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A pipette-based calibration system for fast-scan cyclic voltammetry with fast response times

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Fast-scan cyclic voltammetry (FSCV) is an electrochemical technique that utilizes the oxidation and/or reduction of an analyte of interest to infer rapid changes in concentrations. In order to calibrate the resulting oxidative or reductive current, known concentrations of an analyte must be introduced under controlled settings. Here, I describe a simple and cost-effective method, using a Petri dish and pipettes, for the calibration of carbon fiber microelectrodes (CFMs) using FSCV.

Fast-scan cyclic voltammetry (FSCV) allows for sub-second monitoring of neurotransmitter changes based upon their oxidation and reduction profiles at carbon fiber microelectrodes (CFM). Neurotransmitters such as serotonin, dopamine (DA), norepinephrine, and histamine are subjected to ramping voltages at the carbon surface, leading to unique oxidation and reduction signatures (1–10). Oxidation peak currents are used as a correlate to changing concentrations of analyte, since the oxidation current is linearly correlated to concentration within restricted ranges (1,7,8,11–18). Calibration has previously been achieved through various methods, including flow-injection analysis (FIA) (19,20), capillary electrophoresis (21,22), microfluidic FIA (17), or macrofluidic FIA (23,24) (for a discussion on the difference between micro- and macrofluidic FIA, see Reference 17). Additionally, because fast-scan controlled adsorption voltammetry (FSCAV) aims to analyze preexisting basal DA levels, its calibration is done in scintillation vials of various DA concentrations (25,26). In vitro calibration can also prove useful for preemptively determining what mixed solutions of analytes may look like in vivo (6,8,27,28) or ex vivo (9,21,22,29).

While FIA and microfluidic FIA have been described with sufficient detail to replicate (17,19), both techniques suffer shortcomings that a simpler calibration system could overcome. First, FIA requires costly six-port injector valves under pneumatic or computer control. In addition to injector valves, inert tubing with special connectors and a specialized outlet (for the appropriate positioning of the CFM) (19) are also required. Lastly, a pump is required for FIA to move fluid at the desired rate. Should the tubing become compromised with precipitate or bacteria, this flow rate and the associated bolus of analyte could be disrupted, altering measurements (19). Microfluidic FIA is the cheaper option of the two: it requires open syringes (the flow is driven by gravity) and flow regulator valves (17). However, the microfluidic Y channel requires precision milling, which may not be available to all investigators. Compromised tubing or inaccurate regulators could also pose potential issues. Here, I describe a calibration method using 1 mL of buffer solution per replicate. It has fast response times and uses equipment readily available to most scientists.

All data were collected and analyzed with the Demon Voltammetry setup previously described (30). Data were graphed in Veusz (http://home.gna.org/veusz/), and linear modeling was provided by Past3 (http://folk.uio.no/ohammer/past/). All chemicals were purchased from Sigma Aldrich (St. Louis, MO). CFMs were created as previously described (31); electrode diameter was 7 µm and electrode length was ~200 µm. FSCV parameters were: -0.4 V to 1.3 V and back at 400 V/s; 10 Hz data collection; -0.4 V resting voltage. CFMs were cycled until stable (<0.5 nA background drift) prior to use.

Figure 1A shows a simplified calibration scheme (hereafter termed the "Simple system"). Artificial cerebrospinal fluid (aCSF) (17,31) (125 mM NaCl, 4 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, 0.66 mM NaH_2PO_4, 2 mM Na_2HPO_4, 1 mM glucose, pH 7.4) is placed on the edge of a 35 mm Petri dish (Sigma Aldrich). The top was used, as it is shallower and easier for quick pipetting. The Petri dish lid should be secured (via tape or poster tack) to a flat and level surface to avoid any movement during calibration. The amount of aCSF and analyte stock is dependent upon the desired final concentration. For example, if the final concentration is 1000 nM, 950 µL aCSF is used. A CFM is placed at one end of the crescent of solution, and an Ag/AgCl reference electrode is placed nearby. Two microliters 10 mM DA stock [in 0.1 N perchloric acid (PCA)] is combined with 998 µL aCSF in a 1.5 mL microcentrifuge tube wrapped in aluminum foil. This "new stock" is 20 µM. The new stock (50 µL) is added to the crescent of solution at the end (indicated by a black arrow in Figure 1A) opposite the electrode. Injection of analyte will not diffuse to the electrode position (Figure 1B, asterisk) prior to mixing. Next, the tip of a 1000-µL pipette is placed at the black arrow location, and ~300–400 µL of the solution is repeatedly drawn up and dispensed back into the solution, effectively mixing it (Figure 1C).

The procedure results in an almost instantaneous exposure of the electrode to the final desired concentration of DA,

METHOD SUMMARY

Calibration of fast-scan cyclic voltammetry (FSCV) electrodes is accomplished by adding a dilute stock solution to a small volume of buffer and mixing by pipette. All aspects of calibration are performed by pipette, minimizing cost and complexity.

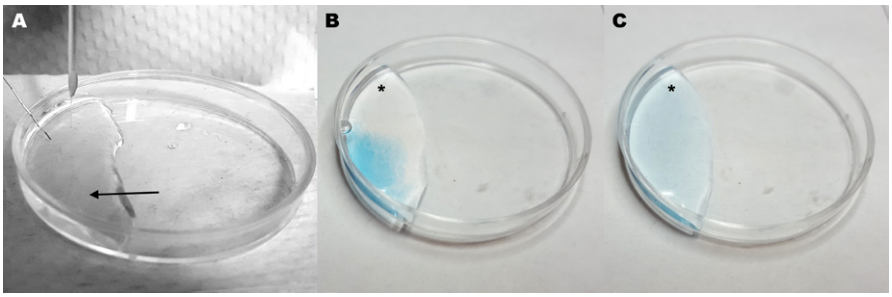

Figure 1. A pipette-based calibration method for fast-scan cyclic voltammetry (FSCV). (A) A carbon fiber microelectrode (CFM) is placed in 950 µL artificial cerebrospinal fluid (aCSF) at one end of the crescent of solution. An Ag/AgCl reference electrode is placed nearby. Fifty microliters 20 µM stock solution [created by adding 2 µL 10 mM dopamine (DA) to 998 µL aCSF] is added to the solution at the black arrow to minimize early diffusion and direct pipetting on the microelectrode. A 1000-µL pipette is used to mix the solution at the black arrow. (B) An injection of dye at the black arrow in (A) shows that injected analyte does not diffuse readily to the electrode location (asterisk). (C) Mixing by a 1000-µL pipette produces a homogenous solution of dye (analyte) throughout.

as is shown in Figure 2A. The background-subtracted 60 s data collection is depicted in false color (31,32). The oxidation and reduction current corresponding to DA is measured along the red line (*y*-axis) within the color plot, further depicted by the red inset voltammogram. The current resulting at the oxidation potential for DA is tracked along the white dashed line and is further depicted by the inset directly above the color plot. Mixing agitation can be seen in Figure 2A as small spikes in the top inset; ceasing of the spikes indicates mixing has stopped. The result of this pipetting is a steady state of DA exposure that is highly repeatable.

Because of the rapid transition of the solution surrounding the CFM from no DA to 1000 nM DA, this calibration technique results in a rapid rise time for the oxidative current. As seen in Figure 2A, the Simple system calibration has an almost immediate rise time (~0.3 s). Over a longer time frame, such as the 60 s seen in Figure 2A, diffusion of DA to the electrode and adsorption lead to an increasing signal (see the slight increase in the black line inset in Figure 2A). This can be seen in other calibration schemes (1,7,17,33,34) and is the result of the adsorption of DA at slower (10 Hz) application frequencies (7) and a negative holding potential (1). Most endogenous or drug-related transient DA events in vivo are <1 s (35), so the sudden rise to peak (~0.3 s) seen in the Simple system should be a good in vitro representation of a CFM's response to sudden changes of DA in vivo. While all experiments were conducted at room temperature (23°C), the Simple system calibration method would theoretically be more sensitive to convective currents than would FIA. Should one wish for a higher temperature solution, a Peltier heater under the Petri dish is feasible with this setup (9).

The size of the oxidation peak of DA is linearly related to the concentration of DA at low concentrations (7), so the Simple system was tested for 50, 100, 200, 500, and 1000 nM DA (5 electrodes; 3 replicates of each concentration). In between each replicate, the previous solution was pipetted off of the Petri dish, and the Petri dish was rinsed with deionized water via pipette. This was done to remove any residual DA between replicates. The results for each electrode are shown in Figure 2B. All 5 electrodes (data are mean ± SEM; error bars are not visible) showed a strong linear fit for increased DA concentrations (R^2 range: 0.9932–0.99785; mean: 0.996478). Data from each electrode were also normalized to peak responses at 1000 nM and then averaged for each concentration. Figure 2C shows that the averaged response to increasing concentrations of DA was a tight linear fit (R^2 = 0.99685), and none of the normalized best-fit lines differed in slope (F = 0.13361; *P* = 0.996478; slope range: 0.92964–0.94608; mean slope: 0.94002).

A disadvantage of the Simple system is that the waveform must either be turned off, or the electrode must be allowed to cycle for a short time once aCSF is returned after the deionized water rinse. A larger Petri dish may be used to set up three calibration stations to expedite the process. There are several advantages to the Simple system. First, it results in a sudden exposure of the CFM

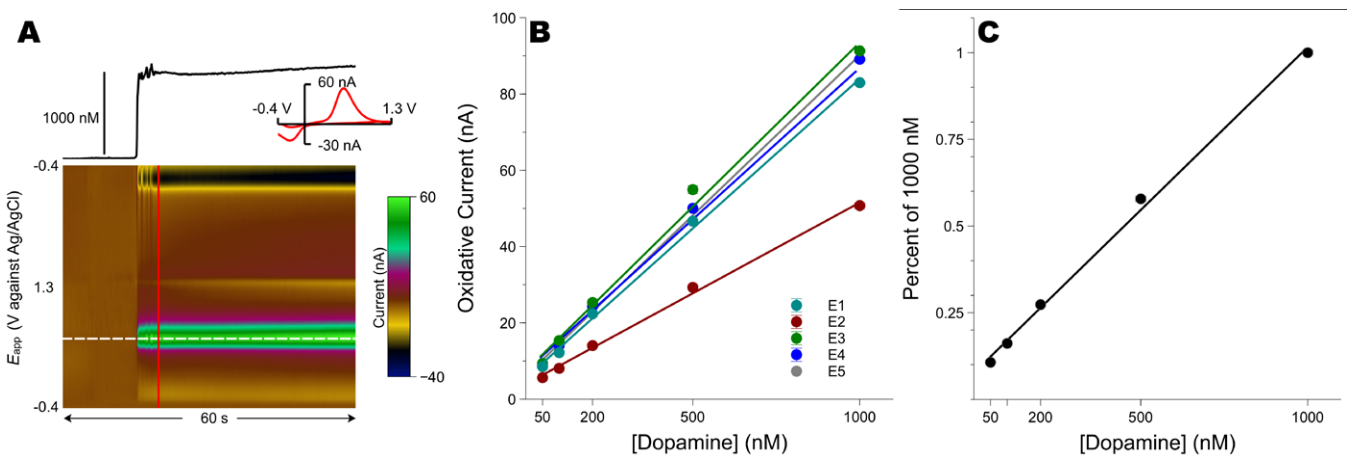

Figure 2. Fast response to dopamine (DA) and linear calibration response. (A) Representative background-subtracted false-color plot of 60 s of recorded calibration data collected at a rate of 10 Hz. Mixing the solution with a 1000-µL pipette resulted in an almost instantaneous exposure of the microelectrode to 1000 nM DA, which can be seen in the color plot by the green oxidation peak and blue/black reduction peak. Data taken along the red line are graphed in the red inset, showing a voltammogram for DA. The current along the oxidation peak for DA (the white dashed line) is graphed in the top inset. It shows a sudden (~0.3 s) transition from 0 to 1000 nM DA. The small peaks represent mixing with the 1000-µL pipette. (B) Five carbon fiber microelectrodes (CFMs) (E1-E5) were exposed to increasing amounts of DA (50, 100, 200, 500, and 1000 nM). The peak DA oxidation shows a linear response to increasing DA concentrations. Lines of best fit are shown, and error bars are not visible. (C) Data from (B) were normalized to the peak response of 1000 nM for each electrode and then averaged. The "Simple system" results in a tight linear averaged response curve, demonstrating its ability to precisely expose CFMs to increasing analyte concentrations. Error bars are not visible.

to analyte at speeds mimicking in vivo DA transients. Second, since the electrode is placed far from the injection site to minimize distortions (early diffusion or pipetting directly onto the CFM), it is not negatively affected by dispersion of analyte in a fluid bolus (19). Third, while electrodes are at risk of being broken when inserted into a microfluidics system or output port of the FIA, the only real risk of electrode breakage in this method is when the electrode is lowered into the Petri dish. Fourth, it uses Petri dishes and pipettes, which are readily available at most institutions, and can be set up in a very short time frame and at minimal cost. Last, the system is easy to clean and replication is quick since there is no loading of injection loops or larger dilutions (e.g., in a volumetric flask); everything is performed by pipette.

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Competing interests

The author declares no competing interests.

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