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Resolving Neurotransmitters Detected by Fast-Scan Cyclic Voltammetry

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Carbon-fiber microelectrodes are frequently used as chemical sensors in biological preparations. In this work, we evaluated the ability of cyclic voltammograms recorded at fast-scan rates to resolve neurochemicals when analyzed by principal component regression. A calibration set of 30 cyclic voltammograms was constructed from 9 different substances at a variety of concentrations. The set was reduced by principal component analysis, and it was found that 99.5% of the variance in the data could be captured with five principal components. This set was used to evaluate cyclic voltammograms obtained with one or two compounds present in solution. In most cases, satisfactory predictions of the identity and concentration of analytes were obtained. Chemical dynamics were also resolved from a set of fast-scan cyclic voltammograms obtained with the electrode implanted in a region of a brain slice that contains dopaminergic terminals. Following stimulation, principal component regression of the data resolved the changes in dopamine and pH that were evoked. In a second test of the method, vesicular release was measured from adrenal medullary cells and the data were evaluated with a calibration set composed of epinephrine and norepinephrine. Cells that secreted one or the other were identified. Overall, the results show that principal component regression with appropriate calibration data allows resolution of substances that give overlapping cyclic voltammograms.

Cyclic voltammetry is infrequently used for chemical analysis in complex mixtures because it provides only limited chemical resolution. A substance's $E°$ is insufficiently unique for molecular identification. In addition, to distinguish between chemical species that are involved in diffusion-controlled, one-electron electrolysis processes, their $E°$'s need to differ by at least 0.118 V.[1] In aqueous solution, the potential limits are less that 2.0 V and so, even under optimum conditions, less than 15 compounds could be resolved. Thus, compared to separation-based methods, the molecular resolving power of cyclic voltammetry is very poor. Despite these limitations, cyclic voltammetry has found considerable use in the measurement of neurotransmitters and related substances in physiological preparations.[2−15] This is because voltammetric measurements allow the rapid concentration dynamics of redox-active species to be followed in situ. No other method offers this quantitative and qualitative information concerning endogenous substances on a millisecond time scale. The most common procedure involves the use of carbon-fiber microelectrodes with fast-scan cyclic voltammetry with scan rates exceeding 100 V s^{-1}.[16−18] Other electrochemical methods have less chemical resolution[19] or low time resolution.[20]

Interpretation of the chemical fluctuations measured in physiological systems with fast-scan cyclic voltammetry requires that its capabilities to resolve chemical substances are critically evaluated. In addition to the peak position, compounds are distinguished by features in their cyclic voltammograms including the relative amplitude of reverse and forward-going peaks, as well as peak shape.[21] These factors arise from the thermodynamic properties of the individual compounds, the rates of electron transfer, and the chemical stability of the electrogenerated products. Many of the target compounds adsorb strongly to carbon surfaces, allowing trace detection. However, adsorption affects the shape of the cyclic voltammogram.[22] Due to the broad oxidation

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waves, differences in E°'s contribute less to distinguishing between compounds than wave shape.

At present, the contribution of a specific compound to the measured signal is determined by matching sample cyclic voltammograms with those from known solutions. The large background current, caused by the high scan rates used, is removed by subtracting a set of cyclic voltammograms recorded at a time where biological activity is minimal.[18] This treatment renders the method a differential one in which only changes in concentration can be characterized. The background-subtracted cyclic voltammograms are then compared to a set obtained from candidate molecules that were recorded in solutions that mimic the physiological environment. One way to examine multiple cyclic voltammograms is with a two-dimensional plot in which the current is encoded in false color and is plotted versus the applied potential and the acquisition time.[23] Comparison of the color plots of authentic species and those present in the biological preparation are then used for identification. However, this approach is subjective since it involves visual matching. The comparison has been automated by using a cyclic voltammogram of a specific substance as a template.[21] Once a match is obtained, the peak current is converted to concentration based on calibrations with the identified substance. This method has the advantage that the correlation between the template and each member of the experimental set can be statistically evaluated. However, it has the disadvantage that it is univariate and it does not distinguish multiple contributors to the electrochemical current.

Principal component regression (PCR)[24] is now routinely used in analytical chemistry,[25−27] including electrochemistry,[28] to analyze complex mixtures whose components generate unresolved signals. In this work, we used this method to evaluate fast-scan cyclic voltammograms. Principal component analysis (PCA) was used to reduce the dimensionality of a calibration set of cyclic voltammograms of nine compounds at multiple concentrations. PCR was used to evaluate experimental data with this calibration set. The approach removes the limitations imposed by the template approach. First, individual components within a mixture of electroactive substances can be determined. Second, a concentration record is obtained for each specific substance evaluated. Third, it allows the resolving power of the method to be evaluated on known samples so that results from physiological preparations can be critically evaluated.

EXPERIMENTAL SECTION

Chemicals. Unless noted, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Solutions were prepared using doubly distilled deionized water (Megapure system, Corning, NY). TRIS buffer solution, pH 7.4, (15 mM TRIS, 126 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 1.2 mM NaH_2PO4, 1.2 mM $MgCl_2$, 2.0 mM Na_2SO_4) was used in calibration experiments and experiments with cells. Artificial cerebral spinal fluid (aCSF) was used in all brain slice experiments; this buffer was made by adding 11 mM glucose to the TRIS buffer. Stock solutions of analyte were prepared in 0.1 N $HClO_4$, and dilute solutions were made in TRIS buffer on the day of use.

Electrodes. Glass-encased carbon-fiber T-650 (Thornel, Amoco Corp., Greenville, SC) microelectrodes were constructed as previously described.[29] Individual carbon fibers were aspirated into glass capillaries (A-M Systems, Carlsborg, WA), and the glass was tapered in a micropipet puller (Narashige, Tokyo, Japan). The carbon fiber was then sealed in the capillary with epoxy (Epon 828 with 14 wt % *m*-phenylenediamine, Miller-Stephenson Chemical Co., Danbury, CT), excess epoxy was removed with acetone, and the assembly was cured (100 °C for 12 h, 150 °C for 1 day). For cylindrical carbon-fiber microelectrodes, the protruding carbon fiber was cut to a length between 50 and 100 μm. Elliptical electrodes were prepared by polishing the fiber down to the glass insulation at 45° on a polishing wheel (Sutter Instrument Co., Novato, CA). Before use, electrodes were soaked in 2-propanol purified with Norit A activated carbon (ICN, Costa Mesa, CA) for at least 10 min,[30] then backfilled with electrolyte (4 M potassium acetate, 150 mM potassium chloride), and fitted with wires for electrical contact. A silver/silver chloride electrode served as the reference.

Data Acquisition. Cyclic voltammograms were acquired using data acquisition hardware and local software written in LabVIEW (National Instruments, Austin, TX).[18,21] The cyclic voltammogram waveform was generated and the voltammetric signal was acquired with an A/D, D/A board, the PCI-6052E (National Instruments). A PCI-6711E D/A board (National Instruments) was used to synchronize waveform application, data acquisition, and stimulation delivery and to trigger the loop injector in the flow injection apparatus. The waveform was then input into a custom-built instrument for application to the electrochemical cell and current transduction (University of North Carolina Department of Chemistry Electronics Facility). The output signal was low-pass filtered at 50 kHz before being digitized. After collection, background subtraction, signal averaging, and digital filtering were all done under software control.

For most experiments, a scan rate of 400 V s^{-1}, a rest potential of −0.4 V versus Ag/AgCl was used between scans, the anodic limit was 1.3 V, and the scans were repeated every 100 ms. This limit provides many of the advantages described previously for neurotransmitter detection,[21] but the signals show less drift. For experiments at adrenal medullary cells, the rest potential was 0.2 V, the anodic limit was 1.6 V, the scan rate was 2000 V s^{-1}, and scans were repeated every 20 ms.

Flow Injection Apparatus. The electrode was positioned at the outlet of a six-port rotary valve.[31] A loop injector was mounted on an actuator (Rheodyne model 5041 valve and 5701 actuator) that was used with a 12-V dc solenoid valve kit (Rheodyne, Rohnert Park, CA) to introduce the analyte to the surface of the electrode. The linear flow velocity (1.0 cm s^{-1}) was controlled with a syringe infusion pump (Harvard Apparatus model 22, Holliston, MA).

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Biological Experiments. Bovine adrenal medullary cells, enriched in either epinephrine or norepinephrine using a Renografin gradient, were cultured as previously described and plated at a density of 3×10^5 cells/plate.[2] Cells were used 3−7 days after culture. The carbon-fiber microelectrode was placed 1 μm from the cell membrane using a piezoelectric manipulator (PCS-1000, Burleigh Instruments, Fishers, NY) on the stage of an inverted microscope (Axiovert 35; Zeiss, Thornwood, NY). Individual cells were stimulated to release by mechanical stimulation; the electrode was lowered onto the cell until the cell was visibly distorted, and then it was moved to a position 1 μm from the cell membrane. This mechanical stimulation caused vesicular release to occur.

For brain slice experiments, male Sprague−Dawley rats (250−300 g; Charles River Laboratories, Wilmington, MA) were decapitated, the brain was rapidly removed, and coronal slices (400 μm thick) containing the striatum were prepared with a vibratome (World Precision Instruments, Sarasota, FL).[6] Slices were placed in a recording chamber and superfused with aCSF maintained at 37 °C. Two twisted wires were used to deliver a constant current stimulation (Neurolog System 800, Hertfordshire, United Kingdom; conditions used: 10 pulse, 10 Hz, biphasic, 300 μA, 0.2 ms for each phase) to a region of the slice 200 μm from the microelectrode. Animal care was in accordance with institutional guidelines.

Data Analysis. The cyclic voltammetry currents were scaled at a single concentration for each compound evaluated, and the responses at other concentrations were adjusted to this scaled value. This accounts for amplitude differences that occur at different electrodes. Principal component procedures were employed using Matlab (The MathWorks, Inc, Natick, MA). PCR was used to construct a calibration set from cyclic voltammograms of known concentrations of a variety of neurochemicals. Calibration data were arranged with each measurement in a single row and the cyclic voltammetric current for each sample (1000 data points) in a column. Data pretreatments such as mean-centering and normalization were not used so the cyclic voltammogram retained its current amplitude. PCA arranges the variance in the calibration data, with the first principal component capturing the most variance. It minimizes correlation between cyclic voltammograms while compressing the data matrix. PCR was then used to create a regression matrix from the calibration data that is used to identify compounds and their concentrations in a cyclic voltammogram recorded in a solution of unknown composition.[24] To accomplish this, the unknown cyclic voltammogram was reduced with the same principle components. Regression analysis was used to quantify samples from biological preparations or for test data.

RESULTS AND DISCUSSION

Feature Extraction. Cyclic voltammograms (400 V s^{-1} scan rate) were collected using the flow injection system to introduce different compounds to a carbon-fiber electrode. Electroactive substances[21] from the following classes of neurochemicals were recorded: neurotransmitters (dopamine, 5-HT), dopamine metabolites (3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid, 3-methoxytyramine (3-MT)), and dopamine's biosynthetic precursor (L-dihydroxyphenylalanine (L-DOPA)), as well as uric acid and ascorbate. Each compound was evaluated at three to six different concentrations that were chosen based on estimates of their in vivo concentrations.[32,33] Cyclic voltammograms of solutions at five different pH values (between 7.1 and 7.7 pH units) were also recorded. Responses occur in these solutions because the structure of the background current is pH dependent, and these changes give a distinct cyclic voltammogram when background subtracted.[30,34] Average voltammetric responses recorded in buffer before the analyte reached the electrode were subtracted from those in the presence of analyte at different concentrations to obtain the 30 cyclic voltammograms used in the calibration set.

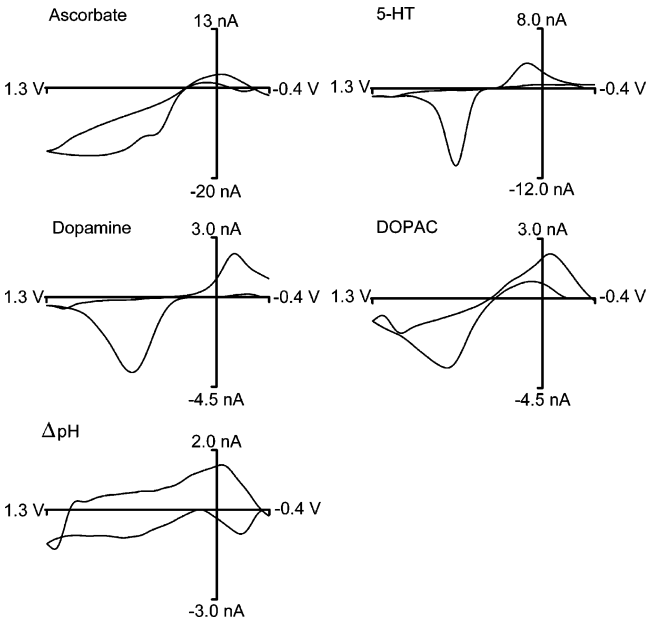

Figure 1. Background-subtracted cyclic voltammograms of five neurochemicals. Results for ascorbate (100 μM), 5-HT (1 μM), dopamine (1 μM), DOPAC (10 μM), and an acidic change in pH (0.1 pH unit) are shown.

The background-subtracted cyclic voltammograms for five of these situations are shown in Figure 1. Usually, peak amplitudes of cyclic voltammograms for equal concentrations of compounds are similar at the same electrode because they differ only by the diffusion coefficient and the number of electrons transferred. However, the neurotransmitters adsorb to carbon-fiber electrodes, enhancing the sensitivity for their detection more than 50-fold.[21,35] The cyclic voltammograms were subjected to PCA to reduce the dimensionality of the data. The projection of a cyclic voltammogram onto each principal component gives it a score. Figure 2A shows the first few eigen values for the corresponding principal components of the data set. These eigen values reflect the amount of variance captured by the individual principal components. Five principal components were adequate to capture >99.5% of the variance in the data. Thus, virtually all of the features of the 1000-point cyclic voltammograms in the calibration set could be represented by 5 data points in the vector space that came from PCA so the calibration set, originally comprising 30 000 data points, was compressed to 150 points.

A cluster plot of the results for these compounds using the first two principal components allows the resolution of these

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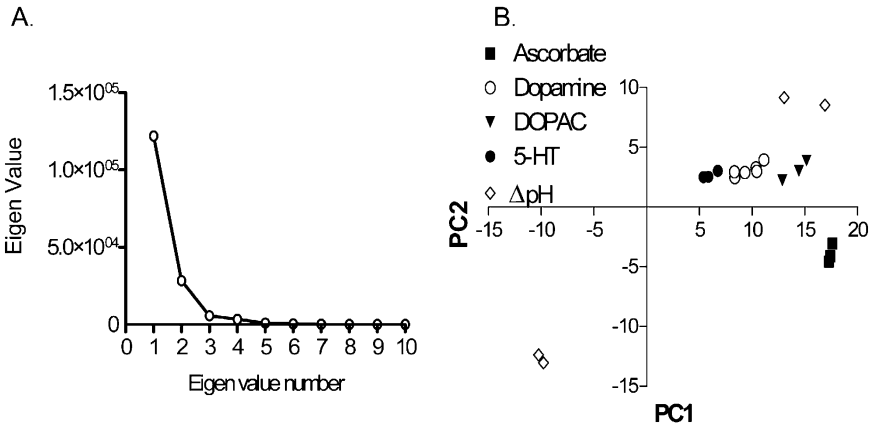

Figure 2. Characteristics of the PCR reduction of data. (A) Variance captured by the method expressed as the eigen value for each principal component. (B) Scores for the first two principal components of the compounds whose cyclic voltammograms are shown in Figure 1.

different compounds to be visualized (Figure 2B). In this case, the background-subtracted cyclic voltammograms were normalized by the area under their peaks to remove the concentration dependence. The cyclic voltammograms measured during changes in pH (open diamonds) give values that are clearly distinct from the other compounds. Basic pH changes appear in the third quadrant, and acidic changes appear in the first quadrant. Ascorbate, whose cyclic voltammogram has a broad oxidation wave and the lack of a reverse wave, is also well separated from the other analytes. The three analytes, whose cyclic voltammograms are most similar, dopamine, 5-HT, and DOPAC, are in the same quadrant but cluster in unique locations.

Identification of Single Components. PCR was used to evaluate concentrations of known solutions to examine its ability to quantitate cyclic voltammetry responses. The data set collected from the nine analytes listed above was used as the calibration set. Background-subtracted cyclic voltammograms were recorded in a separate set of solutions of varying concentrations of each analyte of interest. PCR was then used to evaluate the compound that was present and its concentration. Figure 3 shows the results of such an analysis for the five compounds whose background-subtracted cyclic voltammograms are given in Figure 1. The calibration lines for ascorbate, 5-HT, dopamine, and pH have slopes near unity, indicating appropriate identification of the compounds and their concentrations. In addition, the results for dopamine indicate the high sensitivity of the method. The limit of detection, defined as a signal that is three times the rms noise, was 8 ± 3 nM. The identification of DOPAC was not as good as evidenced by the slope of the calibration curve that deviates significantly from unity. This occurred because the regression analysis assigned part of the signal to 3-MT and L-DOPA, compounds whose cyclic voltammograms have many features similar to those for DOPAC.

In a previous report on the use of PCA to evaluate electrochemical responses, the data were not background subtracted.[36] Because the background was in every recording, it was the major contributor to the first principal component and this was used to remove its contribution. In this work, the background is often 100 times larger than the signal. Thus, small changes in the background can mask chemical changes. For this reason, we elected to use voltammograms for background subtraction that were obtained immediately before a chemical change.

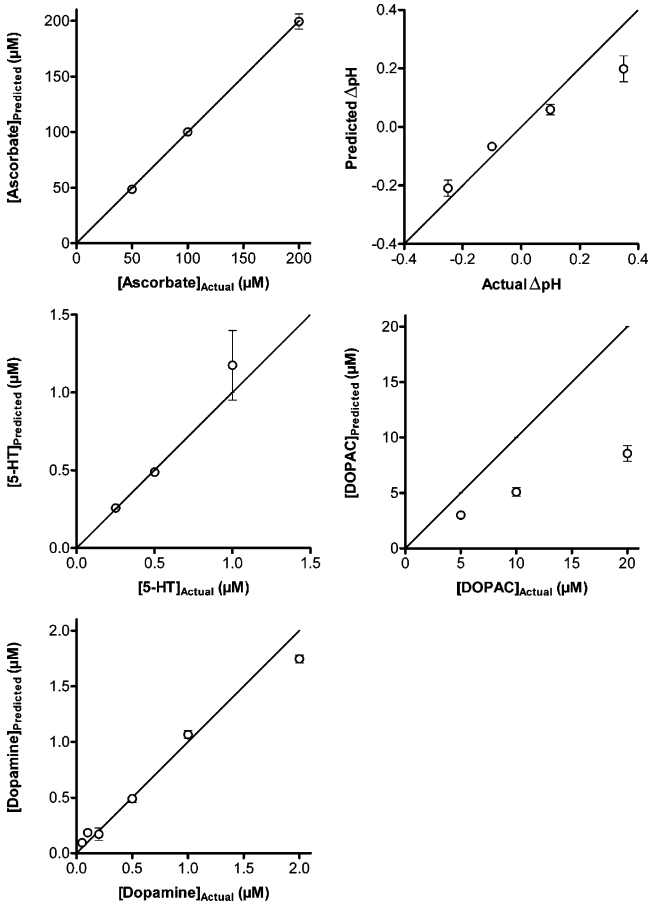

Figure 3. Concentrations evaluated by PCR compared to known concentrations. Error bars represent the standard deviation ($n = 3$). The slopes of the regression lines were determined to be 1.01 ± 0.01 ($r^2 = 0.99$) for ascorbate, 1.2 ± 0.1 ($r^2 = 0.99$) for 5-HT, 0.89 ± 0.05 ($r^2 = 0.98$) for dopamine, 0.67 ± 0.07 ($r^2 = 0.98$) for a change in pH, and 0.37 ± 0.03 ($r^2 = 0.99$) for DOPAC. The diagonal lines are those for unity slope.

Analysis of Mixtures. To further test the capabilities of the PCR analysis, background-subtracted cyclic voltammograms were evaluated from solutions that contained 0.50 μM dopamine and

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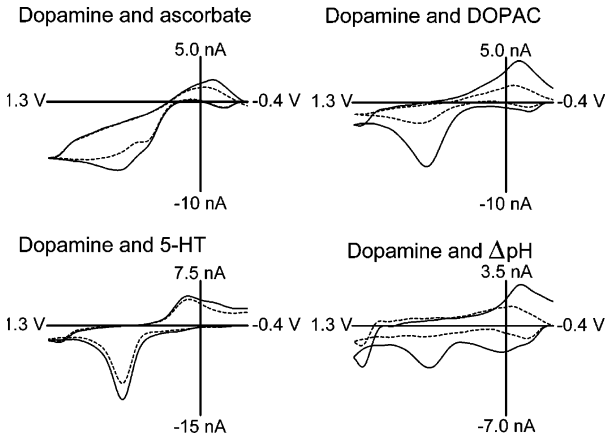

Figure 4. Background-subtracted cyclic voltammograms of mixtures. Each solution contained 0.5 μM dopamine. The mixtures are shown as solid lines, and the pure compounds are dashed lines. Ascorbate is present at 100 μM, DOPAC is present at 10 μM, 5-HT is present at 0.5 μM, and the pH change was an acidic 0.1 pH change.

Table 1. Concentrations Predicted from Cyclic Voltammograms Recorded in Solutions Containing a Mixture of Two Compounds[a]

	ascorbate (100 μM)	DOPAC (10 μM)	5-HT (0.5 μM)	ΔpH (−0.1)
dopamine	0.49 ± 0.04	0.63 ± 0.12	0.53 ± 0.06	0. 46 ± 0.04
5-HT	0.009 ± 0.003	0.007 ± 0.005	0.61 ± 0.05	0.004 ± 0.002
ascorbate	99 ± 3	1 ± 1	1 ± 2	2 ± 1
DOPAC	0.2 ± 0.1	4 ± 2	0.1 ± 0.2	0.3 ± 0.2
ΔpH	0.01 ± 0.01	−0.02 ± 0.01	0.01 ± 0.01	−0.09 ± 0.02

[a] Each solution contained 0.5 μM dopamine and a substance indicated by the column headings. The values in each column are the concentrations determined by PCR analysis. The values in the first four rows of the table are concentrations in micromolar ($n = 3$). The values in the last row are in pH units.

one other analyte (Figure 4). The cyclic voltammograms with dashed lines are those for the specific substance that was present in addition to dopamine, and the cyclic voltammograms recorded in mixtures are shown with the solid line. Dopamine is a target molecule for many neurochemical investigations, and its broad wave (380 mV, full width at half-maximum) sets a limit on its resolution from other substances. The calibration set constructed from nine species was the same as that used in the previous experiments.

The analytes present in the background-subtracted cyclic voltammogram of a mixture of dopamine and ascorbate were correctly identified by PCR. The regression predicted that the concentration was 99 μM for ascorbate (prepared concentration, 100 μM) and 0.49 μM for dopamine. Thus, dopamine can be distinguished from ascorbate with this approach, even when ascorbate is present in a 200-fold excess, whereas traditional electrochemical approaches have had difficulty resolving mixtures of these two compounds.[32,37] The other compounds and their concentrations that were assigned to this mixture are shown in Table 1.

Analysis of the cyclic voltammogram of the mixture containing DOPAC (prepared concentration, 10 μM) and dopamine predicted 4.4 μM DOPAC and 0.63 μM dopamine. As noted above, DOPAC has features in common with several of the compounds in the calibration set that leads to a relatively poor estimation of its concentration, an overestimation of the dopamine concentration (Table 1), and assignment to other compounds. Nevertheless, even in the presence of 20-fold excess DOPAC, dopamine was identified as a major contributor to the signal. Although ascorbate was identified to change with DOPAC and dopamine, as well as in the other solutions summarized in Table 1, the concentration assigned to it corresponds to less that 1% of the voltammetric signal.

For the mixture of dopamine and 5-HT, the concentrations determined were 0.61 μM 5-HT (prepared concentration, 0.5 μM) and 0.53 μM dopamine (Table 1). The concentration of dopamine determined is within 10% of its true concentration. The adsorption of 5-HT is ~5 times greater than for dopamine, but its adsorption magnitude varies slightly between electrodes. In addition, the degree of adsorption progresses with exposure time to 5-HT;[38] all of these factors contribute to the error. For this equimolar mixture, the PCR method does a remarkable job in resolving this mixture because 5-HT dominates the shape (Figure 4). The final test was an acidic change in pH and 0.5 μM dopamine. The determined concentrations were a 0.089 acidic pH change (actual change, 0.10 pH unit) and 0.46 μM dopamine (Table 1). From these results, we conclude that this approach can resolve the major component in mixtures of neurochemicals and provide an estimate for the dopamine concentration with an accuracy of ~10%.

Evaluation of a Temporal Change in Dopamine Concentration. When used in biological preparations, fast-scan cyclic voltammetry is used to evaluate the time course of concentration changes. Therefore, the calibration set was used to evaluate the temporal concentration changes of 0.5 μM dopamine that were obtained during passage through the flow injection system (Figure 5). This was done by determining the scores of the background-subtracted cyclic voltammograms in the data set and evaluating them by PCR with the calibration set.

The cyclic voltammetric responses to a bolus of dopamine are shown by the color representation in Figure 5. The two-dimensional plot shows the current from the 150 background-subtracted cyclic voltammograms in false color with the abscissa as the collection time and the ordinate as the applied potential. The characteristic oxidation (0.6 V) and reduction (−0.2 V) peaks (both versus Ag/AgCl) for dopamine appear as the bolus reaches the electrode, which is taken as time 0. The panel above the color plot shows the current recorded at the peak of the oxidation wave for dopamine.

The PCR results for dopamine, in concentration units, are shown in the right panel. The regression predicts that 88 ± 4% of the oxidative current is due to an increase in the concentration of dopamine, and its maximal concentration was evaluated as 0.53 μM. The remaining signal was assigned to other compounds with 5-HT (8 ± 4%) and DOPAC (3 ± 1%) as the major contributors (Figure 5, right panel). Note, the dopamine concentration trace and the current trace have different shapes, particularly in the descending portion of the trace. Consistent with this, the color plot reveals a shift in the oxidation peak of 104 mV at 5 s when the dopamine is desorbing from the electrode. During the time of this shift, the signal was assigned to DOPAC.

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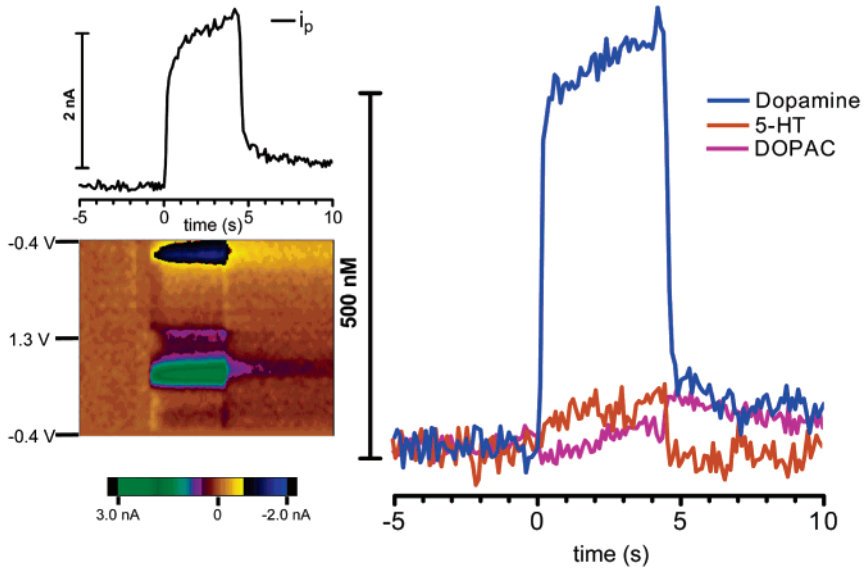

Figure 5. PCR analysis of a temporal change in dopamine concentration. Lower left panel: Color representation of the voltammograms obtained during a bolus (0.5 μM) of dopamine in a flow injection system. The dopamine reached the electrode at $t = 0$ s. The abscissa is time, the ordinate is applied potential, and the current is shown in false color. Upper left panel: the current measured in successive scans at the peak oxidation potential for dopamine. Right traces: predicted dopamine (blue) concentration from PCR. Also shown are the responses assigned to 5-HT (orange) and DOPAC (purple), normalized by the relative sensitivity to dopamine.

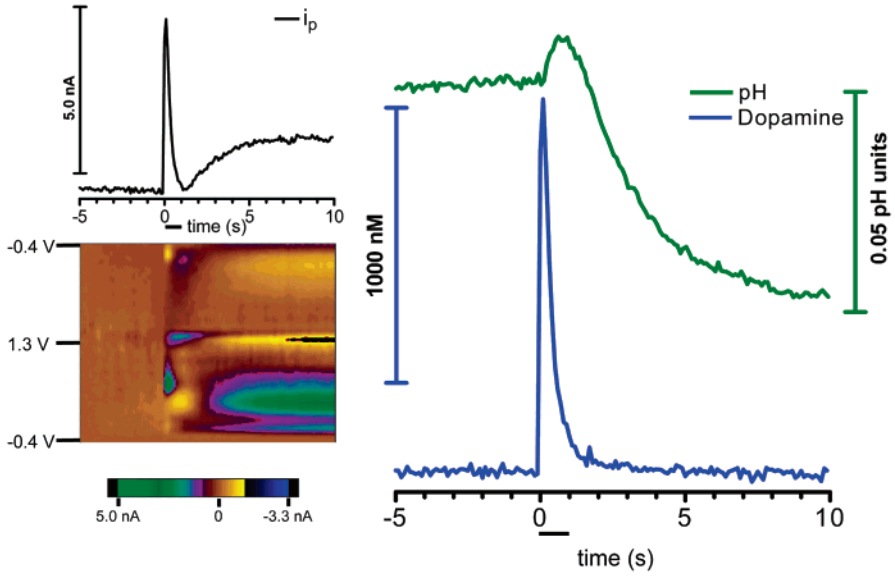

Figure 6. Dopamine release in a brain slice containing the striatum. The electrical stimulation (10 pulses at 10 Hz, 1-s duration) was initiated at $t = 0$ s, as indicated by the solid bar. The color plot (lower left panel) shows the set of background-subtracted cyclic voltammograms that were recorded. Upper left panel: the current at the peak oxidation potential for dopamine. Right panel: the temporal dynamics of dopamine and pH assigned by PCR.

Determination of Dopamine Release from Nerve Terminals in a Brain Slice. An identical analysis with the same calibration set was used to evaluate background-subtracted cyclic voltammograms recorded in a brain slice. In this case, the microelectrode was inserted 100 μm into a section of brain tissue that contains the striatum, a region with multiple dopaminergic release sites. The region near the electrode was electrically stimulated to evoke dopamine release.

A color representation of the cyclic voltammetric data is given in Figure 6. Chemical changes were not seen until the stimulation was applied. The current at the peak oxidation potential for dopamine increased during the stimulation ($t = 0$ s is the time the stimulation began), returned toward its initial value, and then rose again (upper panel, Figure 6). The color plot reveals that a substance other than dopamine is responsible for the second increase in current. PCR analysis of the data set assigned the signal as a composite of a dopamine and a pH change (right panel, Figure 6). Dopamine transiently increases during the stimulation, consistent with its known pattern of evoked release from adjacent nerve terminals, and then decreases in concentration as it is taken up into the terminals by the dopamine uptake transporter.[39] In contrast, the pH shifts to a slightly basic value during the

(39) Jones, S. R.; Gainetdinov, R. R.; Caron, M. G. *Psychopharmacology (Berlin)* **1999**, *147*, 30−32.

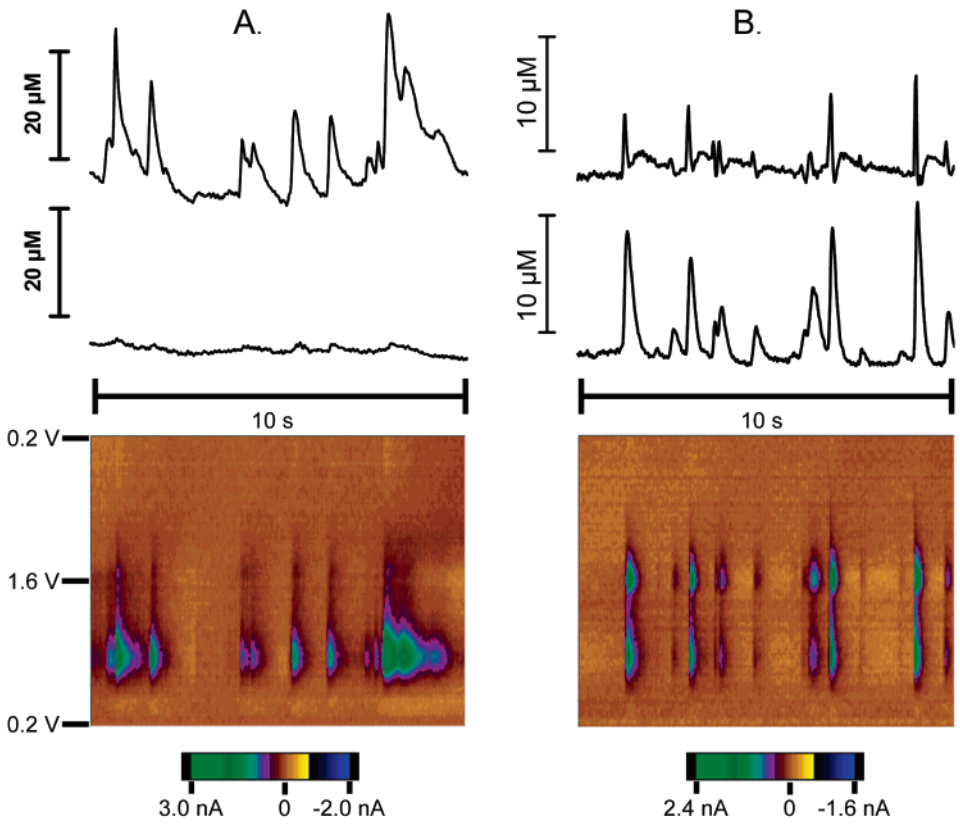

Figure 7. Vesicular release events measured at individual cells. (A) Lower panel: color representation of release from a single cell. The upper trace is the concentration of norepinephrine assigned by PCR, and the middle trace is the epinephrine assignment. (B) Lower panel: release measured at another cell. The upper trace is the norepinephrine prediction, and the middle trace is the epinephrine assignment.

stimulation and is followed by a prolonged acidic change. The pH changes are due to an increase in metabolism in the slice following the stimulation.[40] As in the flow injection experiment, the PCR analysis also assigned a small portion of the signal (<10%) to 5-HT at the time when dopamine was seen. The striatal content of 5-HT is ~8% of the dopamine content[41] and thus could be a contributor. However, since 5-HT was predicted to be in the calibration with dopamine alone, we consider its assignment unreliable.

The chemical assignments of the major contributors, dopamine and pH, were in agreement with the changes seen in the color plot as well as the template method. Individual cyclic voltammograms at the maximum of the dopamine change corresponded to those of dopamine standards as judged by the correlation coefficient (>0.85). The cyclic voltammogram of norepinephrine, another catecholamine neurotransmitter, is identical to that for dopamine.[21] However, because it is present in this brain region only in low concentration, so it was not considered in these analyses.[41] Good fits were obtained for the cyclic voltammograms obtained at later times with those for an acidic change. The utility of the PCR analysis of these data is that it allows separation of these two components throughout the recording interval.

Evaluation of Release from Individual Biological Cells. As another test of the PCR approach, cyclic voltammograms were recorded with a disk-shaped carbon-fiber electrode placed 1 μm from the surface of an adrenal medullary cell. These cells secrete either epinephrine or norepinephrine, depending on their phenotype, through an exocytotic mechanism. Cyclic voltammetry can resolve the contents of each vesicle as release occurs.[42] Both molecules are catecholamines, but norepinephrine has a primary amine on its side chain whereas epinephrine has a secondary amine. This results in a second oxidation wave beyond the catechol oxidation wave for epinephrine, but not norepinephrine, allowing them to be distinguished. Prior work, confirmed by liquid chromatographic analysis of the contents of the secreting cell, has shown that background-subtracted cyclic voltammetry is an effective way to distinguish these compounds during their exocytosis.[2,43]

Release from single vesicles was monitored using fast-scan cyclic voltammetry. Because the voltage range and repetition rate differed from that in the work described above, a different calibration set containing cyclic voltammograms for epinephrine and norepinephrine was constructed and reduced using PCA. Figure 7A shows the background-subtracted cyclic voltammetric data obtained at a single cell in the form of a color plot. The absence of features at 1.6 V indicates that this cell only secretes norepinephrine. The PCR analysis concurred with this (upper trace), assigning 97.8% of the average signal to norepinephrine (lower trace, Figure 7A). Consistent with this, the template approach showed that norepinephrine was the substance detected

(40) Jones, S. R.; Mickelson, G. E.; Collins, L. B.; Kawagoe, K. T.; Wightman, R. M. *J. Neurosci. Methods* **1994**, *52*, 1−10.
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(42) Troyer, K. P.; Wightman, R. M. *J. Biol. Chem.* **2002**, *277*, 29101−29107.
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at the maximum of the release events with a correlation coefficient of 0.78.

Figure 7B illustrates release measured at another cell in which PCR revealed a feature of the data not previously recognized. The color plot shows that each release event was accompanied by current at 1.6 V, indicative of epinephrine secretion. Consistent with this, the PCR analysis assigned the majority of the signal to epinephrine (86%, lower trace, Figure 7B). However, PCR assigned the initial cyclic voltammogram of each exocytotic event to norepinephrine (upper trace, Figure 7B). This assignment seems unlikely because mixing of the contents of an individual vesicle should occur prior to release leading to simultaneous secretion of all of the contents. Therefore, we closely examined the background-subtracted cyclic voltammograms of epinephrine used for calibration. We found that the second oxidation wave was not present on the initial exposure to epinephrine, but only appeared during the second cyclic voltammogram, presumably due to a complex series of steps as it adsorbs onto the electrode. Thus, the unexpected assignment of the PCR analysis is not a failing of the method but rather a result caused by the temporal evolution of the cyclic voltammograms. The template approach showed that norepinephrine was the substance detected at the maximum of the release events with a correlation coefficient of 0.81.

CONCLUSIONS

Principal component regression provides a useful method for the detection of catecholamine changes measured with fast-scan cyclic voltammetry. The PCA data reduction method can accurately reproduce the data set, and PCR proves an effective method for predicting concentration. These methods do not succeed in all cases because of the inherent lack of resolution of the fast-scan cyclic voltammetry technique. However, the methods provide a way to resolve electroactive components in a nonsubjective way and are very effective for the primary components in a composite signal.

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