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Demon voltammetry and analysis software: Analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures

Jordan T. Yorgason^{1,2}, Rodrigo A. España¹, and Sara R. Jones^{1,2}

Jordan T. Yorgason: jyorgaso@wfubmc.edu; Rodrigo A. España: respaña@wfubmc.edu; Sara R. Jones: srjones@wfubmc.edu

¹ Department of Physiology and Pharmacology, Wake Forest University Health Sciences, Medical Center Boulevard, Winston-Salem, North Carolina, 27157, USA

² Neuroscience Program, Wake Forest University Health Sciences, Medical Center Boulevard, Winston-Salem, North Carolina, 27157, USA

Abstract

The fast sampling rates of fast scan cyclic voltammetry make it a favorable method for measuring changes in brain monoamine release and uptake kinetics in slice, anesthetized, and freely moving preparations. The most common analysis technique for evaluating changes in dopamine signaling uses well-established Michaelis-Menten kinetic methods that can accurately model dopamine release and uptake parameters across multiple experimental conditions. Nevertheless, over the years, many researchers have turned to other measures to estimate changes in dopamine release and uptake, yet to our knowledge no systematic comparison amongst these measures has been conducted. To address this lack of uniformity in kinetic analyses, we have created the Demon Voltammetry and Analysis software suite, which is freely available to academic and non-profit institutions. Here we present an explanation of the Demon Acquisition and Analysis features, and demonstrate its utility for acquiring voltammetric data under *in vitro*, *in vivo* anesthetized, and freely moving conditions. Additionally, the software was used to compare the sensitivity of multiple kinetic measures of release and uptake to cocaine-induced changes in electrically evoked dopamine efflux in nucleus accumbens core slices. Specifically, we examined and compared tau, full width at half height, half-life, T₂₀, T₈₀, slope, peak height, calibrated peak dopamine concentration, and area under the curve to the well-characterized Michaelis-Menten parameters, dopamine per pulse, maximal uptake rate, and apparent affinity. Based on observed results we recommend tau for measuring dopamine uptake and calibrated peak dopamine concentration for measuring dopamine release.

Keywords

Demon Voltammetry; cocaine; dopamine; fast scan cyclic voltammetry; software; kinetic analyses; dopamine transporter; Michaelis-Menten

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Corresponding Author: Sara R. Jones, Ph.D., Wake Forest University Health Sciences, Department of Physiology and Pharmacology, Medical Center Boulevard, Winston-Salem, NC 27157, Office: 336-716-8533, Fax: 336-716-8501, srjones@wfubmc.edu.

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1. Introduction

Fast scan cyclic voltammetry (FSCV) is an electrochemical detection technique that is acclaimed for its ability to measure presynaptic monoamine activity with high temporal and spatial resolution. It also provides information about transporter kinetics, as well as presynaptic auto-receptor and hetero-receptor activity (Fawaz et al., 2009; Jones et al., 1999; Jones et al., 1996). In this technique, a potentiostat is used to pass a voltage ramp rapidly across a carbon-fiber electrode, while measuring the resultant current. During this voltage ramp, nearby electroactive chemical species, such as monoamines, adenosine, 3,4-dihydroxyphenylacetic acid (DOPAC) or ascorbate, are oxidized/reduced resulting in changes in current amplitude that are proportional to the concentration of the species (Heien et al., 2003; Swamy and Venton, 2007). The current readout during the voltage ramp is referred to as a voltammogram which is collected and compared across time to determine changes in concentrations of electroactive species. Because of its high temporal resolution, voltammetry is an ideal technique for measuring rapid presynaptic signaling events in the brain during *in vitro*, *in vivo* anesthetized, and freely moving experiments.

One common application of voltammetry methods is to measure dopamine (DA) release and transporter activity in limbic brain regions. By electrically stimulating DA overflow, one can assess exocytotic release during the rising phase of the overflow, and uptake kinetics of DA transporters during the falling phase. The most commonly used kinetic analysis for evaluating changes in DA release and uptake rates uses methods established in anesthetized rats employing electrically evoked DA release (Wightman et al., 1988; Wightman and Zimmerman, 1990). Under these conditions, it is possible to elicit high enough DA concentrations that uptake can be saturated, thus allowing data to be fitted to a Michaelis-Menten kinetic model. Unfortunately, under some conditions, transient DA release events do not reach concentrations that saturate uptake, and therefore Michaelis-Menten-based curve fitting programs cannot be used reliably (Garris et al., 2003; Robinson and Wightman, 2007; Wightman et al., 1988; Wightman and Zimmerman, 1990). Consequently, researchers have turned to other measures to estimate changes in DA uptake, including tau, full width at half height (FWHH), half-life, T_{20} , T_{80} , slope, peak height, calibrated peak dopamine concentration ($[DA_c]$), and area under the curve (AUC). While these methods may be valid, to our knowledge no systematic comparison amongst these measures has been conducted for voltammetry data, and certainly no standard measure has been established. Further, there is currently no comprehensive, commercially available kinetic analysis software program available for voltammetry data, and thus these types of assessments tend to be cumbersome and difficult to compare across laboratories.

Accordingly, we have embarked on an extensive project to develop freely available voltammetry acquisition and analysis software that provides reliable and efficient data analysis. In the present study, we confirmed that our software is compatible with existing hardware and is capable of detecting and quantifying electrically evoked DA release and uptake. The Demon Voltammetry and Analysis software suite (Demon Voltammetry) is available at no cost to academic and nonprofit entities at <http://www.wfubmc.edu/OTAM/Technologies/Computer-Software-and-copyright.htm#Demon>.

2. Material and methods

2.1 Hardware

Two National Instruments data acquisition cards (NI-DAQ; PCI-6711 and PCI-6052e; National Instruments, Austin, TX) were used for interfacing Demon Voltammetry with a Chem-Clamp potentiostat (Dagan Corporation; Minneapolis, MN) for voltammetric

recordings. Other similar NI-DAQ cards may be used as well. The Chem-Clamp was modified to have a gain range of 0.005 to 8.0 mV/pA, and a headstage with 5 MOhm resistance. The NI-DAQ cards contain multiple on-board high speed clocks, and several 16 bit analog outputs/inputs suitable for generating potential sweeps and acquiring voltammograms at high rates (>100kHz) while performing electrical stimulations for evoking DA release. NI-DAQ cards were connected to the potentiostat via specialized breakout boxes created locally from 2 NI-DAQ CB-68LPR screw terminals.

2.2 Software

Demon Voltammetry has been developed for data collection, analysis, and figure making. This suite was written in the graphical programming language, LabView (National Instruments). It contains several new features that were used in this publication as well as many other features which we use regularly. The acquisition portion of Demon Voltammetry contains stimulation settings designed for performing paired pulse stimulations, and burst stimulation trains, which allow the user to designate intervals between either single pulses or configured stimulation trains. Such trains have been shown to be useful for measuring autoreceptor activity (Bao et al., 2010; Kennedy et al., 1992). Demon Voltammetry also incorporates acquisition features for automated data collection, including timed multiple collections, providing controls for specifying collection durations, intervals, and number of files to collect for an experiment. Additionally, the software contains an integrated routine builder which assists in designing more complex experiments with variable stimulation occurrences, collection times, durations, and intervals between files. These parameters are integrated with digital event generation/registration such that TTL pulse information from external devices are recorded and stored within each file. This information is useful for recording behavioral cue presentations and operant responses, and communicating stimulation and collection events to external devices, such as drug self-administration setups. These latter features are critical for drug studies with time collections at different intervals, as well as freely moving experiments where DA transient events, cue-evoked, and electrically stimulated measures are performed in the same animal. Figure 1 shows the Demon Voltammetry user interface for the acquisition portion of the software.

The analysis portion of the software contains many useful tools for examining data, including new peak finding algorithms for detecting evoked and transient neurochemical signals at the click of a button. An additional feature uses a three cursor system for measuring tau, FWHH, half-life, T_{20} , T_{80} , slope, peak height, $[DA_c]$, and AUC. All of these parameters can then be exported to a tab delimited file, compatible with Microsoft Excel® (Redmond, WA). Another part of the analysis software contains a Michaelis-Menten modeling utility, using previously published algorithms to derive information about DA per pulse $[DA_p]$, maximal uptake rate V_{max} , and apparent affinity K_m (Wightman et al., 1988; Wightman and Zimmerman, 1990; Wu et al., 2001).

Additional features not described explicitly in the current publication include a figure making utility that allows for creation of publication quality figures which can be used as-is or exported in various formats for subsequent modification. Additionally, the software includes a well-developed chemometric analysis suite, designed for seamless creation of chemometric training sets and analysis with either principle component or partial least squares regressions (Faber and Rajkó, 2007; Heien et al., 2004; Heien et al., 2005; Hermans et al., 2008; Keithley et al., 2009; Lavine and Workman, 2008). These analyses are extremely useful for extracting information on monoamine concentrations in the presence of interference from pH and other species, especially in freely moving preparations.

2.3 Analysis comparison

Analysis measures were obtained using the newly developed Demon Voltammetry software described in this article. Computations were based on user defined positions on current traces for baseline (Pre-Stim cursor), peak (Peak cursor) and return to baseline (Post-Stim cursor) positions (Figure 2). Tau and half-life values were determined from exponential fit curves based on Peak cursor and Post-Stim cursor positions using a least squares constrained exponential fit algorithm (National Instruments). FWHH was calculated by measuring the time between the rise and decay portions of the current trace at the half-maximal amplitude based on Peak and Post-Stim cursor positions. T_{20} and T_{80} clearance times are based on the time for 20 or 80% decay from peak amplitude, respectively. These measures were also calculated based on Peak and Post-Stim cursor positions. Slope was determined by calculating the negated inverse slope of a line between the T_{20} and T_{80} time point positions. Peak height, (in nA), and $[DA_c]$ (in μM) were designated by the Peak cursor position, while AUC was calculated as the numeric integration of the area between the Pre-Stim and Post-Stim cursors, using a trapezoidal rule. Finally, $[DA_c]$, AUC, slope, and Michaelis-Menten kinetics were calculated from post calibration DA signals obtained from a flow cell.

The Michaelis-Menten based parameters, $[DA_p]$, $V_{\max}$, and K_m , were used to evaluate release and uptake kinetics using the following equation (Wightman et al., 1988; Wightman and Zimmerman, 1990; Wu et al., 2001).

$$d[DA]/dt = f[DA_p] - V_{\max}/((K_m/[DA]) + 1)$$

In this equation, $[DA]$ is the instantaneous extracellular concentration of DA released, f is the stimulation frequency, $[DA_p]$ is the release rate constant (expressed as concentration of DA released per stimulus pulse) and $V_{\max}$ and K_m are Michaelis-Menten uptake rate constants (Figure 2D). The underlying assumptions of this equation are that: 1) individual stimulus pulses release a fixed quantity of dopamine from the presynaptic terminals; 2) uptake is a saturable and not infinite process; and 3) the predominant mechanism responsible for clearing dopamine from extracellular space is uptake via the DA transporter. Changes in release and uptake were obtained by setting baseline K_m values (prior to any drug treatment) to between 0.16 and 0.2 μM and establishing a baseline $V_{\max}$ individually for each subject. Following cocaine bath perfusion, $V_{\max}$ was held constant for the remainder of the experiment and thus cocaine-induced changes in uptake were attributed to changes in apparent K_m . Extracellular concentrations of DA were assessed by comparing the current at the peak oxidation potential for DA in consecutive voltammograms with electrode calibrations of known concentrations of DA (1–3 μM).

2.4 Statistical analyses

All assessments are reported in relation to percent of baseline. To examine the ability of each measure to detect changes in cocaine-induced alterations in DA signaling, planned comparisons were conducted using paired t-tests comparing the effects of individual cocaine concentrations to baseline values. Pearson correlations were conducted to assess the degree to which each of the kinetic measures correlated with well-established Michaelis-Menten parameters. Statistical analyses were performed using Prism 4 (Graphpad Software, La Jolla CA).

2.5 Animals

Male Sprague-Dawley rats were used for all slice and anesthetized FSCV studies. Male C57/Bl6J mice were used for the freely moving FSCV studies. All animals were housed on 12-hr

light-dark cycle with *ad libitum* access to food and water. Experimental protocols adhered to National Institutes of Health Animal Care guidelines and were approved by the Wake Forest University Institutional Animal Care and Use Committee.

2.5 Experimental procedures

2.5.1 In Vitro voltammetry—Rats were euthanized and their brains rapidly removed and prepared as previously described (John and Jones, 2007). Coronal slices (400 μ M) of the striatum were maintained at room temperature in oxygenated (95% O₂–5% CO₂) artificial cerebrospinal fluid (aCSF) which consisted of (in mM) NaCl (126), NaHCO₃ (25), D-glucose (11), KCl (2.5), CaCl₂ (2.4), MgCl₂ (1.2), NaH₂PO₄ (1.2), L-ascorbic acid (0.4), pH adjusted to 7.4. A capillary glass-based carbon-fiber electrode was positioned ~75 μ m below the surface of the slice in the nucleus accumbens (NAc) core. DA release was evoked every 5 min by a 4 ms, one-pulse stimulation (monophasic, 300 μ A) from a bipolar stimulating electrode (Plastics One) placed 100–200 μ m from the carbon-fiber electrode. Drugs were applied by superfusion (1 ml/min) using cumulative increases in cocaine concentration (300 nM, 1 μ M, 3 μ M, 10 μ M, and 30 μ M; John and Jones, 2007; Jones et al., 1995b).

2.5.2 In vivo anesthetized voltammetry—Rats were anesthetized with urethane (1.5 g/kg, i.p.; Sigma-Aldrich, St. Louis, MO, USA) and placed in a stereotaxic apparatus as previously described (España et al., 2010a; España et al., 2010b; España et al., 2008). Rats were implanted with a reference electrode in the left cortex, a bipolar stimulating electrode in the ventral tegmental area (VTA; –5.2 A, +1.1 L, –7.5 V), and a capillary glass-based carbon-fiber electrode in the NAc core (+1.3 A, +1.3 L, –4.5 V). Once a 1 sec, 60 Hz monophasic (4 ms; 300 μ A) stimulation train elicited a robust DA signal, DA release and uptake parameters were recorded.

2.5.3 Freely moving voltammetry—Mice were anesthetized using isoflurane anesthesia and placed in a stereotaxic apparatus. A reference electrode was implanted into the left cortex, a stimulating electrode was placed into the VTA (–3.0 A, +1.0 L, –4.5 V), and a fused silica-based carbon-fiber electrode was placed into the NAc core. Once stimulation parameters were optimized and a 0.4 sec, 60 Hz biphasic (2 ms; 250 μ A) stimulation train elicited a robust DA signal, all implants were cemented into place using acrylic dental cement as previously described (Clark et al., 2010). Mice were tested within 2–7 days following recovery and received identical stimulation parameters to elicit DA release.

2.6 Fast scan cyclic voltammetry

The electrode potential was linearly scanned as a triangular waveform from –0.4 to 1.3 V and back to –0.4 V vs Ag/AgCl using a scan rate of 400 V/s. Cyclic voltammograms were recorded at the carbon fiber electrode every 100 ms by means of a voltammeter/amperometer (Dagan Corporation).

3. Results

To validate the utility of Demon Voltammetry we performed FSCV in the NAc core during *in vitro* slice, *in vivo* anesthetized, and freely moving experiments. As shown in Figure 3, Demon Voltammetry was capable of detecting electrically stimulated DA release under these three conditions. Cyclic voltammograms verify that the electroactive species detected was dopamine, with oxidation and reduction peaks at ~ 600 mV and ~ –200 mV respectively.

Cocaine induced inhibition of DA uptake was measured using *in vitro* voltammetry in brain slices containing the NAc core, a brain region rich in DA terminals, with well characterized

uptake (Garris and Wightman, 1995; Jones et al., 1995a; Jones et al., 1995b; Jones et al., 1996). Figure 4 shows representative electrically evoked (1 pulse) DA traces following application of increasing concentrations of cocaine. To assess the utility of the kinetic measurements in Demon Voltammetry, the current studies also compared the well-documented Michaelis-Menten parameters of $[DA_p]$, V_{max} , and K_m (Wightman et al., 1988; Wightman and Zimmerman, 1990) to results obtained using analyses of tau, FWHH, half-life, T_{20} , T_{80} , slope, peak height, $[DA_c]$, and AUC across increasing concentrations of cocaine (Figure 5). Results indicate that under baseline conditions, slope was the only measure to be correlated with V_{max} , indicating that it might be useful to represent baseline levels of maximal DA uptake (Table I). Following drug application, all measures detected cocaine-induced changes in DA uptake inhibition, with slope being the only measure that failed to detect changes at low concentrations of cocaine. Peak height, $[DA_c]$, and $[DA_p]$ exhibited an inverted U shaped response, reflecting typical reductions in stimulated DA release at high concentrations of cocaine (John and Jones, 2007). AUC also showed a downward deflection, but this effect only occurred at the last concentration examined. Correlations between Michaelis-Menten based parameters and the Demon Voltammetry kinetic measures indicated that tau, FWHH, half-life, T_{20} , T_{80} , and slope were positively correlated with changes in K_m . Although these measures were also correlated with changes in $[DA_p]$, those negative correlations were generally less robust. By comparison, peak height, $[DA_c]$, and AUC did not correlate with K_m , yet both peak height and $[DA_c]$ showed robust positive correlations with $[DA_p]$.

4. Discussion

We have developed Demon Voltammetry and Analysis software to be freely available to academic and non-profit organizations. This software is particularly useful for neurobiological analyses due to its user-friendly analysis tools for measuring parameters relating to release and uptake, as well as for its chemometric analysis capabilities. Additionally, Demon Voltammetry contains useful tools for performing paired pulse stimulations, stimulation trains, automated peak amplitude detection, automated data collection routines, and has integrated digital event generation/registration features. Demon Voltammetry is available at no cost to academic and non-profit entities at <http://www.wfubmc.edu/OTAM/Technologies/Computer-Software-and-copyright.htm#Demon>.

In the current studies, we sought to systematically assess the ability of multiple kinetic measures to detect cocaine-induced alterations in DA signaling and to compare results with well established Michaelis-Menten kinetic models (Wightman et al., 1988; Wightman and Zimmerman, 1990). Tau, FWHH, half-life, T_{20} , and T_{80} , were reliable measures for detecting changes in DA uptake and were strongly correlated with changes in K_m , suggesting that these are accurate measures of DA uptake. Slope did not detect uptake changes at the lowest cocaine concentration, and therefore may not be as useful as the other indices for quantifying uptake changes. Peak height and $[DA_c]$ were reliable measures of cocaine-induced increases in the magnitude of DA efflux across cocaine concentrations, and were strongly correlated with $[DA_p]$, suggesting that these are accurate release measures.

4.1 Michaelis-Menten based measures

The DA transporter is a 12 transmembrane ~ 69kDa enzyme (Kilty et al., 1991) that follows traditional enzyme kinetics, making it a prime candidate for modeling with Michaelis-Menten methods (Wightman et al., 1988; Wightman and Zimmerman, 1990). Indeed, Michaelis-Menten based analyses have been routinely used for modeling changes in DA signaling *in vitro* and *in vivo* (Jones et al., 1995a; Jones et al., 1995b; Jones et al., 1996; Kawagoe et al., 1992; Nah et al., 2009; Robinson et al., 2005; Wightman et al., 1988;

Wightman and Zimmerman, 1990; Wu et al., 2001). Despite its well-established utility, DA concentrations are not always large enough to meet the assumptions of Michaelis-Menten modeling in whole animal preparations, especially in freely moving animals where transient and cue evoked DA release events are frequently low in concentration (Robinson and Wightman, 2007). Therefore, a variety of measures have surfaced over the years to model changes in DA kinetics. To better understand which of the many possible analyses of DA release and uptake best represents cocaine's blockade of DA transport, we compared the kinetic measures included in Demon Voltammetry to the Michaelis-Menten parameters $[DA_p]$, V_{max} , and K_m .

4.2 Release and uptake comparisons

In the present studies we used cocaine as a classical DA transporter inhibitor to produce predictable and well-defined alterations in DA uptake (John and Jones, 2007). These changes in uptake result in a protracted time-course for DA levels to return to baseline. To establish the utility of individual kinetic measures used in the literature to model changes in DA release and uptake, we compared tau, FWHH, half-life, T_{20} , T_{80} , and slope to traditional parameters obtained from Michaelis-Menten modeling. These studies demonstrated that tau, FWHH, half-life, T_{20} , and T_{80} highly correlate with cocaine-induced changes in apparent K_m , indicating that these measures accurately represent changes in uptake. Although slope was also correlated with K_m , this measure was unable to detect changes in uptake at the lowest concentrations of cocaine, displayed the greatest degree of variation between subjects, and differed substantially from the other indices at modeling changes in DA signaling at higher concentrations. Nevertheless, under basal conditions when stimulation intensity is sufficient to produce zero-order kinetics, slope may be a useful measure to detect V_{max} when calculated over the linear portion of the falling phase of the curve, preferably during the initial half of the descent. AUC appears to be a contaminated measurement, as it was useful at showing expected changes following cocaine application, but was not significantly correlated with either $[DA_p]$ or K_m . Based on these observations, and the fact that tau is derived from an exponential curve fit that encompasses the majority of the DA clearance curve, we recommend it as an initial and accurate measure for changes in DA uptake.

Another aspect of DA signaling that is altered by cocaine is the magnitude of evoked DA overflow, which reflects both release and uptake. At low concentrations of cocaine (0.3–3 μ M), DA efflux increases relative to baseline, whereas at higher concentrations (10–30 μ M) DA efflux is reduced. To model these changes in amplitude we examined peak height and $[DA_c]$ and compared these measures to the Michaelis-Menten parameter, $[DA_p]$. Both peak height and $[DA_c]$ were useful for tracking the well-documented changes in DA amplitude across cocaine concentrations (John and Jones, 2007). Additionally, peak height and $[DA_c]$ were highly correlated with changes observed using $[DA_p]$, indicating that these indices would be useful as measurements of DA release. Given that $[DA_c]$ takes electrode sensitivity into account with a calibration, we recommend this as a more reliable index of release.

4.3 Conclusions

The current studies describe a new FSCV acquisition and analysis software suite, Demon Voltammetry and Analysis software, which is freely available to the public. Using this software we successfully detected evoked DA release in the NAc core during *in vitro*, anesthetized *in vivo*, and freely moving experiments. Additionally, we conducted a detailed comparison of multiple kinetic parameters and we recommend tau for measuring DA uptake, and $[DA_c]$ for measuring DA release. These findings confirm the utility of Demon Voltammetry for FSCV acquisition and analysis of DA signaling under various experimental

conditions. Further the information provided concerning the various indices for measuring release and uptake will help to clarify when it is most appropriate to use a particular kinetic parameter to describe specific pharmacological effects.

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Figure 1.

Graphical user interface. Shown is the Demon Voltammetry user interface for the acquisition portion of the program. The graph on the left shows an oscilloscope containing the background current. The chart on the right displays background subtracted current vs time data during a collection. From this main front panel, the user can specify acquisition settings, designate stimulation and command voltage parameters, and access the analysis portion of the program.

Figure 2.

Measures of release and reuptake. Shown are depictions of multiple measures of evoked release and uptake overlaid upon a representative baseline DA overflow curve obtained during an *in vitro* experiment. **(A)** Shows the example positions for the Pre-Stim (green), Peak (magenta), and Post-Stim (red) cursors which are used to calculate the various measures described in this article. **(B)** Shows peak height, half-life, area under the curve (AUC), and tau. The green decay line denotes the exponential fit from which half-life and tau are derived. **(C)** Shows T₂₀, T₈₀, and full width at half height (FWHH). Slope is derived by calculating the negative inverse of the slope of a line created between the T₂₀ and T₈₀ time point positions. **(D)** The red dashed line denotes the Michaelis-Menten fit used to model changes in [DA_p], V_{max}, and K_m. In this example, DA release was elicited by a single-pulse stimulation, therefore, [DA_p] coincides with the peak height. The descending portion of the curve is best represented by two separate measures of uptake, V_{max} and K_m. The initial decay after stimulation is primarily regulated by the maximal rate of uptake (V_{max}), whereas the latter portion is largely driven by the apparent affinity of DA for the transporter (K_m).

Figure 3.

Evoked DA release using Demon Voltammetry. Shown are concentration-time plots (upper), color plots (lower) and cyclic voltammograms (insets) from (A) an *in vitro*, (B) an *in vivo* anesthetized, and (C) a freely moving FSCV experiment. Red bars below concentration-time plots indicate the timing of electrical stimulation. Concentration-time plots represent the concentration of DA over time. Color plots depict the voltammetric current (color in the z-axis) plotted against the applied potential (y-axis) and the acquisition time (x-axis; color plots were magnified to better show DA spikes). Cyclic voltammograms depict two current peaks, one at ~ 600 mV (positive deflection) for DA oxidation and one at ~ -200 mV (negative deflection) for reduction of DA-o-quinone. Peak positions identify the substance oxidized as DA.

Figure 4. Cocaine elicits concentration-dependent effects on DA signaling. Shown are representative *in vitro* slice data for a cocaine concentration response curve within the NAc core. Voltammetric signals were plotted every 100 ms, in response to a single pulse electrical stimulation (300 μ A, 2 ms pulse width). Note that at low concentrations, cocaine increases evoked DA release and inhibits DA uptake, while at high concentrations, evoked DA release is reduced.

Figure 5.

Measures of evoked DA release and uptake. Shown are the mean $\pm$ SEM for (A) area under the curve (AUC), peak height, [DA]_p and [DA]_c (B) tau, T₂₀, T₈₀, and slope, and (C) half-life, full width at half height (FWHH) and K_m. * indicates a significant difference ($P \leq 0.05$) from baseline for all measures depicted across graphs (A–C). † indicates that AUC was significantly different ($P \leq 0.05$) from baseline but not the other measures depicted in (A). + indicates that tau, T₂₀, and T₈₀, but not slope, were significantly different ($P \leq 0.05$) from baseline in (B).

Table I

Correlations between Demon Voltammetry measures and Michaelis-Menten parameters

Measure	Baseline $V_{\max}$	Apparent K_m	$[DA_p]$
tau	-0.4414	*** 0.9989	* -0.7311
FWHH	-0.5233	*** 0.9976	* -0.7090
Half-life	-0.4407	*** 0.9989	* -0.7307
T₂₀	-0.5200	*** 0.9988	* -0.7324
T₈₀	-0.5420	*** 0.9675	-0.7002
Slope	* 0.6718	*** 0.9901	* -0.7991
Peak height	0.2657	-0.55772	*** 0.9535
[DA_c]	0.5262	-0.56369	*** 0.9613
AUC	0.01692	0.6706	-0.1373

Tau, full width at half height (FWHH), half-life, T₂₀, and T₈₀, were positively correlated with K_m , suggesting that these are accurate measures of DA uptake. Slope was also correlated with K_m but was unable to detect uptake changes at the lowest cocaine concentrations (see figure 5). Peak Height and [DA_c] were positively correlated with [DA_p] suggesting that these are accurate measures of release. Area under the curve (AUC) was not significantly correlated with either K_m or [DA_p].

* (P ≤ 0.05);

*** (P ≤ 0.0001).