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Dopamine Detection with Fast-Scan Cyclic Voltammetry Used with Analog Background Subtraction

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Fast-scan cyclic voltammetry has been used in a variety of applications and has been shown to be especially useful to monitor chemical fluctuations of neurotransmitters such as dopamine within the mammalian brain. A major limitation of this procedure, however, is the large amplitude of the background current relative to the currents for the solution species of interest. Furthermore, the background tends to drift, and this drift limits the use of digital background subtraction techniques to intervals less than 90 s before distortion of dopamine signals occurs. To minimize the impact of the background, a procedure termed analog background subtraction is reported here. The background is recorded, and its inverse is played back to the current transducer during data acquisition so that it cancels the background in subsequent scans. Background drift still occurs and is recorded, but its magnitude is small compared to the original background. This approach has two advantages. First it allows the use of higher gains in the current transducer, minimizing quantization noise. Second, because the background amplitude is greatly reduced, principal component regression could be used to separate the contributions from drift, dopamine, and pH when appropriate calibrations were performed. We demonstrate the use of this approach with several applications. First, transient dopamine fluctuations were monitored for 15 min in a flowing injection apparatus. Second, evoked release of dopamine was monitored for a similar period in the brain of an anesthetized rat. Third, dopamine was monitored in the brain of freely moving rats over a 30 min interval. By analyzing the fluctuations in each resolved component, we were able to show that cocaine causes significant fluctuations in dopamine concentration in the brain while those for the background and pH remain unchanged from their predrug value.

Fast-scan cyclic voltammetry (FSCV) is useful to follow rapid chemical changes.[1] It has been used in several applications including evaluating rapid electron-transfer kinetics,[2–4] monitoring biological microenvironments,[5,6] and chromatographic detection.[7,8] FSCV has been particularly useful for monitoring fluctuations of neurotransmitter concentrations in vitro[9,10] and in vivo.[11–13] In all of these applications, microelectrodes are used because small currents are generated even at high scan rates, which minimizes distortion of the cyclic voltammograms by ohmic drop. Except for nanosecond time-domain experiments,[14,15] the instrumentation can be simple, consisting of a waveform generator and a current transducer.

A shortcoming of FSCV is that the background current greatly exceeds the faradaic current from redox reactions of solution species. The background is composed of current required to charge the double layer and current arising from redox reactions of surface-attached functional groups. The magnitude of both of these sources is directly proportional to scan rate, whereas the current arising from a diffusion-controlled electrochemical reaction is proportional to the square root of the scan rate.[16] Thus, optimum ratios of the faradaic to background current are not achieved with FSCV. Several methods have been used to minimize the contribution of the background. Early experiments used two separate, but identical, electrochemical cells, one cell containing the background solution and the other containing the sample in the background solution, and the signals were subtracted by analog means.[17] Data digitizers simplified this procedure by allowing subtraction of recordings from the same electrochemical cell of the background solution and an identical solution containing the electroactive

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species.[18] This digital subtraction procedure is the one used by most investigators in this field. More recently, Yoo and Park[19] described a method to subtract background signals electronically during a fast potential sweep using a customized waveform. The applied waveform was selected so that it generated a flat background current. The current was then converted into a voltage and subtracted with a voltage limiter. This enabled amplification of the background-subtracted current yielding an improved dynamic range, minimizing quantization noise, and improving detection limits for the catecholamine neurotransmitter dopamine. However, information was only obtained on the anodic sweep and the cathodic current was discarded.

In this work we present an improved background subtraction approach. First, a background cyclic voltammogram is recorded. Next, it is played from a digital-to-analog converter back into the current-to-voltage transducer during collection of subsequent voltammograms. Because the analog playback is an inverted version of the background, its addition at the transducer input cancels the background in a manner that is analogous to that used in some types of noise cancellation headphones. This procedure has two advantages for monitoring low concentrations. First, the gain of the analog-to-digital converter can be increased, diminishing quantization noise. Second, elimination of the majority of the background current allows the drift of the electrode response, which has a much smaller amplitude, to be recorded and accounted for with principal component analysis (PCA). Background currents at carbon-fiber microelectrodes during FSCV drift over a minute time scale,[20,21] limiting the time over which digital background subtraction is useful. Even after a conditioning period of several minutes, background drift is sufficient that subtraction fails to yield a recognizable voltammetric signal for voltammograms separated by more than 90 s.[22] With the subtraction of the signal before digitization, and extraction of the drift components with PCA, undistorted signals could be monitored for more than 30 min, a 20-fold increase over existing methods. When used with carbon-fiber microelectrodes implanted in the rat brain, this procedure could be used to evaluate the effects of the drug of abuse, cocaine, on the extracellular levels of dopamine.

EXPERIMENTAL SECTION

Chemicals. All chemicals for flow injection analysis were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Solutions were prepared using doubly distilled water (Megapure system, Corning, NY). Flow injection analysis was done in a Tris buffer solution, pH 7.4, containing 15 mM Tris, 140 mM NaCl, 3.25 mM KCl, 1.2 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 1.2 mM $MgCl_2$, and 2.0 mM Na_2SO_4. This buffer mimics the ionic environment present in cerebral spinal fluid. Stock solutions of dopamine were prepared in 0.1 M $HClO_4$, and were diluted to the desired concentration with Tris buffer on the day of use.

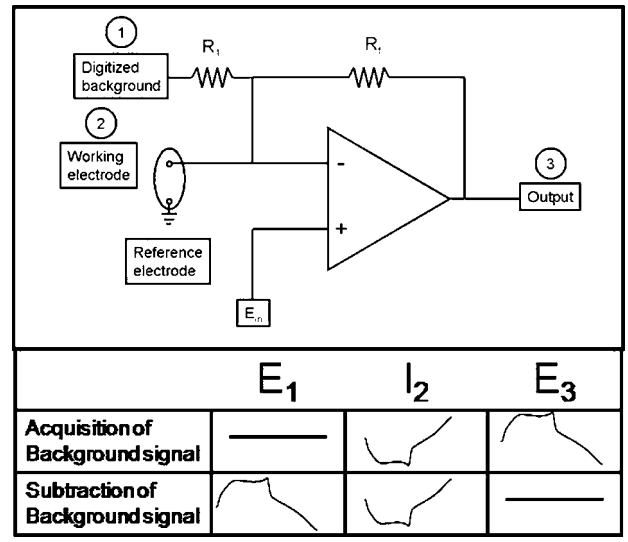

Figure 1. Electronic setup for analog background subtraction. The background is subtracted in a two-step process. First step: acquisition of background signal. The waveform is applied to E_{in} while the other input (E_1) is disconnected and the current at the working electrode (I_2) is transduced to a voltage (E_3). Second step: subtraction of background signal. The triangular waveform is applied to E_{in} while the background signal which was recorded during the first step (E_3) is applied to input (E_1). The current obtained at the working electrode (I_2) is canceled out at the summing point resulting in a flat signal at the output (E_3).

Data Acquisition and Analog Background Subtraction. Cyclic voltammograms were acquired and analyzed using locally constructed hardware and software written in LabVIEW (National Instruments, Austin, TX) that has been described previously.[23,24] Data was acquired with a commercial interface (PCI-6052, 16 bit, National instruments, Austin TX) with a personal home computer. For fast-scan cyclic voltammetric measurements, the rest potential was held at −0.4 V versus Ag/AgCl. Triangular excursions of the working electrode potential were made to 1.3 V from −0.4 V at a scan rate of 400 V/s and repeated at a frequency of 10 Hz. The measurements were conducted inside a grounded Faraday cage to minimize electrical noise.

Figure 1 shows a simplified schematic of the current-to-voltage transducer used in this work. The triangular waveform (E_{in}) is applied to the noninverting input of the operational amplifier. The working electrode is connected to the inverting input, and the reference electrode is held at ground. This arrangement is advantageous because it can be used with multiple electronic devices (ion-selective electrodes, single-unit recording electrodes, etc.) while all are biased against the same reference electrode. The inverting input of the amplifier is also connected to a resistor (R_1, impedance equal to R_f, the feedback resistor) into which the background signal, collected and digitized from previous scans, E_1, can be input. The output of the amplifier, E_3, is given by

$$E_3 = E_{in} - (E_1/R_1 + I)R_f \quad (1)$$

where I is the working electrode current.

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The background correction process consists of two steps. First, the background signal is recorded while the digitized background signal input is disconnected ($E_1 = 0$ V). Then, the digitized signal measured is solely due to the background signal and the applied voltage:

$$E_3 = E_{in} - (IR_f) \quad (2)$$

The negative sign next to the second term on the right-hand side is due to the inverting property of the operational amplifier. Next this signal is applied as E_1 in synchrony with E_{in}, and the resultant output from the operational amplifier has both the applied voltage and background signal removed yielding a flat line. If the background changes from the value stored in E_1, for example, due to drift, the output will deviate from a flat line.

The triangular waveform and the digitized background must be applied synchronously to provide appropriate removal of the background. However, digital-to-analog converters typically have a microsecond time lag when switching between two output channels. This time delay can cause spurious results in the form of sharp spikes, particularly at the beginning and switching potentials where the rate of background change is the greatest. To account for the time lag the digitized background signal was time-shifted with software until the current spikes were minimized.

Flow Injection Analysis. In some experiments a flow injection analysis apparatus was used to expose the electrode to both background and sample. The electrode was positioned at the outlet of a six-port rotary valve. A loop injector mounted on an actuator (Rheodyne model 5041 valve and 5701 actuator) and controlled by a 12 V dc solenoid valve kit (Rheodyne, Rohnert Park, CA) was used to introduce a bolus of analyte to the electrode. The flow rate was controlled by a syringe infusion pump (2 mL/min, Harvard Apparatus model 22, Holliston, MA).

Electrode Preparation. The microelectrodes were fabricated as previously described[25] with T-650 carbon fibers (Thornel, Amoco Corp., Greenville, SC, 5 μm diameter). A single fiber was aspirated into a glass capillary and pulled on a micropipette puller (Narashige, Tokyo, Japan). The carbon fiber was cut so that approximately 50 μm protruded from the glass seal. The microelectrodes were backfilled with electrolyte solution (4 M potassium acetate, 150 mM potassium chloride), and wires were inserted into the capillary for electrical contact. Before use, electrodes were soaked in isopropyl alcohol purified with Norit A activated carbon (ICN, Costa Mesa, CA) for at least 20 min.[26] Before recording, electrodes were cycled at a repetition rate of 60 Hz for 15 min from −0.4 to 1.3 V for 15 min followed by 30 min of cycling at 10 Hz with the same scan parameters. Electrochemical activation was conducted in Tris buffer solution or in vivo in the indicated brain region where the measurement was conducted.

For all measurements Ag/AgCl reference electrodes were used.

Quantization Noise Analysis. To evaluate quantization noise, a triangular waveform at 400 V/s from −0.4 to 1.3 V was applied to an equivalent electrochemical cell consisting of a resistor (1 MΩ) and capacitor (400 pF) in series. The values for resistance and capacitance mimic those of the microelectrodes used in this work. After the background signal was removed, the input range of the analog-to-digital converter was increased and the noise level in three different systems was measured as a standard deviation. The noise values were then normalized to the original noise obtained with the lowest gain.

In Vivo Measurements in Anesthetized Rats. Male Sprague−Dawley rats (225−350 g; Charles River, Wilmington, MA) were anesthetized with urethane (1.5 g/kg, ip) and placed in a stereotaxic frame (Kopf, Tujunga, CA). A heating pad (Harvard Apparatus, Holliston, MA) maintained body temperature at 37 °C. Holes were drilled in the skull for the working, reference, and stimulating electrodes using coordinates (relative to bregma) from the brain atlas of Paxinos and Watson.[27] The carbon-fiber microelectrode was placed in the striatum at coordinates that are given relative to bregma: anterior/posterior (AP) +1.2 mm, medial/lateral (ML) +2.0 mm, and dorsal/ventral (DV) −4.5 mm. A stimulating electrode was placed in the vicinity of the dopamine cell bodies in the substantia nigra: AP −5.2 mm, ML +1.0 mm, and DV −7.5 mm. A Ag/AgCl reference electrode was inserted in the opposite hemisphere. The DV position of both the stimulating and working electrodes was adjusted to find sites that supported maximal dopamine release. The stimulation consisted of 40 biphasic pulses delivered at 60 Hz frequency (±125 μA, 2 ms per phase).

In Vivo Measurements in Freely Moving Rats. Male Sprague−Dawley rats implanted with a jugular vein catheter (300−450 g, Charles River Laboratories, Wilmington, MA) were housed individually on a 12:12 h light cycle with ad libitum access to food and water. Procedures were as described previously.[28] Surgical implantation of electrodes was done at least 2 days prior to experiments. Rats were anesthetized with ketamine hydrochloride (100 mg/kg ip) and xylazine hydrochloride (20 mg/kg im) and placed in a stereotaxic frame for implantation surgery. A Ag/AgCl reference electrode was chronically implanted in the forebrain and secured in place with dental cement. A guide cannula (Bioanalytical Systems, West Lafayette, IN) was positioned above the contralateral nucleus accumbens (1.7 mm AP, 0.8 mm ML, −2.5 mm DV relative to bregma). A bipolar stimulating electrode was lowered toward the VTA (−5.4 mm AP, 1.0 mm ML, −8.0 mm DV). All items were affixed to the skull with machine screws and cranioplastic cement and secured in place.

Following surgery, rats were allowed to recover and received acetaminophen administered orally every 12 h. During measurements the rats were in an operant box containing an infusion pump for drug delivery through the jugular catheter. The electrodes were connected to a lightweight amplifier that was connected to a swivel that linked to the remaining electronics. Rats were acclimated to the recording chamber for 1 h. Once baseline voltammetric recordings were made, the rats were given a 6 s infusion of either saline or cocaine (3 mg/kg).

Histological Verification of Electrode Placement. The tip of the carbon fiber is too small (5 μm diameter) to be seen histologically. Therefore, following experiments each rat was deeply anesthetized with urethane and a constant current was

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applied to an electrode to lesion the area. The animal was then perfused transcardially with phosphate-buffered saline, followed by a 4% formalin solution. The brain was extracted and stored in a 4% formalin solution. The fixed tissue was frozen and sliced on a cryostat in 40 μm sections. Placement of the electrode tip in the target regions was verified by microscopic inspection of the section.

Data Analysis. Data analysis was performed with local software written in LabVIEW (National Instruments and MATLAB (MathWorks, Natick, MA)). Principal component regression (PCR) was conducted as described previously.[29] In this procedure, a calibration set of cyclic voltammograms obtained at different concentrations was evaluated to find the relevant eigenvectors (principal components) that described the data using Malinowski's F-test ($p = 0.05$).[30,31] Only a few eigenvectors were needed, and thus the dimensionality of the 1000 point cyclic voltammograms was reduced considerably. The principal eigenvector captured the maximum variation in the calibration set, the second eigenvector captured variance not described by the first eigenvector, and so on.

For experiments in vitro and in anesthetized animals, calibration sets for dopamine and pH changes were constructed using the flow injection apparatus with concentrations ranging from 0.5 to 2 μM and pH excursions of 0.2 pH units, respectively. For freely moving animals, the calibration sets for dopamine were constructed from electrically evoked stimulations with varying number of stimulation pulses and converted to concentration based on standards obtained in the flow injection apparatus.[22] Calibrations for pH changes during in vivo experiments were also obtained from pH shifts following electrical stimulation.[32] The calibration set for background drift was constructed from the cyclic voltammograms obtained for the background drift 10 min before and for 10 min after each of the recordings. These data were used to compute a regression matrix that provides the scaling factors for the retained principal components.

The cyclic voltammograms obtained during the experiments were resolved into components at their respective concentrations using the eigenvectors and the regression matrix. In other words, the following equation was evaluated by regression:

$$\mathbf{C} = \mathbf{F_{cal}A} \tag{3}$$

where **C** is a matrix of concentration values of l components, **A** is a matrix of the currents at each potential, and $\mathbf{F_{cal}}$ is a regression matrix that relates the projection of the currents on their relevant principal components to concentration values.[33] In this study, **A** was an $n \times m$ matrix containing cyclic voltammograms measured at n potentials and m time points, **C** was an $l \times m$ concentration matrix of l components, and $\mathbf{F_{cal}}$ was an $l \times n$ matrix calculated using the in vivo calibration set of dopamine, pH, and background change.

This procedure results in separate traces for each chemical species used in the calibration set and an error signal, the residual. The results of the analysis were accepted as long as the residual was smaller than the level set by the 95% confidence limit. Noise and other interferences that are represented by the discarded eigenvectors constitute the residual.

Quantitative Evaluation of the Temporal Changes of Each Component. Although the PCR procedure described above provides concentrations of the relevant species, it is also of interest to examine fluctuations in the signal for each species. To do this, the contribution to the whole cyclic voltammogram of each species at each time point needs to be resolved. The cyclic voltammetric currents can be regenerated if concentration values are known according to

$$\mathbf{A} = \mathbf{KC} \tag{4}$$

where **K** is an $n \times l$ matrix containing pure component data with calibration factors at each measurement value.[33] In this study, eq 4 was used to regenerate the cyclic voltammetric contribution of each analyte and the background as explained below. **K** contained pure component cyclic voltammograms with a calibration factor at each potential, relating concentration units into units of current. The concentration values used in eq 4 were obtained from PCR using eq 3.

By substituting eq 3 into eq 4 it can be seen that **K** is the inverse of $\mathbf{F_{cal}}$. However, since $\mathbf{F_{cal}}$ is not a square matrix, its inverse cannot be calculated. Instead, the transpose of $\mathbf{F_{cal}}$ ($\mathbf{F_{cal}^T}$) was used to determine the pseudoinverse using singular value decomposition according to

$$\mathbf{K} = \mathbf{VS^{-1}U^T} \tag{5}$$

where **V** was a matrix containing the principal components of the row space of $\mathbf{F_{cal}^T}$, **U** was a matrix containing the principal components of the column space of $\mathbf{F_{cal}^T}$, and $\mathbf{S^{-1}}$ is the inverse of a diagonal matrix that contained the singular values that quantify the importance of each principal component contained in **U** and **V**.[34] $\mathbf{F_{cal}}$ used in eq 5 is from the PCR algorithm in eq 3. The transpose of $\mathbf{F_{cal}}$ was used in order to ensure **K** was nonsingular.

Once **K** was obtained, cyclic voltammograms at m time points for the jth component ($\mathbf{A}_j$) were generated according to

$$\mathbf{A}_j = \mathbf{k}_j\mathbf{c}_j \tag{6}$$

where $\mathbf{k}_j$ and $\mathbf{c}_j$ are the single-species cyclic voltammograms containing calibration factors and concentration traces for the jth component, respectively. With eq 6 it is possible to separate the measured cyclic voltammograms into the contributions from each species. The amount of charge in each cyclic voltammogram due to each species was determined as Q_j by summing the absolute value of the current at each potential:

$$Q_j = \sum |i_j|\Delta t \tag{7}$$

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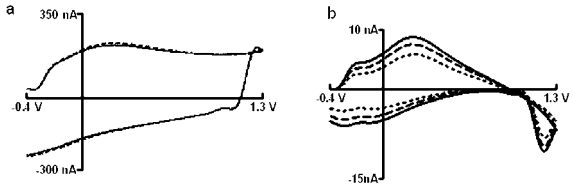

Figure 2. Background changes measured at a carbon-fiber microelectrode. Panel a: background signal obtained after conditioning with triangular cycling from −0.4 to 1.3 V vs Ag/AgCl repeated at a frequency of 60 Hz for 15 min following by cycling at a frequency of 10 Hz for 30 min (solid line) or for 60 min (dashed line). Panel b: cyclic voltammograms obtained for changes in background current after analog background subtraction. The background current was subtracted after conditioning from −0.4 to 1.3 V vs Ag/AgCl for 15 min repeated at 60 Hz and 30 min at 10 Hz. The cyclic voltammograms were obtained 10 (dotted line), 20 (dashed line), and 30 min (solid line) after the analog background subtraction.

where i_j is the current at each potential for a single component and Δt is time between data points.[16] The standard deviation of the charge was determined over 3 min intervals before, during, and after cocaine administration. Slow changes for each component were minimized by fitting a quadratic equation to the data and subtracting this curve from the experimental data. In this way, high-frequency fluctuations can be examined. Differences in the standard deviations were evaluated with a one-way ANOVA using MATLAB.

RESULTS AND DISCUSSION

Noise Reduction. Because of the large amplitude of the background current, the current-to-voltage transducer normally has to be used with a relatively low gain so that the signal remains on scale. For example, the background shown in Figure 2a requires an input range of ±350 nA. When recorded on this scale, the drift appears small; however, it is larger than the measured signals. For example, the sensitivity for dopamine is ~7 nA/μM, and biologically meaningful concentrations of dopamine monitored during behavior are ~50 nM,[35] yielding 350 pA excursions. When the uncorrected background signal is digitized with a 16 bit analog-to-digital convertor, the minimum resolvable current will be (700/2^{16}) nA or 10.7 pA. Because each value of the analog-to-digital converter is separated by this magnitude, this results in quantization noise. A reduction in the background amplitude allows the gain to be increased, lowering the quantization noise. The theoretical improvement is given by the dashed line in Figure 3 that is taken from the manufacturer's specifications of the interface board.[36]

The background is eliminated by the analog background subtraction procedure. Thus, the gain can be increased considerably and the current can be recorded with a ±15 nA range. This 24-fold increase in gain reduces the quantization noise proportionately. As shown in Figure 3, the measured noise levels off at around 35% of the original noise at the highest gain settings. At these settings other noise sources predominate over quantization noise.

Drift of the Background Current. The analog background subtraction procedure employs a background signal that was

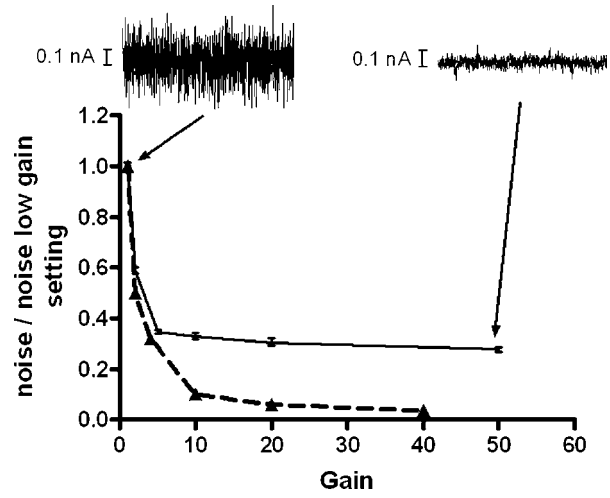

Figure 3. Noise characterization. Plot of noise measured at different input ranges of the analog-to-digital converter. The data is normalized for the noise level measured at the highest input range. Solid line: noise level measured in the system. Dashed line: quantization noise of analog-to-digital converter according to specifications (ref 36). Insets show cyclic voltammograms recorded to determine the noise levels at the highest input range and the lowest input range.

captured in a single time interval. Thus, background drift still contributes to the recorded signal. Background drift at carbon-fiber electrodes has been noted before.[20] The initial changes in the background current are large, but become smaller as cycling is continued. To minimize the effect of drift, the carbon fiber is typically cycled with the triangular wave repetitively before measurements are made to accelerate the transition to a more stable background. With the waveform used in this work, the drift was in the form of an increasing background current, which we attribute to a continual oxidation of the carbon surface with each triangular sweep. The rate of drift sets the time limit over which changes can be monitored with digital subtraction and FSCV. In measurements of dopamine in vivo, we have restricted measurement times to less than 90 s because of the distortion of background-subtracted cyclic voltammetric recordings by the drift.[22]

In this work, electrodes were conditioned by cycling for 15 min with a triangular waveform from −0.4 to 1.3 V and back versus Ag/AgCl at a repetition rate of 60 Hz. Further conditioning was then done at 10 Hz for the next 30 min. Then, the analog subtraction procedure was initiated and recordings commenced. Signals recorded at three intervals after initiation of analog background subtraction are shown in Figure 2b. Their amplitude is much smaller than the whole background, but their increase with time is clearly evident. These cyclic voltammograms reveal that the magnitude of the drift is potential dependent. An oxidation peak around 0.2 V versus Ag/AgCl emerges that appears surface associated. In addition, there is a current spike at the anodic switching potential that is distorted by the filtering employed. Thus, even after 45 min of preconditioning, the current at the carbon-fiber electrode has not reached an equilibrium condition. In prior work, compensation for background drift has been achieved by subtracting current at a potential where the analyte is not electroactive from the current at a potential where the

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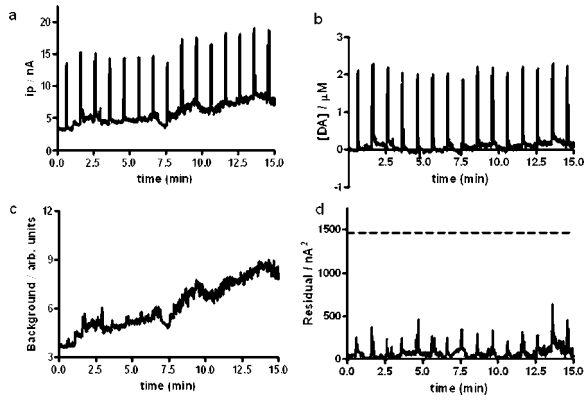

Figure 4. Consecutive injections of dopamine in a flow injection apparatus monitored with FSCV. Dopamine (2 μM) was injected for 5 s every min. (a) Current monitored in successive voltammograms at the potential where dopamine is oxidized. (b) Temporal response of dopamine extracted from the cyclic voltammograms using principal component regression (PCR). (c) The background drift extracted from the same data by PCR. (d) Residual obtained for these data following PCR. The dashed line indicates the 95% confidence interval.

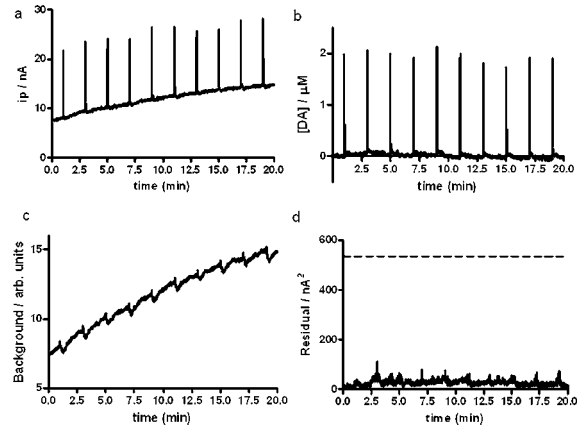

Figure 5. Dopamine release measured in vivo during electrical stimulation. A carbon-fiber microelectrode was placed in the caudate putamen, and the stimulating electrode was placed in the substantia nigra of an anesthetized rat. The electrical stimulation (40 biphasic pulses at 60 Hz, $\pm$125 μA, 2 ms per phase) was repeated every 2 min. (a) Current recorded in successive cyclic voltammograms at the potential where dopamine is oxidized. (b) Dopamine concentration extracted from the set of cyclic voltammograms with principal component regression (PCR). (c) Background drift extracted from the same set. (d) Residual remains below the 95% confidence interval (dashed line) during the entire recording interval.

analyte is electroactive.[37] The present results show that this is an inappropriate method because of the potential dependence of the background changes.

PCR has been used to remove the background from cyclic voltammograms.[38] Previously, this approach did not improve our results because the background signal dwarfed the faradaic signals.[29] However, with the analog background subtraction the only component of the background that is evident is the drift whose amplitude is much nearer that of the recorded dopamine signals. Since the drift has a potential dependent shape, a calibration set for this component to be used in PCR can be obtained from cyclic voltammograms recorded before and after an interval when data of interest is collected. This approach was evaluated in a flow injection analysis experiment with the electrode cycling at 10 Hz for 15 min. At 1 min intervals, 2 μM dopamine was injected for 5 s into the system. Figure 4a shows the current from successive voltammograms recorded at the dopamine oxidation potential. Upward drift is clearly apparent as are the superimposed rapid changes arising from the dopamine injections. This set of cyclic voltammograms was evaluated by PCR with a calibration set of cyclic voltammograms recorded for dopamine and another set recorded of the drift. This procedure separates the cyclic voltammograms into two different components (Figure 4, parts b and c) and also generates a residual (Figure 4d). The baseline for the dopamine channel is essentially flat (Figure 4b), while the drift is assigned by the regression to the background (Figure 4c). The dashed line in Figure 4d represents the 95% confidence limit, and the residual remains well below that limit. Indeed, we found that dopamine could be monitored in this way for longer than 1 h before the residual value crossed the 95% confidence interval. The residual (Figure 4d) shows a spike at the beginning of every dopamine injection. This arises from a slight change in shape of the dopamine signal as it adsorbs to the electrode.[29] However, this error is never higher than 5% of the total signal measured throughout the whole data collection.

Monitoring Dopamine Concentrations in the Brain of Anesthetized Rats. To test the method in vivo, a carbon-fiber microelectrode was implanted in the caudate putamen of an anesthetized rat, and stimulations of cell bodies of dopaminergic neurons were repeated every 2 min. The results were quite similar to those obtained in the flow injection analysis system. When the current was monitored in successive cyclic voltammograms that had been analog background subtracted, there was an increase in oxidation current during every stimulation, but these changes were superimposed on an upward drift. With suitable calibration sets, PCR could resolve the background drift from the dopamine concentration (Figure 5, parts a and b), and the residual remained low over the 20 min interval. In contrast, with the traditional method of digital subtraction of the background, signals could be continuously monitored for only 90 s because the residual exceeded the 95% confidence limit.

Monitoring Dopamine in Ambulatory Rats. As a second in vivo test of the analog subtraction procedure, we measured changes in the brain following an intravenous dose of cocaine (3 mg/kg) administered to ambulatory rats, inexperienced to cocaine, and compared the responses to those following saline. Cocaine is a dopamine uptake blocker and increases extracellular dopamine concentration in the striatum.[22] While early microdialysis studies suggested that dopamine levels were elevated for hours,[39,40] recent results have shown that the dopamine concentrations in the nucleus accumbens are elevated for around 20 min[41] and closely follow the time course of elevated cocaine levels in the brain.[42] In prior cyclic voltammetric recordings using digital background correction we were only able to capture the initial

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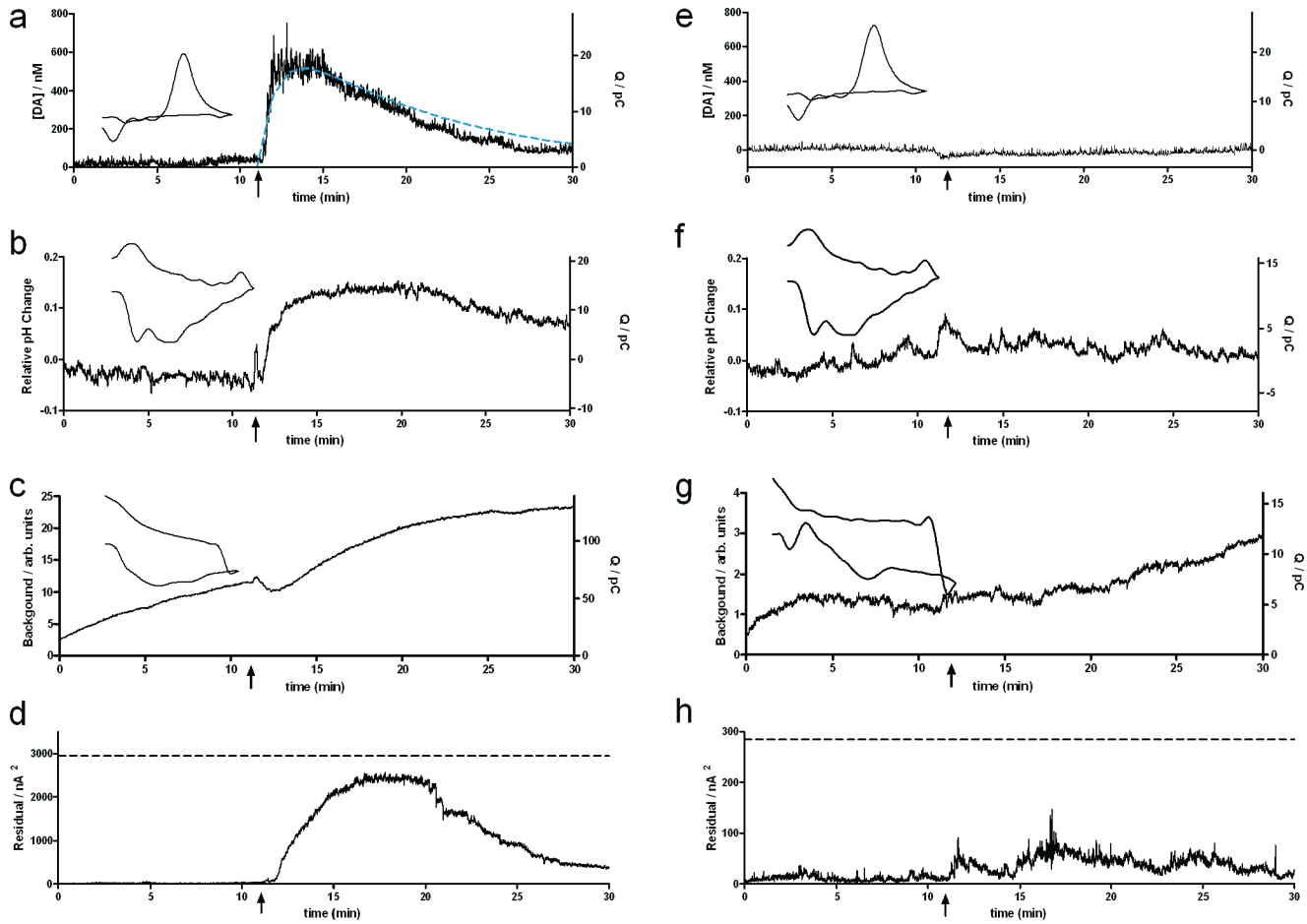

Figure 6. In vivo responses to intravenous injections of cocaine (3 mg/kg) and saline. Cyclic voltammograms were recorded in the nucleus accumbens of an ambulatory rat. Analog background subtraction was applied at $t = 0$. Left panels show traces extracted with principal component regression (PCR) for cocaine injection at approximately 11 min (indicated by arrow). Right panels show traces extracted with PCR for saline injection at approximately 11 min (indicated by arrow). Insets show a representative cyclic voltammogram that was used for the calibration set. (a and e) Dopamine concentrations: in panel a the concentration trace for dopamine is overlaid with concentration trace for cocaine in the brain (dashed line) obtained by pharmacokinetic modeling (ref 42). (b and f) pH change extracted by PCR. (c and g) Background drift extracted by PCR. For panels a−c and e−g, the right axis shows the Q_j values. (d and h) Residual remains within the 95% confidence interval indicated by the dashed line.

rise of dopamine to a maximum before the 90 s analysis window dictated by the drift precluded further analysis.[22]

Cyclic voltammograms were collected for 30 min after analog background subtraction with cocaine administered at 11 min into the recording time. PCR was conducted on these data similarly to the flow injection and stimulation experiments, except that the responses to pH changes were also included in the calibration set. The resulting concentration trace for dopamine (Figure 6a) shows a long-term concentration change occurring within 1 min after the cocaine infusion. The observed time course matches well recent microdialysis recordings[41] as well as with the pharmacokinetic model[42] for cocaine concentrations (dashed line, Figure 6a). Also time-locked with cocaine administration, the pH of the brain region changes to more basic values and then returns to near starting levels. When viewed on a 90 s time scale, much smaller shifts in an acidic direction were observed.[22] Shifts in extracellular pH are highly complex; they reflect the balance of local metabolism and blood flow.[32] The residual increases after the cocaine injection, although it does not cross the 95% confidence level. The cyclic voltammogram of the background shift differs from that in vitro (Figure 2), but it increases throughout the experiment with a small dip at the time of cocaine administration. In addition, the residual increases following cocaine. These changes may indicate that other electroactive chemical substances, not included in the training set, change in response to cocaine. Alternatively, the increased dopamine concentration may have altered the background, causing the shift.

Responses following saline injections were also examined. As apparent in Figure 6 (right panels) no significant change in dopamine concentration or pH shifts could be observed following saline injection. However, background drift was still observed and could be accounted for. The traces shown in Figure 6 were collected at a 10 Hz sampling rate. Accordingly, all traces presented here have a resolution of 100 ms, and the time traces of Figure 6 consist of a total of 18 000 measurements. For microdialysis the fastest sampling rates for dopamine have reported to be on the order of 1−2 min[41,43,44] resulting in around 20 sampling points per 30 min trace.

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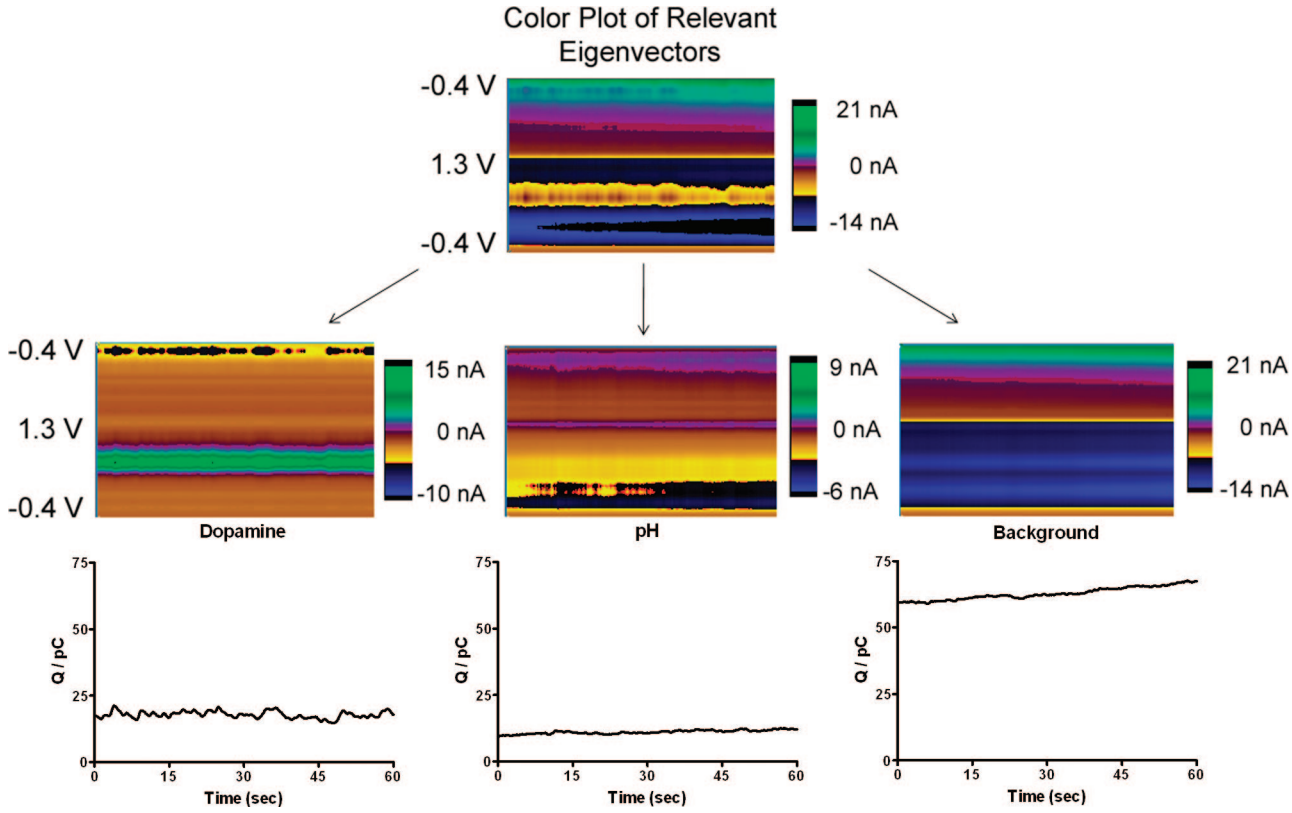

Figure 7. Construction of single-component color plots and traces. Data recorded in the nucleus accumbens of an ambulatory rat 2 min after cocaine administration. In the color representations the current is shown in false color with time on the *x*-axis, potential on the *y*-axis. Top panel: color representation of the recorded data after discarding the nonretained principal components. Middle panels: color representations of single-component cyclic voltammetric data. Bottom panels: Q_j values for each of the species.

Both saline and cocaine experiments were replicated in three animals. All showed similar traces to those shown in Figure 6. However, the amplitude showed differences between each experiment. The maximal change in dopamine concentration after cocaine administration ranged from 100 to 600 nM, values that are higher than reported from microdialysis studies. Because of the relatively large size of microdialysis probes, a trauma layer forms around the probe[45,46] which might explain differences between voltammetric recordings and microdialysis recordings.[47,48] In addition, the microelectrode samples a much smaller area than the microdialysis probe, which reveals the microheterogeneity of the brain.

Investigation of Chemical Fluctuations. The dopamine trace exhibits an increase in fluctuations immediately after cocaine administration (Figure 6a), a change that is not apparent in the pH, background, or residual responses. These cocaine-induced concentration fluctuations have been reported before and appear to arise because of cocaine's unique actions on dopamine neurons.[22,49] We use the term fluctuations to indicate that the dopamine trace contains unique information about the effects of cocaine on the neurotransmitter release process. Alternatively, the increased fluctuations would be termed noise if they do not contain useful information.[50]

To quantify the fluctuations for each species, we reconstructed the charge contributed by each species (dopamine, pH, and background) to each cyclic voltammogram for the data from three animals using the **K** matrix approach. Charge was chosen because each species contributes to the cyclic voltammogram at all potentials, even though the value at some potentials may be zero. An example is shown for a 1 min segment recorded at the time of the maximum dopamine change following cocaine (Figure 7, minutes 12−13 from Figure 6a). The color plot in the top panel of Figure 7 shows the cyclic voltammograms after the unused eigenvectors have been discarded. This can be broken down into a set of distinct cyclic voltammograms for each species that are shown in the form of color plots (Figure 7, lower panels). These plots are a representation of the $\mathbf{A}_j$ matrices of the current values. The Q_j of each reconstructed cyclic voltammogram was determined, and the standard deviation of these values over a 3 min interval was determined.

The data for dopamine, pH, and the background signal were evaluated in this way before cocaine administration, immediately after cocaine infusion, and 20 min after cocaine administration, as shown in Figure 8. Before cocaine administration the standard deviation for the dopamine, pH, and background signals were similar when evaluated in this way. Consistent with this, examination of the cyclic voltammetric data revealed that only one of the recordings from the three animals showed naturally occurring

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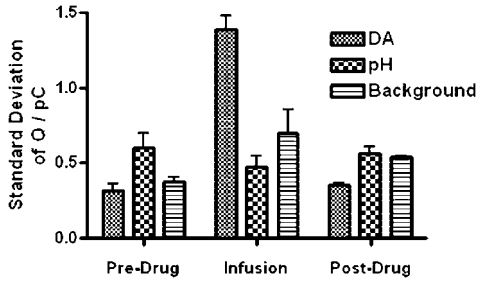

Figure 8. Standard deviation of Q_j with cocaine. Standard deviations of the Q_j values are shown for dopamine (DA), pH change, and background drift. Data were collected for 3 min predrug, for 3 min immediately after cocaine infusion, and for 3 min 20 min after cocaine administration ($n = 3$ animals; error bars: standard error of the mean).

dopamine fluctuations before cocaine. After cocaine administration the fluctuations significantly ($p < 0.001$) increased only in the dopamine signal. Thus, even though both a pH change and a background shift occurred following cocaine administration, an increase in their fluctuations did not occur. Twenty minutes after cocaine administration, the fluctuations in all three signals were not statistically ($p > 0.05$) different to that before cocaine. This analysis reveals that the short-term effect of cocaine is to cause increased dopamine fluctuations, and this effect is not seen in the other signals from the same recordings. This confirms previous qualitative observations[22] and lays the groundwork for more sophisticated analysis of cocaine evoked changes in dopamine neurotransmission. Neurotransmitter release is expected to be stochastic, dynamic, and fluctuating because it occurs as a result of actions potentials. In contrast, pH values are regulated by the balance between metabolism and blood flow, processes that occur on a slower time scale.

CONCLUSIONS

In this study we have demonstrated an analog method to subtract the background currents that occur during cyclic voltammetry at high scan rates. This subtraction enables the use of higher gains before the analog-to-digital conversion, lowering quantization noise. Furthermore, accounting for background changes with PCR allows FSCV measurements to be made for longer times. This enables dopamine and pH concentrations to be monitored over time windows that previously were accessible only to microdialysis experiments but with a time resolution that is 600 times greater. With this high time resolution we were able to monitor short-term fluctuations in dopamine concentrations. In the results of this study, these are clearly superimposed on the rise in dopamine following cocaine infusion. Further characterization is required to understand the origin of these chemical fluctuations.

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