbard AT, Fukunaga K, Blanchard RJ. Brain Research. 1976; 114:346–352. [PubMed: 963554]
17. Lane RF, Hubbard AT. Analytical Chemistry. 1976; 48:1287–1293. [PubMed: 952392]
18. Osteryoung JG, Osteryoung RA. Analytical Chemistry. 1985; 57:101A–110A.

19. Murphy MM, Odea JJ, Arn D, Osteryoung JG. Analytical Chemistry. 1989; 61:2249–2254.
20. Oldham KB, Zoski CG. Analytical Chemistry. 1983; 55:1990–1992.
21. McMahon, G. Analytical Instrumentation: A Guide to Laboratory, Portable and Miniaturized Instruments. 1. J. Wiley & Sons; Chichester, England ; Hoboken, NJ: 2007. p. xxip. 296p. 8
22. Batchelor-McAuley C, Yang MJ, Hall EM, Compton RG. Journal of Electroanalytical Chemistry. 2015; 758:1–6.
23. Bilewicz R, Osteryoung RA, Osteryoung J. Analytical Chemistry. 1986; 58:2761–2765.
24. He PX. Analytical Chemistry. 1995; 67:986–992.
25. Howell JO, Kuhr WG, Ensman RE, Wightman RM. Journal of Electroanalytical Chemistry. 1986; 209:77–90.
26. Takmakov P, Zachek MK, Keithley RB, Walsh PL, Donley C, McCarty GS, Wightman RM. Analytical Chemistry. 2010; 82:2020–2028. [PubMed: 20146453]
27. Dayton MA, Brown JC, Stutts KJ, Wightman RM. Analytical Chemistry. 1980; 52:946–950.
28. Strein TG, Ewing AG. Analytical Chemistry. 1992; 64:1368–1373.
29. Bath BD, Martin HB, Wightman RM, Anderson MR. Langmuir. 2001; 17:7032–7039.
30. Li XC, Majdi S, Dunevall J, Fathali H, Ewing AG. Angew Chem Int Edit. 2015; 54:11978–11982.
31. Zhang B, Heien MLAV, Santillo MF, Mellander L, Ewing AG. Analytical Chemistry. 2011; 83:571–577. [PubMed: 21190375]
32. Huang XS. Materials. 2009; 2:2369–2403.
33. McCreery RL. Chemical Reviews. 2008; 108:2646–2687. [PubMed: 18557655]
34. Hafizi S, Kruk ZL, Stamford JA. J Neurosci Methods. 1990; 33:41–9. [PubMed: 2232859]
35. Patel AN, Tan SY, Miller TS, Macpherson JV, Unwin PR. Analytical Chemistry. 2013; 85:11755–11764. [PubMed: 24308368]
36. Mcdermott MT, Mccreery RL. Langmuir. 1994; 10:4307–4314.
37. Mitchell EC, Dunaway LE, McCarty GS, Sombers LA. Langmuir. 2017; 33:7838–7846. [PubMed: 28715197]
38. Gerhardt GA, Oke AF, Nagy G, Moghaddam B, Adams RN. Brain Res. 1984; 290:390–395. [PubMed: 6692152]
39. Vreeland RF, Atcherley CW, Russell WS, Xie JY, Lu D, Laude ND, Porreca F, Heien ML. Analytical Chemistry. 2015; 87:2600–2607. [PubMed: 25692657]
40. Pihel K, Walker QD, Wightman RM. Analytical Chemistry. 1996; 68:2084–2089. [PubMed: 9027223]
41. Leszczyszyn DJ, Jankowski JA, Viveros OH, Diliberto EJ, Near JA, Wightman RM. J Biol Chem. 1990; 265:14736–14737. [PubMed: 2394692]
42. Pihel K, Schroeder TJ, Wightman RM. Analytical Chemistry. 1994; 66:4532–4537.
43. Bull DR, Palij P, Sheehan MJ, Millar J, Stamford JA, Kruk ZL, Humphrey PPA. J Neurosci Meth. 1990; 32:37–44.
44. Street SE, Walsh PL, Sowa NA, Taylor-Blake B, Guillot TS, Vihko P, Wightman RM, Zylka MJ. Mol Pain. 2011; 7
45. Petrovic J, Walsh PL, Thornley KT, Miller CE, Wightman RM. Endocrinology. 2010; 151:1773–1783. [PubMed: 20181796]
46. Miles PR, Mundorf ML, Wightman RM. Synapse. 2002; 44:188–97. [PubMed: 11954051]
47. Sun M, Kaplan SV, Gehringer RC, Limbocker RA, Johnson MA. Anal Chem. 2014; 86:4151–4156. [PubMed: 24734992]
48. Brodnik ZD, Espana RA. Neurosci Lett. 2015; 606:129–34. [PubMed: 26321152]
49. Sabeti J, Gerhardt GA, Zahniser NR. Neurosci Lett. 2003; 343:9–12. [PubMed: 12749985]
50. Rodeberg NT, Johnson JA, Bucher ES, Wightman RM. ACS Chem Neurosci. 2016; 7:1508–1518. [PubMed: 27548680]
51. Garris PA, Kilpatrick M, Bunin MA, Michael D, Walker QD, Wightman RM. Nature. 1999; 398:67–69. [PubMed: 10078530]

52. Rodeberg NT, Johnson JA, Bucher ES, Wightman RM. ACS Chemical Neuroscience. 2016; 7:1508–1518. [PubMed: 27548680]
53. Michael DJ, Joseph JD, Kilpatrick MR, Travis ER, Wightman RM. Analytical Chemistry. 1999; 71:3941–3947. [PubMed: 10500480]
54. Kissinger PT, Heineman WR. J Chem Educ. 1983; 60:702–706.
55. Sanford AL, Morton SW, Whitehouse KL, Oara HM, Lugo-Morales LZ, Roberts JG, Sombers LA. Analytical Chemistry. 2010; 82:5205–5210. [PubMed: 20503997]
56. Schmidt AC, Wang X, Zhu YT, Sombers LA. Acs Nano. 2013; 7:7864–7873. [PubMed: 23941323]
57. Takmakov P, Zachek MK, Keithley RB, Bucher ES, McCarty GS, Wightman RM. Anal Chem. 2010; 82:9892–900. [PubMed: 21047096]
58. Siegel, GJ. Basic Neurochemistry: Molecular, Cellular, and Medical Aspects. 6. Lippincott-Raven Publishers; Philadelphia: 1999. p. xxip. 1183
59. Kile BM, Walsh PL, McElligott ZA, Bucher ES, Guillot TS, Salahpour A, Caron MG, Wightman RM. ACS Chemical Neuroscience. 2012; 3:285–292. [PubMed: 22708011]
60. Nguyen MD, Venton BJ. Comput Struct Biotec. 2015; 13:47–54.
61. Chen BT, Avshalumov MV, Rice ME. J Neurophysiol. 2001; 85:2468–76. [PubMed: 11387393]
62. Ray PD, Huang BW, Tsuji Y. Cellular signalling. 2012; 24:981–990. [PubMed: 22286106]
63. Bao L, Avshalumov MV, Patel JC, Lee CR, Miller EW, Chang CJ, Rice ME. Journal of Neuroscience. 2009; 29:9002–9010. [PubMed: 19605638]
64. Spanos M, Gras-Najjar J, Letchworth JM, Sanford AL, Toups JV, Sombers LA. ACS Chemical Neuroscience. 2013; 4:782–789. [PubMed: 23556461]
65. Roberts JG, Hamilton KL, Sombers LA. Analyst. 2011; 136:3550–3556. [PubMed: 21727955]
66. Roberts JG, Toups JV, Eyualem E, McCarty GS, Sombers LA. Analytical Chemistry. 2013; 85:11568–11575. [PubMed: 24224460]
67. Smith SK, Lee CA, Dausch ME, Horman BM, Patisaul HB, McCarty GS, Sombers LA. ACS Chem Neurosci. 2017; 8:272–280. [PubMed: 27984698]
68. Dengler AK, Wightman RM, McCarty GS. Anal Chem. 2015; 87:10556–10564. [PubMed: 26375039]
69. Walters SH, Robbins EM, Michael AC. ACS Chem Neurosci. 2016; 7:662–667. [PubMed: 26886408]
70. Buxton RB, Frank LR. J Cerebr Blood F Met. 1997; 17:64–72.
71. Sheth SA, Nemoto M, Guiou M, Walker M, Pouratian N, Toga AW. Neuron. 2004; 42:347–355. [PubMed: 15091348]
72. Graeff FG, Guimaraes FS, DeAndrade TGCS, Deakin JFW. Pharmacol Biochem Be. 1996; 54:129–141.
73. Grome JJ, Harper AM. J Cerebr Blood F Met. 1983; 3:71–77.
74. Zimmerman JB, Wightman RM. Analytical Chemistry. 1991; 63:24–28. [PubMed: 1810167]
75. Bucher ES, Fox ME, Kim L, Kirkpatrick DC, Rodeberg NT, Belle AM, Wightman RM. J Cerebr Blood F Met. 2014; 34:1128–1137.
76. Cheer JF, Wassum KM, Wightman RM. J Neurochem. 2006; 97:1145–1154. [PubMed: 16686693]
77. Hobbs CN, Johnson JA, Verber MD, Wightman RM. Analyst. 2017; 142:2912–2920. [PubMed: 28715004]
78. Dankoski EC, Wightman RM. Frontiers in integrative neuroscience. 2013; 7:44. [PubMed: 23760548]
79. O'Connor JJ, Kruk ZL. British journal of pharmacology. 1992; 106:524–532. [PubMed: 1504738]
80. Jackson BP, Dietz SM, Wightman RM. Analytical Chemistry. 1995; 67:1115–1120. [PubMed: 7717525]
81. Hashemi P, Dankoski EC, Petrovic J, Keithley RB, Wightman RM. Analytical Chemistry. 2009; 81:9462–9471. [PubMed: 19827792]
82. Dankoski EC, Carroll S, Wightman RM. J Neurochem. 2016; 136:1131–1141.
83. Swamy BEK, Venton BJ. Analyst. 2007; 132:876–884. [PubMed: 17710262]

84. Hashemi P, Dankoski EC, Lama R, Wood KM, Takmakov P, Wightman RM. Proc Natl Acad Sci U S A. 2012; 109:11510–5. [PubMed: 22778401]
85. Keithley RB, Takmakov P, Bucher ES, Belle AM, Owesson-White CA, Park J, Wightman RM. Anal Chem. 2011; 83:3563–71. [PubMed: 21473572]
86. Oh Y, Kim DH, Shin H, Park C, Chang SY, Blaha CD, Bennet KE, Kim IY, Lee KH, Jang DP. Int J Electrochem Sc. 2015; 10:10061–10073.
87. Ross AE, Venton BJ. Analytical Chemistry. 2014; 86:7486–7493. [PubMed: 25005825]
88. Murphy NP. ACS Chem Neurosci. 2015; 6:94–107. [PubMed: 25585132]
89. Schmidt AC, Dunaway LE, Roberts JG, McCarty GS, Sombers LA. Analytical Chemistry. 2014; 86:7806–7812. [PubMed: 24967837]
90. Bledsoe JM, Kimble CJ, Covey DP, Blaha CD, Agnesi F, Mohseni P, Whitlock S, Johnson DM, Horne A, Bennet KE, Lee KH, Garris PA. J Neurosurg. 2009; 111:712–723. [PubMed: 19425890]
91. Chang SY, Kim I, Marsh MP, Jang DP, Hwang SC, Van Gompel JJ, Goerss SJ, Kimble CJ, Bennet KE, Garris PA, Blaha CD, Lee KH. Mayo Clinic Proceedings. 2012; 87:760–765. [PubMed: 22809886]
92. Pinnacle Technology Inc. [accessed Nov 1, 2017] Wireless Fast Scan Cyclic Voltammetry System. https://www.pinnaclet.com/fscv-systems-wireless-rats.html
93. Amos AN, Roberts JG, Qi LJ, Sombers LA, McCarty GS. Ieee Sens J. 2014; 14:2975–2980.
94. Dorta-Quinones CI, Wang XY, Dokania RK, Gailey A, Lindau M, Apsel AB. Ieee T Biomed Circ S. 2016; 10:289–299.
95. Bozorgzadeh B, Schuweiler DR, Bobak MJ, Garris PA, Mohseni P. Ieee T Biomed Circ S. 2016; 10:654–667.
96. Lee KH, Lujan JL, Trevathan JK, Ross EK, Bartoletta JJ, Park HO, Paek SB, Nicolai EN, Lee JH, Min HK, Kimble CJ, Blaha CD, Bennet KE. Scientific reports. 2017; 7
97. Clark JJ, Sandberg SG, Wanat MJ, Gan JO, Horne EA, Hart AS, Akers CA, Parker JG, Willuhn I, Martinez V, Evans SB, Stella N, Phillips PE. Nat Methods. 2010; 7:126–129. [PubMed: 20037591]
98. Kim S, Bhandari R, Klein M, Negi S, Rieth L, Tathireddy P, Toepper M, Oppermann H, Solzbacher F. Biomedical Microdevices. 2009; 11:453–466. [PubMed: 19067174]
99. Wise KD. IEEE engineering in medicine and biology magazine : the quarterly magazine of the Engineering in Medicine & Biology Society. 2005; 24:22–9.
100. Wisniewski N, Moussy F, Reichert WM. Fresenius' journal of analytical chemistry. 2000; 366:611–21. [PubMed: 11225773]
101. Zachek MK, Park J, Takmakov P, Wightman RM, McCarty GS. Analyst. 2010; 135:1556–1563. [PubMed: 20464031]
102. Burmeister JJ, Moxon K, Gerhardt GA. Anal Chem. 2000; 72:187–192. [PubMed: 10655652]
103. Webster RA, Watkins JD, Potter RJ, Marken F. Rsc Adv. 2012; 2:4886–4890.
104. DeWaele M, Oh Y, Park C, Kang YM, Shin H, Blaha C, Bennet KE, Kim IY, Lee KH, Jang DP. Analyst. 2017:4317–4321. [PubMed: 29063091]
105. Hermans A, Keithley RB, Kita JM, Sombers LA, Wightman RM. Analytical Chemistry. 2008; 80:4040–4048. [PubMed: 18433146]
106. Meunier CJ, Roberts JG, McCarty GS, Sombers LA. ACS Chemical Neuroscience. 2017; 8:411–419. [PubMed: 28044445]
107. Harris, DC. Quantitative chemical analysis. 8. W. H. Freeman and Co.; New York: 2010. p. 1 v(various pagings)
108. Rodeberg NT, Johnson JA, Cameron CM, Saddoris MP, Carelli RM, Wightman RM. Analytical Chemistry. 2015; 87:11484–11491. [PubMed: 26477708]
109. Day JJ, Roitman MF, Wightman RM, Carelli RM. Nat Neurosci. 2007; 10:1020–1028. [PubMed: 17603481]
110. Kopin IJ. Pharmacological reviews. 1985; 37:333–64. [PubMed: 3915090]
111. Schmidt-Rohr K, Chen Q. Nat Mater. 2008; 7:75–83. [PubMed: 18066069]
112. Hashemi P, Walsh PL, Guillot TS, Gras-Najjar J, Takmakov P, Crews FT, Wightman RM. ACS Chem Neurosci. 2011; 2:658–666. [PubMed: 22125666]

113. Zhang QH, Liu JW, Sager R, Dai LM, Baur J. Compos Sci Technol. 2009; 69:594–601.
114. Borman RP, Wang Y, Nguyen MD, Ganesana M, Lee ST, Venton BJ. ACS Chemical Neuroscience. 2017; 8:386–393. [PubMed: 28196418]
115. Johnson JA, Rodeberg NT, Wightman RM. ACS Chemical Neuroscience. 2016; 7:349–359. [PubMed: 26758246]
116. Roberts, S., Everson, R. Independent Component Analysis: Principles and Practice. Cambridge University Press; Cambridge ; New York: 2001. p. xiip. 338
117. Gorban, AN., Kégl, B., Wunsch, DC., Zinovyev, AY. Principal Manifolds for Data Visualization and Dimension Reduction. Springer-Verlag Berlin Heidelberg; Berlin, Heidelberg: 2007. p. XXIVp. 340
118. Rebec GV, Christensen JR, Guerra C, Bardo MT. Brain Res. 1997; 776:61–67. [PubMed: 9439796]
119. Sombers LA, Beyene M, Carelli RM, Wightman RM. Journal of Neuroscience. 2009; 29:1735–742. [PubMed: 19211880]
120. Owesson-White CA, Roitman MF, Sombers LA, Belle AM, Keithley RB, Peele JL, Carelli RM, Wightman RM. J Neurochem. 2012; 121:252–262. [PubMed: 22296263]
121. Shou MS, Ferrario CR, Schultz KN, Robinson TE, Kennedy RT. Analytical Chemistry. 2006; 78:6717–6725. [PubMed: 17007489]
122. Atcherley CW, Laude ND, Parent KL, Heien ML. Langmuir. 2013; 29:14885–14892. [PubMed: 24245864]
123. Abdalla A, Atcherley CW, Pathirathna P, Samaranayake S, Qiang B, Pena E, Morgan SL, Heien ML, Hashemi P. Anal Chem. 2017; 89:9703–9711. [PubMed: 28795565]
124. Gardier AM, David DJ, Jego G, Przybylski C, Jacquot C, Durier S, Gruwez B, Douvier E, Beauverie P, Poisson N, Hen R, Bourin M. J Neurochem. 2003; 86:13–24. [PubMed: 12807420]
125. Bungay PM, Newton-Vinson P, Isele W, Garris PA, Justice JB. J Neurochem. 2003; 86:932–946. [PubMed: 12887691]
126. Chen G, Ewing AG. Critical reviews in neurobiology. 1997; 11:59–90. [PubMed: 9093814]
127. Sombers LA, Hanchar HJ, Colliver TL, Wittenberg N, Cans A, Arbault S, Amatore C, Ewing AG. Journal of Neuroscience. 2004; 24:303–309. [PubMed: 14724228]
128. Ales E, Tabares L, Poyato JM, Valero V, Lindau M, de Toledo GA. Nat Cell Biol. 1999; 1:40–44. [PubMed: 10559862]
129. Wang K, Xiao T, Yue Q, Wu F, Yu P, Mao L. Anal Chem. 2017; 89:9502–9507. [PubMed: 28776368]
130. Wang Y, Noel JM, Velmurugan J, Nogala W, Mirkin MV, Lu C, Guille Collignon M, Lemaitre F, Amatore C. Proc Natl Acad Sci U S A. 2012; 109:11534–11539. [PubMed: 22615353]
131. Pathirathna P, Yang YY, Forzley K, McElmurry SP, Hashemi P. Analytical Chemistry. 2012; 84:6298–6302. [PubMed: 22856609]
132. Harrington JM, Bargar JR, Jarzecki AA, Roberts JG, Sombers LA, Duckworth OW. Biometals. 2012; 25:393–412. [PubMed: 22187125]
133. Roberts JG, Voinov MA, Schmidt AC, Smirnova TI, Sombers LA. J Am Chem Soc. 2016; 138:2516–2519. [PubMed: 26840154]
134. Schluter EW, Mitz AR, Cheer JF, Averbeck BB. PloS one. 2014; 9:e98692. [PubMed: 24921937]
135. Min HK, Ross EK, Jo HJ, Cho S, Settell ML, Jeong JH, Duffy PS, Chang SY, Bennet KE, Blaha CD, Lee KH. The Journal of Neuroscience. 2016; 36:6022–9. [PubMed: 27251623]
136. Kasasbeh A, Lee K, Bieber A, Bennet K, Chang SY. Stereotactic and functional neurosurgery. 2013; 91:141–147. [PubMed: 23445903]
137. Jones LJ, McCutcheon JE, Young AMJ, Norton WHJ. Frontiers in behavioral neuroscience. 2015; 9
138. Pyakurel P, Privman Champaloux E, Venton BJ. ACS Chem Neurosci. 2016; 7:1112–9. [PubMed: 27326831]
139. Makos MA, Kim YC, Han KA, Heien ML, Ewing AG. Anal Chem. 2009; 81:1848–54. [PubMed: 19192966]

140. Smith AR, Garris PA, Casto JM. J Chem Neuroanat. 2015; 66–67:28–39.
141. Kuhr WG, Wightman RM, Rebec GV. Brain Res. 1987; 418:122–8. [PubMed: 3499205]
142. Belle AM, Owesson-White C, Herr NR, Carelli RM, Wightman RM. ACS Chem Neurosci. 2013; 4:761–71. [PubMed: 23480099]
143. Takmakov P, McKinney CJ, Carelli RM, Wightman RM. Review of Scientific Instruments. 2011; 82
144. Kirkpatrick DC, McKinney CJ, Manis PB, Wightman RM. Analyst. 2016; 141:4902–4911. [PubMed: 27314130]
145. Clark LC Jr, Lyons C. Annals of the New York Academy of Sciences. 1962; 102:29–45. [PubMed: 14021529]
146. Lugo-Morales LZ, Loziuk PL, Corder AK, Toups JV, Roberts JG, McCaffrey KA, Sombers LA. Anal Chem. 2013; 85:8780–8786. [PubMed: 23919631]
147. Asri R, O'Neill B, Patel JC, Siletti KA, Rice ME. Analyst. 2016; 141:6416–6421. [PubMed: 27722568]
148. Lee HM, Giguere PM, Roth BL. Drug Discov Today. 2014; 19:469–473. [PubMed: 24184433]
149. Li C, Sugam JA, Lowery-Gionta EG, McElligott ZA, McCall NM, Lopez AJ, McKlveen JM, Pleil KE, Kash TL. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2016; 41:2122–2132. [PubMed: 26792442]

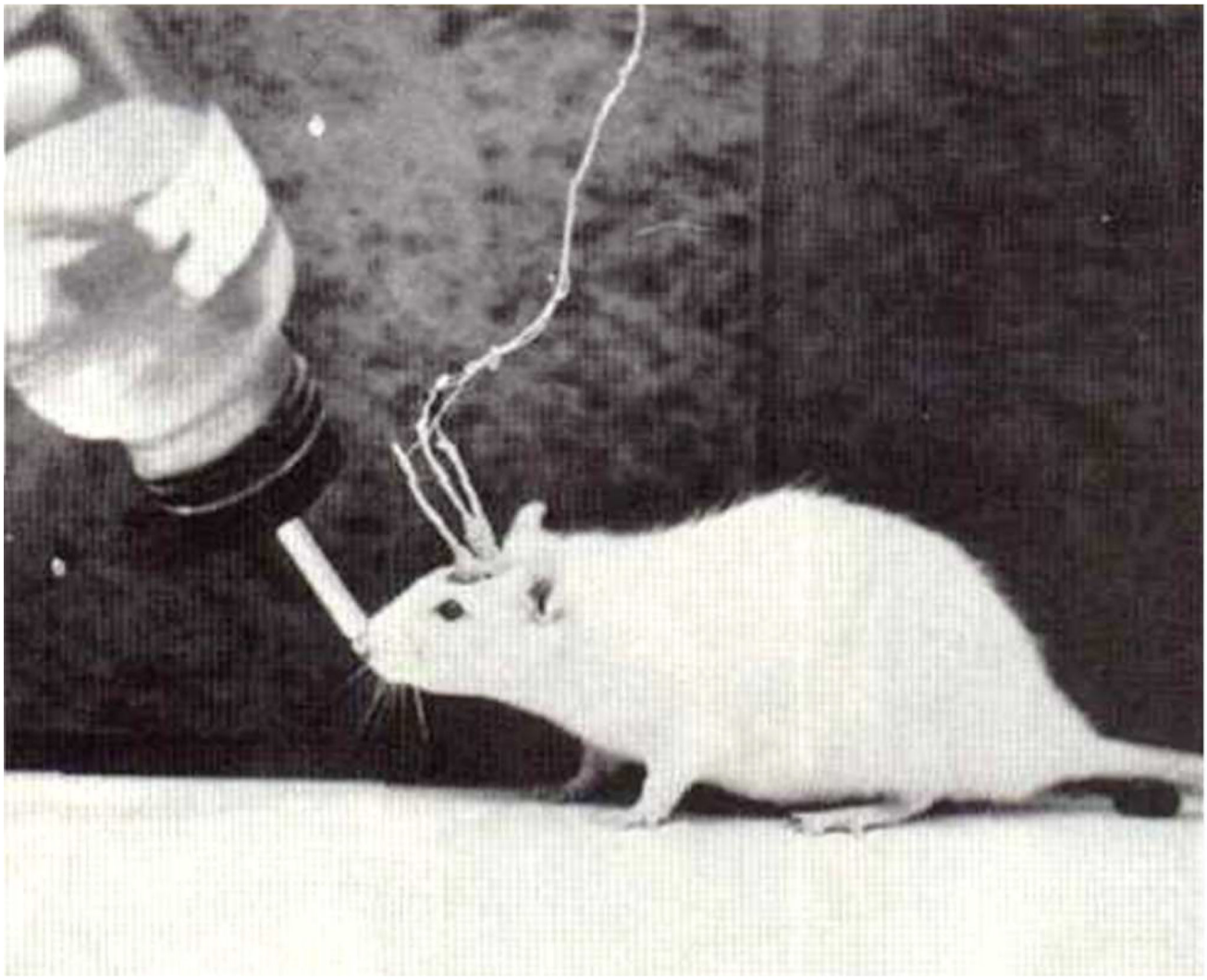


Figure 1.
The Origin of *In Vivo* FSCV. Rat with chronically implanted graphite paste electrodes to "see what we can see". Reproduced from Adams, R. N. Analytical Chemistry 1976, 48, 1126–1138. (ref 11). Copyright 1976 American Chemical Society.

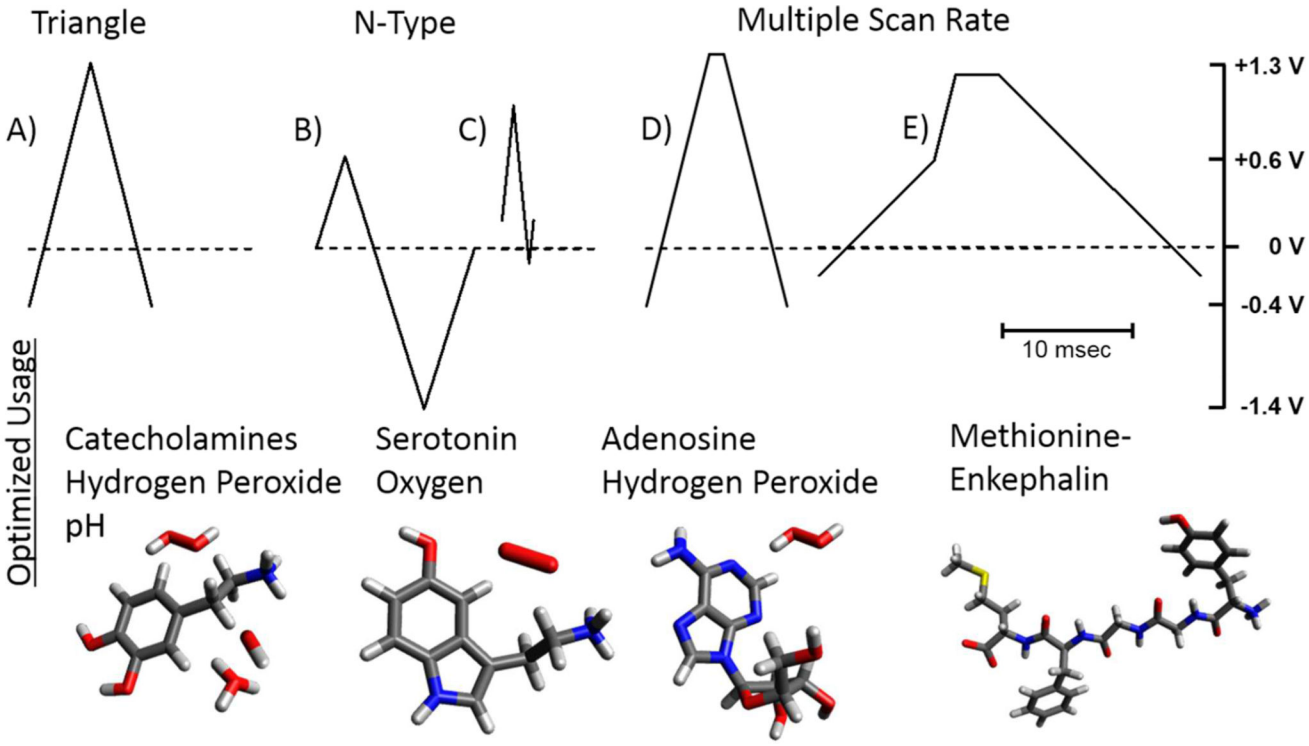


Figure 2.

Common FSCV Waveforms. A) Triangular waveform. B and C) 'N-shaped' waveforms with various holding potentials and wavelimits. D) Sawhorse and E) multiple-scan-rate, modified sawhorse waveforms. Below) List of analytes typically targeted with each optimized waveform.

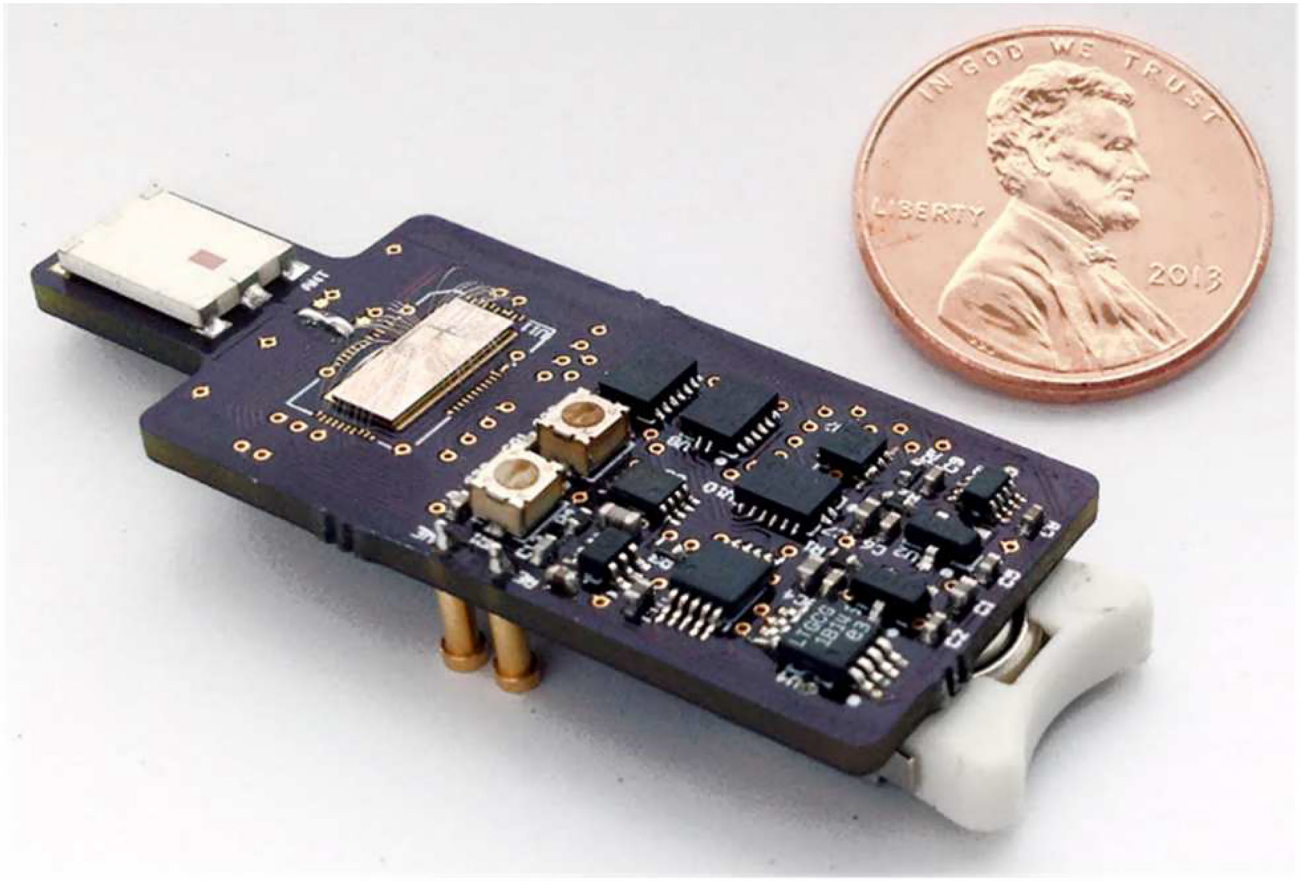


Figure 3.

Ultracompact Wireless FSCV Device. Complete integrated device with potentiostat, ADC, analog background subtraction, impulse transmitter, and power supply. Reproduced from Dorta-Quinones, C. I.; Wang, X. Y.; Dokania, R. K.; Gailey, A.; Lindau, M.; Apsel, A. B. IEEE Transactions on Biomedical Circuits and Systems 2016, 10, 289–299 (ref 94). Copyright 2016, with permission from IEEE.

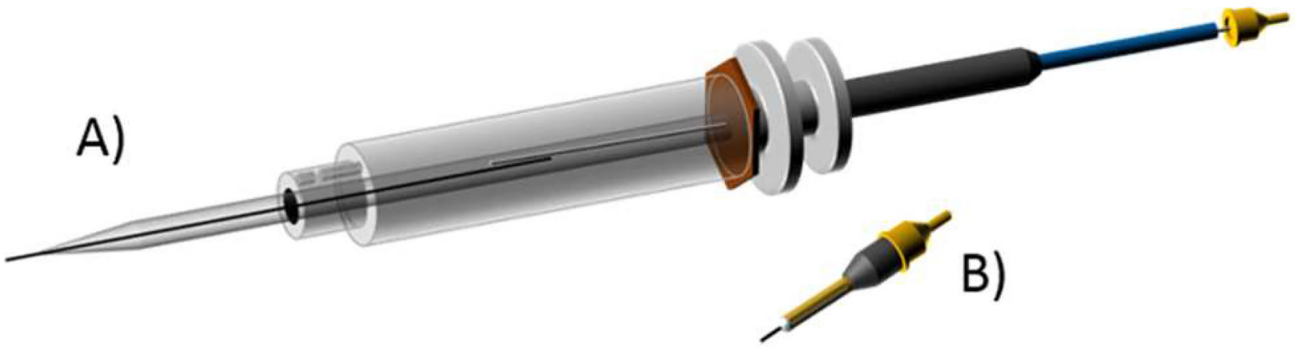


Figure 4.
Carbon-Fiber Microelectrodes for FSCV. A) Microdrive manipulator loaded with a glass-insulated carbon-fiber microelectrode. B) Fused-silica insulated carbon-fiber microelectrode for long-term implantation.

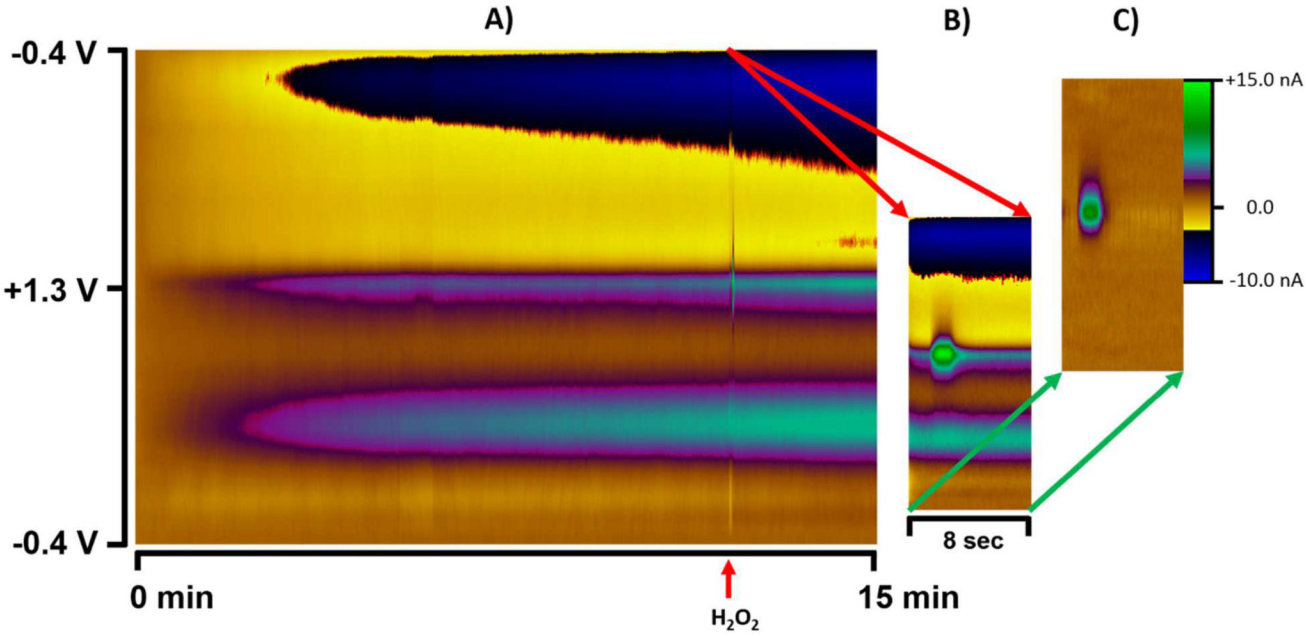


Figure 5.

Electrode Drift. A) Color plot of 15 minutes of voltammetric data collected in a flow cell on the benchtop. 160 μM H_2O_2 was introduced to the electrode surface at 12 minutes (red arrow). The background selected for subtraction was at time zero. B) A closer look at 8 seconds of the color plot during H_2O_2 exposure. C) Appropriate correction for drift clarifies the H_2O_2 signal. Note: this electrode was intentionally not fully conditioned to emphasize drift.