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Improved Calibration of Voltammetric Sensors for Studying Pharmacological Effects on Dopamine Transporter Kinetics in Vivo

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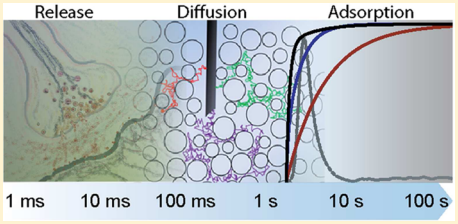
ABSTRACT: The distribution and density of neurons within the brain poses many challenges when making quantitative measurements of neurotransmission in the extracellular space. A volume neurotransmitter is released into the synapse during chemical communication and must diffuse through the extracellular space to an implanted sensor for real-time in situ detection. Fast-scan cyclic voltammetry is an excellent technique for measuring biologically relevant concentration changes in vivo; however, the sensitivity is limited by mass-transport-limited adsorption. Due to the resistance to mass transfer in the brain, the response time of voltammetric sensors is increased, which decreases the sensitivity and the temporal fidelity of the measurement. Here, experimental results reveal how the tortuosity of the extracellular space affects the response of the electrode. Additionally, a model of mass-transport-limited adsorption is utilized to account for both the strength of adsorption and the magnitude of the diffusion coefficient to calculate the response time of the electrode. The response time is then used to determine the concentration of dopamine released in response to salient stimuli. We present the method of kinetic calibration of in vivo voltammetric data and apply the method to discern changes in the K_M for the murine dopamine transporter. The K_M increased from 0.32 ± 0.08 μM (n = 3 animals) prior to drug administration to 2.72 ± 0.37 μM (n = 3 animals) after treatment with GBR-12909.



KEYWORDS: *Dopamine, electrochemistry, in vivo, calibration, hindered diffusion, kinetics*

Dopamine is an important volume neurotransmitter that is involved in reward based behavior, decision making, and locomotion.[1] The neurochemical phenomena underlying dopamine neurotransmission occur rapidly and at low concentrations.[2,3] Fast-scan cyclic voltammetry (FSCV) has been used to measure dopamine because it is fast, sensitive and provides a chemical signature for analyte identification.[4] The signals measured with FSCV arise from a complex combination of mass-transport, adsorption, and electrochemical process occurring at the surface of carbon-fiber microelectrodes.[5,6] Despite the widespread use of carbon-fiber microelectrodes,[7−11] they are hand fabricated, and standardization has not yet been achieved. Thus, quantitative in vivo experimentation demands individual calibrations of these sensors.[12] We seek here to improve upon current calibration methods by developing a mathematical model to account for mass-transport-limited adsorption to aid in quantifying dopamine concentrations and concentration changes in vivo.

Background-subtracted fast-scan cyclic voltammetry (FSCV) has been extensively used to measure dopamine because it is capable of collecting data rapidly (subsecond).[5] The analytical signal observed in FSCV experiments has been shown to be dependent on dopamine adsorption.[6] However, the analytical advantage of adsorption comes at a price; to accurately determine the concentration of analyte in the brain, the electrode surface must be at equilibrium with its surrounding environment. With FSCV, the rapid, continuous application of triangular waveforms (100 ms intervals) can prevent this equilibrium from being achieved, especially when mass transport to the electrode is slow.[5,13] In the brain, diffusion is the main mode of mass transport for volume neurotransmitters and is markedly slower than diffusion in free solution due to volume exclusion and high tortuosity.[14,15] This causes a problem with in vivo FSCV measurements as the required time for sensors to reach equilibrium is often longer than the duration of neurotransmitter release and clearance. Indeed, in vivo amperometry experiments, which use sensors with faster response times, are suggestive of this problem.[10]

Traditionally, to quantify in vivo measurements using FSCV, researchers have depended on a calibration using flow-injection analysis (FIA), to simulate concentration changes as required by background subtraction.[16] Typically, FIA employs a pneumatically actuated six-port HPLC valve to inject a bolus of 1 μM dopamine solution onto the electrode at room temperature. The sensitivity (nA/μM) is quantified by measuring the current after the resultant signal has reached a steady state ($>t_{90\%}$). Clearly, the intercellular environment of neuronal tissue is different in terms of temperature, density,

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viscosity, tortuosity, and chemical interferences from that of a flow cell.[16,17] Previous efforts to correct for these problems have used convolution theory, however this work was limited by the requirement of using a flow cell.[5,6] Additionally, an analytical solution for mass-transport-limited adsorption at cylindrical microelectrodes does not exist, leading researchers to rely on the assumption that the bulk concentration remains constant.

In this work, we overcome previous limitations by using Fast-scan controlled-adsorption voltammetry (FSCAV),[13] which is performed without the use of a flow cell and at body temperature (37 °C). A model for hindered diffusion was developed to demonstrate the time for dopamine adsorption to reach equilibrium is increased in a tortuous environment such as the brain. An implicit-finite difference model was coupled to the experiments and used to solve both the mass transfer and adsorption partial differential equations simultaneously so that dynamic concentration changes are accurately tracked at the electrode surface. To account for the movement of mass (flux) to the electrode a kinetic calibration (takes into account both diffusion and the strength of adsorption) was developed. Using this model, experimentally determined electrode parameters, and convolution theory were used to calibrate electrodes and determine the concentration of electrically evoked and pharmacologically induced dopamine transients. It is important to note that convolution theory requires a linear response function. As such the kinetic calibration can only be used for calibrations of dopamine concentrations in the linear range (<2 μM) of the adsorption isotherm.[18] Application of the kinetic calibration to in vivo FSCV data allowed the determination enzyme kinetics for the dopamine uptake transporter (DAT) before and after drug administration to validate the calibration. The kinetic calibration presented here provides a robust yet facile method for analyzing in vivo FSCV data without introducing calibration bias.

RESULTS AND DISCUSSION

The Rate of Dopamine Adsorption at Microelectrodes Is Hindered in Tortuous Environment. A suspension of lipid-coated silica particles was used as a model system to determine whether the diffusion of dopamine in the brain could bring an electrode to equilibrium surface concentration in the time frame of neurotransmitter release events.[6,19] With varied volume fractions of particles present in Tris buffer, the effect of excluded volume on mass transport is measured using FSCAV without the use of a flow cell. Mass-transport-limited adsorption was studied by comparing results of FSCAV experiments in free solution and in a solution with hindered diffusion (both at 37 °C) to standard flow injection experiments performed at 25 °C. (Figure 1) Flow-injection analysis for FSCV introduces convective flow, which causes electrodes to reach a steady state within one second (Figure 1 black trace). When convection is eliminated and diffusion dominates mass-transport, the carbon-fiber microelectrode approaches equilibrium in free solution approximately 5 times longer than in a flow cell (Figure 1 blue trace). With this model system, where diffusion of dopamine is hindered by the tortuosity of the solution, the equilibrium surface concentration (Γ_{eq}) at the electrode is not achieved prior to 20 s (Figure 1 red trace). While this model system does not provide a quantitative measure of hindered diffusion in the brain, it allows for a qualitative understanding of electrode response when dopamine diffusion is hindered. When compared to the time scale

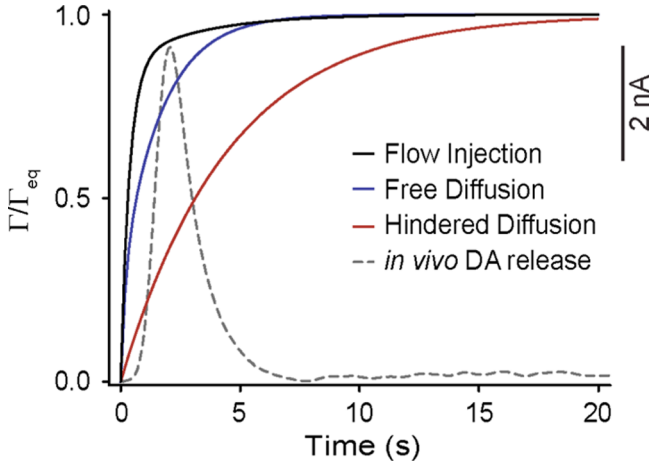

Figure 1. Comparison of mass-transport-limited electrode response times to 1 μM dopamine to a typical stimulated dopamine release events observed in vivo. Electrode response times were measured using FSCAV for the same electrode under multiple experimental conditions and are expressed as the fraction of equilibrium surface concentration. An in vivo dopamine release profile (gray dashed trace) is shown for qualitative comparison in the time dimension ($t_{10-90\%}$ = 1.8 s). In situ flow-injection analysis of dopamine (black trace) exhibits rapid response times due to convective mass transport ($t_{10-90\%}$ = 1.6 s). Free diffusion of the same concentration of dopamine in Tris buffer at 37 °C results an increased response time ($t_{10-90\%}$ = 3.5 s). When diffusion is hindered at 37 °C by increasing solution viscosity with 20% (w/v) sucrose and excluding volume using 40% (v/v) lipid-coated glass beads, the response time further decreases ($t_{10-90\%}$ = 9.8 s). Under these brainlike conditions, the equilibrium surface coverage of dopamine is not achieved during the period of release-and-reuptake. Flow-injection calibrations thus tend to result in underestimates of actual in vivo dopamine concentration profiles and overestimate the duration of release events.

of a stimulated dopamine release event monitored by in vivo FSCV (Figure 1 gray dashed line), it is apparent that equilibrium surface concentration of the electrode is not reached over the apparent duration of the release event.

This experiment illustrates that mass transport of dopamine at a microelectrode surface is hindered in brainlike environments and the time to reach Γ_{eq} is much longer than the duration of a stimulated release event. Meaning that the response time of the electrodes is very close to the duration of transient concentration changes that are observed in vivo such that the electrode does not reach equilibrium. Simply stated, when the time-scale of biological events are shorter than the time required to reach equilibrium, calibrations taken under steady-state conditions are inaccurate.

Implicit Finite Difference Simulation Reveals the Effect of Mass Transport on Dopamine Measurements. A previously validated implicit finite difference simulation was used to calculate the amount of time required to reach equilibrium surface concentration as a function of diffusion and adsorption processes.[13] The magnitude of the adsorption rate constant dictates the flux of analyte to the electrode; however, as such, if the rate of mass transfer is slow, the magnitude of this flux is limited. In the model using a diffusion coefficient of ~1.0 $\times 10^{-6}$ cm^2/s the flux is unaffected by adsorption rate constants above ~0.1 cm/s, indicating that the systems is mass-transport limited rather than under kinetic control. To ensure the model was mass-transport limited, the dopamine adsorption rate constant was cautiously set to 10 cm/s. Additionally, the

concentrations measured are in the linear portion of the Langmuir isotherm for dopamine (< 2 μM). This model verifies that the signal response of electrochemical measurements of dopamine is affected by adsorption and mass transport (Figure 2). By normalizing the instantaneous surface concentration (Γ)

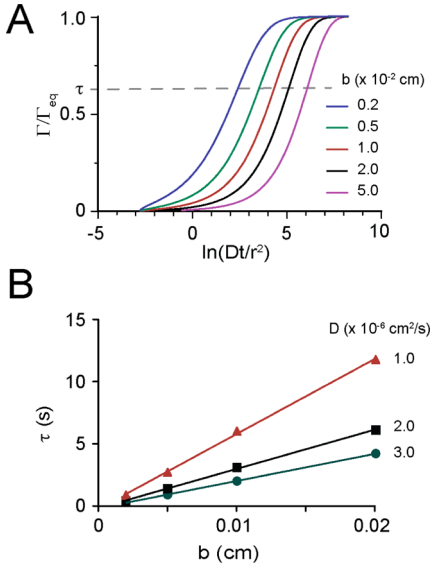

Figure 2. Effect of resistance to mass transfer and adsorption on sensor response. Mass transport affects the temporal response of a cylindrical electrochemical sensor. (A) The ratio of adsorbed dopamine (Γ_{DA}) to the equilibrium surface concentration (Γ_{eq}) is a function of molecular diffusion, time, and electrode radius, which can be universally expressed as a dimensionless parameter (Dt/r^2). Each trace represents the response for a sensor with different adsorption properties (b). The normalized surface coverage is on the ordinate and the natural logarithm of the dimensionless parameter Dt/r^2 on the abscissa. The horizontal dashed line represents the value for τ, taken at ~0.63 or $1 - 1/e$ (the point where $t = \tau$). (B) The electrode time constant is also dependent on the rate of diffusion. The relationship of b to τ is shown for different diffusion coefficients. Red trace, $\tau = 606b - 0.28$; black trace, $\tau = 320b - 0.20$; and blue trace, $\tau = 220b - 0.20$.

to Γ_{eq}, it is clear that the time required for dopamine adsorption to reach equilibrium depends upon a dimensionless parameter $\ln(Dt/r^2)$ (Figure 2A). This dimensionless parameter includes mass transport to the electrode and takes into account the analyte diffusion coefficient (D), the radius of the electrode (r), and time (t). Each subsequent curve in Figure 2A shows the behavior of Γ/Γ_{eq} by varying the strength of adsorption (b, where $\Gamma = b[DA]$) with the time required to reach equilibrium increasing as b increases. Values of b (0.002–0.05 cm) were chosen to encompass the typical values measured for carbon-fiber microelectrodes.[2,18,20,21] When using carbon-fiber microelectrodes, the rate of mass-transport-limited adsorption is dictated by the radius. Additionally, in electrochemistry, it is common to express the surface coverage in mol/cm^2 and concentration in mol/cm^3. As such, the relationship of surface concentration to bulk concentration when working in the linear range of the Langmuir isotherm scales by its adsorption strength (b) in terms of centimeters. For real experimental systems, electrode radii vary from 1 to 18 μm[22] and diffusion coefficients range from 6×10^{-6} to 0.6×10^{-6} cm^2/s for small molecule neurotransmitters.[23−25]

For the measurement of dopamine at a 3 μm radius (r) carbon-fiber microelectrode with an adsorption strength (b) of 5×10^{-3} cm and a diffusion coefficient (D) of 2×10^{-6}, the temporal response ($t_{10-90\%}$) is ~4 s. For molecules with greater adsorption strengths such as serotonin, $t_{90\%}$ can be expected to increase. To calculate the time to reach equilibrium, the model uses known values for b, D, and r to calculate the surface concentration over time, and a value for τ can be determined as shown by the horizontal gray dashed line ($\tau = 1 - 1/e$, Figure 2A). These times are much greater than the time to reach equilibrium in a flow cell where convective forces increase the flux of neurotransmitter to the electrode surface.

This model was then used to generate a relationship for b and τ for different rates of diffusion (Figure 2B). The diffusion coefficient for dopamine in the brain has been previously measured to be between 0.6 and 2×10^{-6} cm^2/s.[23−25] The lower the magnitude of the diffusion coefficient, the longer the temporal response. To show the best case scenario, the value of 2×10^{-6} cm^2 was chosen for this work. The modeled relationship for this diffusion coefficient is illustrated in in Figure 2B (black). Using a value of D from the literature[23] and an experimentally determined value of b (the adsorption strength was determined by FSCAV measurements of 1 μM dopamine, see Methods section), a value for τ can be determined and an electrode response function can be generated for use in developing in vivo calibrations. When taken together, Figures 1 and 2 demonstrate that mass transport and sensor size have a significant effect on the temporal response of sensors. Therefore, when the time scale of biological events is similar to the time response of the sensor, a more comprehensive approach to calibration of analytical signals arising from in vivo concentration transients is required. This empirically determined value of τ is used herein for the kinetic calibration of in vivo measurements of dopamine.

Determination of the Electrode Response Function Using Fast-Scan Controlled-Adsorption Voltammetry. For measurements of biogenic amines using fast-scan cyclic voltammetry, the magnitude of the measured current depends on the amount of analyte adsorbed to the surface. In typical FSCV experiments, the waveform is applied every 100 ms, this cycling limits the amount of time for adsorption to occur. During the voltammogram, dopamine is oxidized to dopamine-*o*-quinone, which can then desorb from the electrode surface. This process was previously characterized and modeled by Bath et al.,[5] with one compelling piece of evidence for this process being that the magnitude of the oxidation peak is greater than the reduction peak, where if this species was surface confined, the peaks would be identical in magnitude. Commonly, once the application of the triangle waveform has finished, the potential is held at −0.4 V to reduce dopamine oxidation products back to dopamine to promote dopamine adsorption.[26] To accurately quantify the strength of adsorption when continuously applying a triangle waveform at 10 Hz, FSCAV was modified such that instead of holding the potential constant (−0.4 V) during the delay time, a triangle waveform (−0.4 to 1.3 V at 1200 V/s) was applied every 10 ms (Figure 3A). Effectively mass-transport-limited adsorption using typical FSCV parameters for dopamine detection can be studied in three steps: (1) apply the waveform at 100 Hz to minimize adsorption, (2) apply the waveform at 10 Hz to allow adsorption to occur until a steady state is achieved, and (3) return the waveform application frequency to 100 Hz, to measure the amount of dopamine accumulated on the electrode. This effect is compared to FSCAV with a constant potential in Figure 3B. The adsorption strength for FSCAV,

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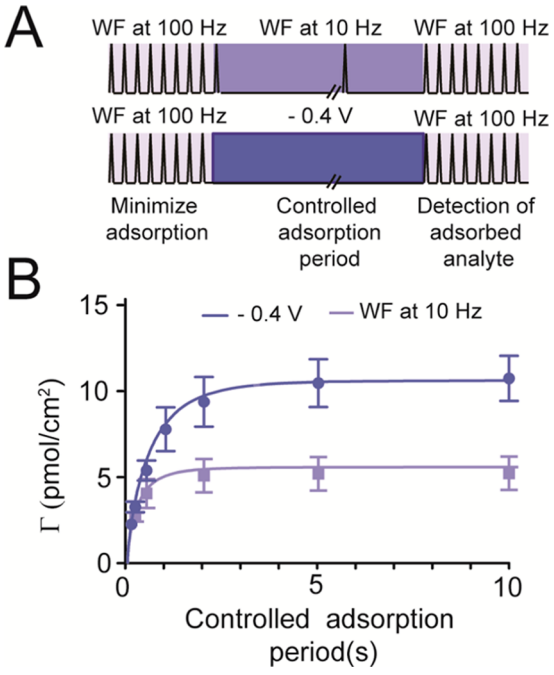

Figure 3. Experimental determination of electrode response function. Fast-scan controlled-adsorption voltammetry was expanded to investigate the adsorption strength. (A) To more closely mimic in vivo FSCV, FSCAV was performed in a beaker submersed in a 37 °C water bath and the waveform was applied every 100 ms during the delay (dark blue trace) instead of a constant potential (−0.4 V, light blue trace). (B) To compare the effects applying a continuous waveform application (dark blue trace) or a constant potential (light blue trace) on the magnitude of dopamine adsorption, the controlled adsorption period was varied from 0.2 to 20 s and the amount of adsorbed dopamine (Γ) was measured.

where a triangle waveform was applied during the rest period (10 Hz application frequency), was determined to be 5.5 ± 0.9 × 10^{-3} cm (±SEM, n = 3 electrodes) which at a concentration of 1 μM, corresponds to a surface concentration of 5.5 pmol/cm^2, a factor of ∼2 less than when a constant potential is applied. Using a previously validated model that was developed using Comsol 4.3,[13] the resultant data was fit with a diffusion coefficient of 6.0 × 10^{-6} cm^2/s at 37 °C.[24] The model assumes diffusion is directly from the solution to the electrode surface. From this novel use of FSCAV, τ for the average Nafion-coated T-650 was determined to be 1.5 ± 0.1 s (±SEM, n = 3 electrodes).

Calibration of FSCV Measurements of Dopamine Release in Vivo Reveals Increased Concentrations and Faster Release and Reuptake. The analytical signal obtained from in vivo electrochemical experiments is a convolution of the electrode response function and the concentration profile arising from release, mass transport, and reuptake of neurotransmitters. In the brain, neurotransmitter concentration profiles are unknown and the goal of in vivo electrochemical experiments is to measure them. The electrode response and the analytical signal may be decoupled by convolution theory, giving rise to a higher fidelity measurement of changes in concentration over time..

The process for deconvolving the analytical signal and the electrode response involves several relatively simple steps: (1) Perform FSCAV in known solutions of dopamine to determine b, (2) determine the magnitude of the diffusion coefficient D, (3) use the data in Figure 2B to determine τ, (4) generate the response function from these parameters, (5) convert the collected current versus time trace to surface concentration (Γ) versus time, and (6) deconvolve Γ versus time with the electrode response function. This enables a quantitative determination of the amount of material released and the time scale of the event.

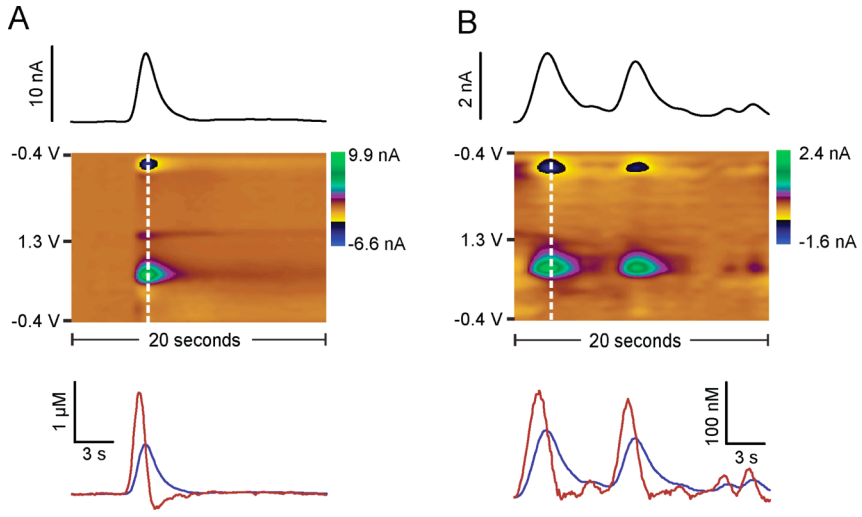

Figure 4. Deconvolution of in vivo data to quantify changes in dopamine concentration. Application of the kinetic calibration to in vivo signals allows for improved quantitative analysis. (A) Dopamine release was evoked by electrical stimulation and measured. Representative color plots are shown with time on the abscissa, voltage on the ordinate, and current in false color. Cyclic voltammograms are collected every 100 ms, at 400 V/s by scanning the potential from −0.4 to 1.3 to −0.4 V. Uncalibrated current versus time traces selected at the peak oxidation potential of dopamine are shown above the color plots (black traces). Kinetic calibration of the surface concentration versus time traces was performed using an electrode response function corresponding to b = 5.45 × 10^{-3} cm (red trace). The concentration profile resulting from equilibrium-based calibration is displayed for comparison (blue trace). (B) Pharmacologically induced dopamine transients were measured. Application of the kinetic calibration to these data reveal increased concentrations, shorter durations, and possibly additional transients (red trace) when compared to the concentration profile resulting traditional flow-cell calibration (blue trace).

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This kinetic calibration was applied to in vivo measurements of electrically stimulated and spontaneous (transient) dopamine release in the nucleus accumbens of the mouse brain (Figure 4). As shown in Figure 4A-B, FSCV measurement of a stimulated release event recorded a concentration change lasting ~5 s with a maximal concentration of 900 nM using a flow-injection calibration (of 10 nA/μM).[17,27] However, the kinetic calibration indicates that the maximal concentration of dopamine released was 1.76 μM and the extracellular concentration was back to baseline in ~1 s. Interestingly, the concentration undershoot following the peak in the kinetically calibrated data is only found in traces resulting from electrically evoked dopamine and may provide insight complexities in dopamine metabolism which have been shown previously.[28]

The kinetic calibration presented here is also suitable for quantification of the transient release of dopamine. A combination of GBR-12909 (dopamine uptake inhibitor) and raclopride (D_2 antagonist) were used to slow clearance of dopamine following tonic firing, increasing the amplitude of tonic release events in anesthetized animals, which improves detectability.[29] By this data it may also be inferred that blocking the DAT with GBR-12909, inhibits the concentration undershoot that is visible in Figure 4A. A representative 20 s period showing transient release is shown in Figure 4. The current versus time trace at the peak oxidation potential (660 mV versus Ag/AgCl) and the voltammogram of dopamine are shown above the color plot. Two current transients in this window meet the signal-to-noise criterion ($S/N > 3$) and were selected for analysis. Quantification of these peaks using the kinetic calibration resulted in concentrations of 350 and 330 nM with widths of ~3 s, whereas the flow-cell calibration underestimates the concentrations at 210 and 190 nM and their duration remains unchanged with a duration of 6 s (Figure 4B).

Pharmacologically Inhibited Dopamine Reuptake Kinetics Are Observable Only When Calibration by Deconvolution Is Used. Electrically evoked dopamine release was measured from multiple animals before and after treatment with GBR-12909 to determine kinetic parameters for the dopamine transporter (Figure 5). As expected, treatment with GBR-12909 increases the concentration and duration of dopamine release (Figure 5A). This is observed regardless of the calibration method used. The kinetic calibration was calculated using an experimentally determined adsorption strength (b) of 0.00545 cm. By separating the response of the electrode from the measured release event using the kinetic calibration, a more accurate determination of DAT kinetics following the previous model described by Wu et al. is performed.[30] GBR-12909 is known to be a competitive binding inhibitor for DAT, which should not affect V_{max}. Thus, V_{max} for DAT was assumed to be constant before and after drug administration. The V_{max} values for the postdrug samples were calculated prior to drug delivery for each rat with average reported as 3.7 ± 0.13 μM s^{-1} for n = 3 rats (±SEM). Data processed with the kinetic calibration show that K_m increases from 0.32 ± 0.08 μM (±SEM, n = 3) prior to drug administration to 2.72 ± 0.37 μM (±SEM, n = 3) after treatment with 10 mg/kg GBR-12909 (t_2 = 10.47; $p < 0.001$). The values of K_m and V_{max} determined predrug are similar to previously reported values.[31] When using a flow cell calibration factor, no significant change in K_m was observed (t_2 = 0.51; $p > 0.05$), showing that a flow cell calibration factor does not account for the electrode response time and, because of this, changes in transport kinetics are masked (Figure 5B).

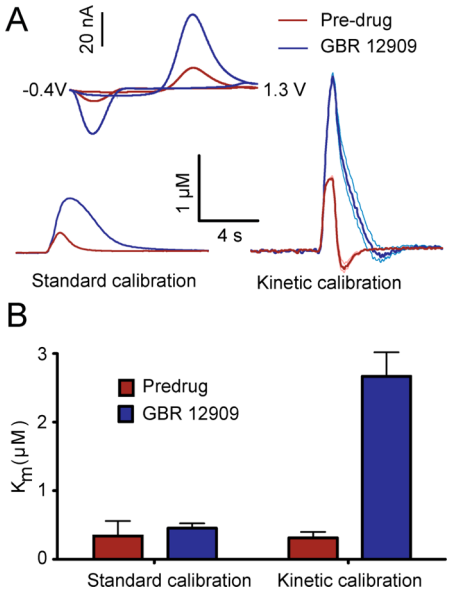

Figure 5. Kinetic calibration for improved characterization of biological processes. The dopamine reuptake inhibitor GBR-12909 was administered and the effects of uptake and release were studied. (A) Cyclic voltammograms of dopamine before (red trace) and after (blue trace) treatment are shown. The dopamine concentration profiles obtained from flow-cell calibration (left) and the kinetic calibration (right) are shown for comparison. The lighter traces represent 1 standard deviation on the error associated with the calibration factor (b). (B) These concentration profiles were fit to the Michaelis–Menten model. Upon treatment with GBR-12909, a significant increase in K_M was observed when kinetic calibration was applied to the data ($p < 0.001$). No significant difference was observed when using standard calibrations ($p > 0.05$).

SUMMARY AND CONCLUSIONS

In vivo electrochemical measurements of neurotransmitters such as dopamine depend on mass-transport-limited adsorption to an electrochemical sensor. In the brain, mass transport is slowed with minimal convection and hindered diffusion due to obstructions posed by cell bodies and projections. Current calibration strategies of electrochemical sensors use a sensitivity parameter that is based on measurements made at a steady-state surface concentration. We have demonstrated that this equilibrium condition is not achieved within the time frame of typical release events given a tortuous environment where diffusion is obstructed. To overcome this problem, a novel calibration strategy was developed using a model of mass-transport-limited adsorption and convolution theory to decouple the response time of the electrode from the analytical signal. Through this process, the kinetic calibration is used to determine the concentration of dopamine released in response to salient stimuli. In general, the result is that the measured neurotransmitter release events are greater in the magnitude and shorter in duration than previously measured. This impacts the interpretation of pharmacological effects on enzymes related to dopamine metabolism. We show that the kinetic calibration's advantage of decoupling the electrode response from the biological processes is the ability to accurately fit neurotransmitter concentration decays to Michaelis–Menten kinetics. This improves upon current methods of determining dopamine reuptake kinetics in vivo as the convoluted signal masks the subtle changes arising from pharmacological challenge. The kinetic calibration is a facile and robust means

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for accurately quantifying transient changes in neurotransmitter release when measured with fast-scan cyclic voltammetry.

METHODS

Chemicals. Dopamine hydrochloride, trizma hydrochloride (Tris), sucrose, potassium chloride, and calcium chloride were purchased from Sigma-Aldrich (St. Louis, MO). Sodium chloride was purchased from EMD (Gibbstown, NJ). Sodium phosphate and perchloric acid were purchased from Mallinckrodt (Phillipsburg, NJ). GBR-12909 dihydrochloride was obtained from Tocris Bioscience (Ellisville, MO), and raclopride tartrate was obtained from Sigma-Aldrich (St. Louis, MO). A 1.0 mM stock solution of dopamine was prepared in 0.1 N $HClO_4$ and diluted to the desired concentration in pH = 7.4 Tris buffer (15 mM Tris, 126 mM NaCl, 2.5 mM KCl, and 1.2 mM $CaCl_2$) immediately prior to experiments. All water was purified to a resistivity of 18.2 MΩ·cm (Milli-Q Gradient A10, EMD Millipore).

In Situ Model of the Hindered Diffusion in the Brain. Nonporous hollow-silica particles with an average diameter of 11 μm (Discovery Scientific, Kelowna, BC) were used to simulate the volume exclusion observed in brain tissue. To minimize adsorption of dopamine to the silica, a lipid coating was applied. L-α-Phosphatidylcholine lipids (egg PC) derived from chicken egg (Avanti Polar Lipids, Alabaster, AL) were dissolved in chloroform and portioned out into 4 mL glass vials. The solvent was evaporated under a stream of argon, and the lipid residue was lyophilized for 1 h. The lipid film was rehydrated in Tris buffer for 4 h followed by vortexing for 30 s. The total lipid concentration was 10 mg/mL in the Tris buffer. One gram of silica particles was added to 4 mL of this solution, and the suspension was mixed using magnetic stirring and stored overnight at 4 °C. Excess liquid was removed and the remaining bead-containing fraction was lyophilized prior to use. The dry lipid-coated particles were then suspended in Tris buffer containing 20% (w/v) sucrose at the desired percent weight. The addition of sucrose increased the viscosity of the solution to minimize bead settling during the experiment.

Electrode Fabrication. Cylindrical carbon-fiber microelectrodes were prepared as previously described.[13] Briefly, a single T-650 carbon fiber (Cytec Thornel, Woodland Park, NJ) was aspirated into a 0.68 mm I.D. glass capillary (A-M Systems, Inc., Sequim, WA). Capillaries were subsequently heated and pulled to a fine seal using a PE-2 pipet puller (Narishige, Japan) and then cut to 40−50 μm in length. Electrodes were then coated with Nafion (Ion Power, DE) which was deposited electrochemically as previously described.[32] A Ag/AgCl reference electrode was prepared by soaking a silver wire (0.25 mm, Alfa Aesar) in chlorine bleach.

Electrochemical Measurements. Data were collected using custom hardware and software written in house using LabVIEW 2009 (National Instruments, Austin, TX). The voltammetric waveform was applied, and the data was acquired using a PCIe-6341 DAC/ADC Card (National Instruments). Fast-scan controlled-adsorption voltammetry (FSCAV) was performed as previously described.[13] Briefly, a CMOS precision analog switch, ADG419 (Analog Devices), was implemented and controlled with the PCIe-6341 National Instruments interface card.[13] Delay times for controlled adsorption were varied from 0.2−20 s to provide sufficient time for the dopamine surface concentration to reach equilibrium. For in situ FSCAV experiments, the electrodes were placed directly into a scintillation vial containing the desired analyte concentration and samples were placed in a temperature-controlled water bath at 37 °C to match the temperature present during in vivo experiments. A modification of previously described FSCAV experiments was implemented to replicate how mass transport-limited adsorption is affected when a waveform is applied every 100 ms. In this experiment, a 10 Hz waveform application was used during the controlled adsorption period rather than a constant potential.

Flow cell FSCV was performed in a manner similar to previous work.[16] A bolus of 1.0 μM dopamine was injected for 20 s via a pneumatically actuated 6-port HPLC injection valve (VICI, Houston, TX). Continuous flow was delivered via a PHD 2000 syringe pump (Harvard Apparatus, Holliston, MA). Adsorption of dopamine to the electrode in the flow cell was measured over time by applying the waveform at 1200 V/s every 100 ms.

Animals. Surgery was performed on 15−25 g male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) housed in a 12/12 h light-dark cycle. Food and water were offered ad libitum. Animals were anesthetized using 25% urethane in saline (i.p.) and mounted in a stereotax for surgery (David Kopf Instruments, Tujunga, CA). Stereotaxic coordinates were referenced from bregma. After holes for the stimulating electrode (1.06 mm posterior, 1.25 mm lateral) and working electrode (1.10 mm anterior, 1.30 lateral) were drilled, a 0.2 mm bipolar stainless steel stimulating electrode (Plastics One, Roanoke, VA) was lowered into the medial forebrain bundle (5.00 mm ventral). A carbon-fiber microelectrode was lowered into the nucleus accumbens (4.3 mm ventral). A Ag/AgCl reference electrode was implanted into the contralateral hemisphere. A waveform was applied at 400 V/s for stimulated and tonic release studies and 1200 V/s for pharmacological studies at a frequency of 10 Hz. An isothermal heating pad (Braintree Scientific, Braintree, MA) maintained body temperature at 37 °C. Dopamine release was electrically stimulated with 40 pulses (biphasic pulse, ± 350 μA, 4 ms in width) delivered at 60 Hz through a constant-current stimulus isolator (NL800A, Neurolog, Medical Systems Corp., Great Neck, NY). Mouse surgery and handling were in agreement with The Guide for the Care and Use of Laboratory Animals, approved by Wayne State University's the Institutional Animal Care and Use Committees (IACUC). A pharmacological challenge was used to modulate dopamine release and reuptake. GBR 12909 and raclopride tartrate were delivered via intraperitoneal injection in saline at 10 and 2 mg/kg, respectively, and delivered at a volume of 0.1 mL/20 g body weight.

Modeling. A previously developed, implicit finite-difference simulation written in COMSOL Multiphysics 4.3 (Comsol Inc., Los Angeles, CA) was used to solve the time-dependent adsorption equation, based on adsorption strength (b) and the magnitude of the diffusion coefficient (D).[13] The output of this model is the surface concentration of an analyte (Γ) versus time. As the time increases, the surface concentration increases until equilibrium conditions are met. An exponential growth function was fit to the modeled Γ versus time trace to determine the time constant of an electrode response (τ).

Convolution Theory/Electrode Response Calculation and Application to Uptake Kinetics. Faradaic current (i) measured during in vivo electrochemical experiments is proportional to the Γ for an adsorbing analyte such as dopamine. Thus, the measured current versus time trace can be converted to a Γ versus time trace (the analytical signal). By integrating the oxidation current in the cyclic voltammogram and using Faraday's law, the surface concentration (Γ) can be determined. The analytical signal is the convolution of a concentration profile and the response function of the electrode as defined by[33]

$$g(t) \otimes h(t) = S(t) \tag{1}$$

where $g(t)$ is the response function of the electrode, $h(t)$ is the concentration profile, and $S(t)$ is the analytical signal.

Software was written in house using LabVIEW 2009 to convolve and deconvolve data that has been stored in ASCII tab-delimited text files. Deconvolving the analytical signal with the electrode response (division of the Fourier transforms of the analytical signal by the electrode response function) reveals the concentration profile from the collected data.

The response of the electrode to an impulse function was modeled by an exponential decay

$$g(t) = A_0\, e^{-t/\tau} \tag{2}$$

where t is time, τ is the time constant of the electrode response, and the coefficient (A_0) is a scalar factor. To determine A_0, the instantaneous surface concentration was defined by eq 3.

$$C\int_0^\infty g(t)\, dt = \Gamma\Delta t \tag{3}$$

Under equilibrium conditions, the integral of the response function (eq 5) must be equal to $\Gamma\Delta t/C$ which can be reduced to $b\Delta t$. As the definite integral from 0 to ∞ of the response function is equal to $A_0\tau$, the value for A_0 may be calculated as

$$A_0 = b\frac{\Delta t}{\tau} \tag{4}$$

where Δt is the time between data points. By measuring b for an electrode via a 10 Hz FSCAV experiment and understanding the diffusion coefficient for dopamine in vivo, τ may be determined. In this work, the values of τ were determined from a simulation with a diffusion coefficient of 2×10^{-6} cm^2/s[17] and a b value of 0.00545 ± 0.00095 cm (SEM, n = 3 electrodes). While there is great variation in the length of the electrode, b is a ratio of the surface concentration (determined from Faraday's law and incorporates electrode area) to bulk concentration so that the variation in length is inherently account for and an average b can be used. Deconvolved concentration profiles were fit to Michaelis−Menten kinetics.[30] The starting concentration is taken from the input, deconvolved data, and the instantaneous velocity of the Michaelis−Menten reaction is calculated from

$$V_t = \frac{V_{max}[S]_t}{k_m + [S]_t} \tag{5}$$

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