A Flexible Software Platform for Fast-Scan Cyclic Voltammetry Data Acquisition and Analysis

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Abstract

Over the last several decades, fast-scan cyclic voltammetry (FSCV) has proved to be a valuable analytical tool for the real-time measurement of neurotransmitter dynamics *in vitro* and *in vivo*. Indeed, FSCV has found application in a wide variety of disciplines including electrochemistry, neurobiology and behavioral psychology. The maturation of FSCV as an *in vivo* technique led users to pose increasingly complex questions that require a more sophisticated experimental design. To accommodate recent and future advances in FSCV application, our lab has developed High Definition Cyclic Voltammetry (HDCV). HDCV is an electrochemical software suite, and includes data acquisition and analysis programs. The data collection program delivers greater experimental flexibility and better user feedback through live displays. It supports experiments involving multiple electrodes with customized waveforms. It is compatible with TTL-based systems that are used for monitoring animal behavior and it enables simultaneous recording of electrochemical and electrophysiological data. HDCV analysis streamlines data processing with superior filtering options, seamlessly manages behavioral events, and integrates chemometric processing. Furthermore, analysis is capable of handling single files collected over extended periods of time, allowing the user to consider biological events on both sub-second and multi-minute time scales. Here we describe and demonstrate the utility of HDCV for *in vivo* experiments.

Introduction

Electrochemistry is widely employed for the detection of neurotransmitters at single biological cells, in brain slices, and *in vivo*.[1] The original impetus for the development of *in vivo* measurements of neurotransmitters was the detection of dopamine.[2] Today, however, this approach is used not only for the detection of catecholamines but also for compounds as diverse as adenosine,[3] oxygen,[4] histamine, and 5-hydroxytryptamine.[5] Although several voltammetric techniques have been evaluated for *in vivo* use, fast-scan cyclic voltammetry (FSCV) is particularly useful because it combines high sensitivity (nanomolar range) with sufficient features to identify the substances detected. To follow the rapid concentration fluctuations of neurotransmitters, cyclic voltammograms are generated at high scan rates (>100 V/s), allowing data collection on the millisecond time scale. At these high rates, very

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large background currents are generated. This charging current can be removed by analog or digital background subtraction, yielding cyclic voltammograms of the analytes of interest.[6]

The rapid scan rates and the large number of data points that are collected both require a computer for waveform generation and data acquisition. Typically, at least 10 cyclic voltammograms (CVs) are recorded per second with each containing 1000 scanned potentials within the voltammetric sweep. Furthermore, multiple electrodes can also be used, and the electrochemical experiments may be combined with other electrical measurements (ion selective electrodes, amperometry, electrophysiology, etc.).[4,7,8] Multifunctional data acquisition systems from several vendors that include analog and digital inputs and outputs and clocks are readily available and allow such measurements. Thus, electrochemical instrumentation has advanced considerably from 1983 when one of the first commercially available computer controlled potentiostats, the BAS 100, was introduced.[9] Our own lab,[10] as well as others,[11] have generated a series of programs for neurotransmitter measurements as documented elsewhere.[12]

Here we describe some of the features of our HDCV ('High Definition Cyclic Voltammetry'), our latest software package for high speed acquisition and analysis of electrochemical data. HDCV serves as an upgrade and replacement for TarHeelCV, the previous program distributed by our group for FSCV data acquisition and analysis.[13] These new programs incorporate features that were not previously present. The data acquisition program enables simultaneous collection from up to 16 electrodes, allowing use with arrays.[14] Multiple waveforms, optimized for each analyte, can be used simultaneously, and the waveforms can have any shape. Data is digitally background subtracted and displayed in real time as it is collected. The program allows concurrent electrochemical and electrophysiological recording at a single electrode. In the electrophysiological recordings, the firing of individual neurons is measured in the form of single units. Importantly, it allows continuous data collection for several hours. Moreover, the program is compatible with behavioral experiments in which items such as levers, tone generators, or other binary output devices, can be connected to digital input signal lines and user-mapped into the data flow. All of the analog and digital data are clocked in lockstep with a common hardware clock. The data analysis program streamlines the analysis of large amounts of data, facilitates signal-averaging of events marked by the digital signals, performs current artifact removal, and provides digital filtering. Principal component regression is incorporated to extract signals of specific analytes. In this manuscript we illustrate several of these capabilities with examples from ongoing experiments.

Experimental Section

Hardware

All events configured through HDCV employ a single PCIe-6363 X-series data acquisition card (National Instruments, Austin, TX) rather than the three required with TarheelCV. (It is expected that future versions of HDCV will allow the use of USB-connected DAQ hardware.) A custom-built breakout box (UNC Department of Chemistry Electronics Design Facility) provides access to 4 analog outputs, 16 differential analog inputs, and 40 digital I/O lines. For single electrode experiments, the maximum sampling rate is 2 MHz. For multiple electrodes, the acquisition card's single instrumentation amplifier limits the maximum sampling rate to 1 MHz divided by the number of channels. Another digital line (Port2/Line 0) gates output signals through the breakout box. This line is held high when HDCV is initialized. Signal routing is otherwise fully configurable through the acquisition program.

Software

The HDCV electrochemical software suite was written in LabVIEW (National Instruments, Austin, TX). HDCV consists of two independent executable programs for data acquisition and analysis.

(a) HDCV Data Acquisition—HDCV Data Acquisition controls the hardware, while also collecting and displaying data in real-time. Low-level hardware control is achieved through an interface that allows the selection of a device and the assignment of its analog and digital lines to specific functions in the software. The interface allows the user to customize analog and digital input/output (I/O) parameters, adjust analog input gain settings, and assign names to the I/O lines. Analog and digital lines are synchronized via a signal generated by the DAQ hardware clock. The timing of this clocking signal is defined by the CV frequency.

Analog outputs can be assigned to one of three functions: waveform generation, electrical stimulation, or analog background subtraction. For waveform generation, the shape of the applied voltage can be designed from preset options (triangle, sine, square and sawtooth), designed arbitrarily by piecewise mathematical construction, or loaded from a text file. Additional inputs provide control over the voltage limits, scan-rate, application frequency and sampling rate. Stimulation trains are defined by pulse duration, polarity (mono and biphasic), frequency, and number. The stimulus onset is delayed from the beginning of the voltage waveform by a user-defined time to prevent overlap. These trains can be triggered internally at a user-defined time or externally by a digital input. For analog background subtraction, an inverted voltage proportional to the electrode charging current is output. In a manner similar to noise-cancelling headphones, this signal is applied to the inverting input of the headstage amplifier decreasing the amount of background recorded. This prevents saturation of the headstage amplifier and allows the data acquisition card voltage range to be reduced to decrease quantization noise.[6]

Bidirectional digital lines are used to control and monitor binary output external devices. Two digital lines are reserved to output the CV frequency signal and the timing signal for electrochemistry/electrophysiology experiments so that other instruments can be synchronized with HDCV. Other digital outputs can be set to change state once per file or once per waveform application. Other ports can be specified as digital inputs, which the program will record.

Data are collected and saved as files in single run, multiple run, or continuous modes. Single run mode is designed for short duration (seconds to several minutes) collection periods. Multiple run mode allows automatic collection of multiple experiments (separated by a specified rest time) that are saved in one file. Continuous mode allows for minutes to hours of data acquisition and is designed for recordings during behavioral experiments.

Acquired data are stored in two files. The main file, with a .hdcv extension, contains a header with the operating parameters and the current values for each cyclic voltammogram. The transition times of digital input lines are recorded in a companion text file with a .dig extension.

(b) HDCV Analysis—HDCV Analysis is used for post-processing data (including files generated by TarheelCV). Data are displayed in a color plot with currents at all potentials shown as a function of acquisition time.[15] Current at each potential is background subtracted from a user-defined time point and is visualized in false color. At a user-selected time and potential, two supporting plots display current versus time and its corresponding cyclic voltammogram. These representations are controlled by the main cursor placement within the color plot. An additional display maps the timing of digital input signals. (For backward

compatibility with TarheelCV, the electrochemical and digital data files are concatenated into HDCV format using the Data Converter program included in the executable package).

HDCV analysis offers several digital filtering and smoothing options including a two dimensional Fast Fourier Transform (FFT) algorithm. On-screen deglitching tools allow for easy removal of undesired electrical artifacts (glitches). If digital inputs are recorded, they serve as time points to clip periods of data into time bins. Such changes are saved in an analysis work file, preserving the original data.

Concentration information can be extracted from files in one of two ways. If the data represent the response of a single analyte, the user may input a calibration factor to convert currents into concentrations. Principal component regression (PCR) is used for more complex *in vivo* experiments.[16] HDCV performs PCR analysis on data based on a user-built training set of CV standards. Concentrations determined by either of these methods can be averaged across multiple files, runs, or time bins.

Experimental setup

Four major components are required for combined electrochemical/electrophysiological (echem/ephys) experiments (Supporting Information Figure S-1). These include two computer stations for separate acquisition of echem/ephys data, a custom-built headstage amplifier that interfaces with a universal electrochemical instrument (UEI) mainframe (UNC Department of Chemistry Electronics Shop), and a commercially available operant conditioning system (Med. Associates, Inc., St. Albans, VT). HDCV software controls the voltammetric acquisition parameters, collects current responses, and synchronizes experimental timing through the PCIe-6363 in the electrochemistry computer. The output waveform is reduced to one third to utilize the full DAC bit depth and low-pass filtered to reduce quantization noise before application via a swivel commutator (Med. Associates, Inc.) to the headstage amplifier. Stimulation waveforms are configured within HDCV, converted to current by a pair of optically isolated stimulators (Digitimer Ltd., Letchworth, U.K.) and routed to the headstage. The Med Associates operant conditioning system generates digital time stamps indicating behavioral events such as a lever press. These are delivered to the PCIe-6363 via an 8 bit interface, synchronizing the echem/ephys measurements with behavioral events.

In combined echem/ephys experiments, the voltage of the electrode floats between voltammetric recordings to allow recording of electrical activity from nearby cells. This is controlled by a digital output that triggers a CMOS analog switch on a modified headstage (UNC Department of Chemistry Electronics Design Facility) that alternates between a current-to-voltage amplifier for FSCV, and a voltage-follower for single unit recordings, circuitry described previously.[17] Electrophysiology is collected on a computer with *Plexon* Neurosurgery Workstation software (Plexon Inc., Dallas, TX).

Experimental procedures

Experiments shown here are used to illustrate the features of HDCV. The experimental details have been described in detail elsewhere.[18,19]

Results and Discussion

Programming techniques

LabVIEW is a programming language optimized for data acquisition and processing. The data acquisition program of HDCV, in particular, involves several interesting coding techniques. The main program is written as four loops operating in parallel. The data

acquisition loop has the highest priority, and runs once per CV. The data collected are fed through queues to two separate processing loops that run at lower priority so data acquisition is not delayed. The analog data loop trims away unwanted data points (the interval between voltammograms), maintains real-time graphs, and stores the data to a file. The digital data loop runs only once per transition on any digital input line, and likewise maintains graphs and stores the data to a second file. The fourth loop, the event loop, handles user interactions.

Timing control

Traditional LabVIEW programming provides a way for an input task to clock an output task so that the two proceed in lockstep, data point by data point. This technique was extended to maintain four tasks in parallel: analog input (the master) clocking analog output, digital input, and digital output. This provides far higher temporal resolution for the digital input lines than TarHeelCV, which sampled them only once per CV scan. It also provided a useful organizing principle for the entire program.

Active/passive configuration routines

HDCV has extensive configuration screens, because numerous parameters for experiment control are provided to the user. Each of these is implemented as a LabVIEW VI (virtual instrument), an interaction screen with associated code, and does far more than collect parameters from the user. The full process entails: read a configuration file, display its parameters, allow the user to change them, post-process the parameters into a form more useful for program operation, and update the configuration file. In some cases postprocessing reveals errors that could only have been revealed by configuring the hardware. Reading the configuration file and postprocessing its contents needs to be done on every startup whether or not the user intends to make any changes.

To implement this scheme, we used an approach we term Active/Passive routines in which each configuration VI can be called in either mode. In active mode, it opens and waits for the user to change and confirm the settings. In passive mode, it invisibly loads and postprocesses the configuration file. When encountering certain errors that would prevent meaningful operation, it can become active, displaying a message and waiting for the user to resolve the problem. These VIs are called in active mode when the program is first run, or upon user request.

Setup control structure

The experiment setup VI, in particular, presented interesting problems. It uses an interface with nine tabs, many of which lead to complex, full-screen pages that display many parameters and graphs. Several of these pages have internal states (useful for maintaining graphs) that need to be "unpacked" from the raw parameters when the page is presented, and "packed away" when the page is closed. A natural program structure would be to have a Case structure with a separate case for each page in the user interface, and within each, an Event structure to handle events relevant to that page (an Event structure is a Case-like control structure that waits for a user interface event and then executes the appropriate case when an event takes place).

In HDCV the program structure was designed in the following way. A single Event structure in a loop fields all events for the entire VI. It translates each event type into a textual code. Each event is passed via queue to the main operating loop. The main operating loop consists of a primary Case structure, with a case for each page in the user interface. Within each case is an inner Case structure, with a case for each event relevant to that page. The Event structure takes special action when the user switches tabs, and generates two event codes.

The first, "Leaving", is directed to the page that has been open, and allows its code to pack away any data for that page as needed. The second, "Entering", is directed to the page that is being opened, and allows its code to make preparations. This last feature alone was so powerful as to make the entire technique worthwhile. The code organization that resulted from allowing each page to "own" a case in the primary Case structure has proved vastly useful for the understanding and readability of the code.

Data storage

HDCV uses the binary data file format provided by LabVIEW. Data are organized into "chunks", one per run of a Multiple run experiment, one per experiment otherwise. Each chunk is stored as a three-dimensional array, indexed by CV, channel (for multichannel data collection experiments), and data point. Writing the 3-D array after data collection is vulnerable to data loss in case of system failure during long experiments. To avoid this problem, the 3-D array is written into the file piecemeal, one "slice" per CV, as it is collected. Each slice can be viewed as a two-dimensional array, indexed by channel and data point. At the end of the experiment, the number of CVs is plugged into the 3-D array header, completing the file.

The same technique is used when reading the data for long experiments. No portion of the analysis process could handle all the data for a multiple hour experiment at once without exceeding available memory. The user specifies the time region of interest (up to 5 minutes long), and analysis reads the data only for that time region.

Real-Time Data Analysis and Acquisition

The HDCV acquisition program has several live data displays that provide immediate user feedback. These include oscilloscopes for the analog and digital signals, a current-time chart, and a live color chart. These run continuously during waveform application and are active even when data storage is not occurring.

The live color chart is particularly useful since the entire experiment can be viewed with the voltage scan mapped along the ordinate, and the time of CV acquisition shown along the abscissa. Oxidation and reduction currents are shown in a 3: −2 false color scale to enhance peak contrast.[15] Hence, the color chart provides both qualitative and quantitative data assessment. The flux of multiple analytes, potential-dependent noise, and electrode stability can be monitored continuously. This type of immediate feedback assists with trouble-shooting and experimental optimization.

To illustrate this feature, Figure 1 shows data obtained during acquisition with a carbon-fiber electrode in the nucleus accumbens of an anesthetized rat. FSCV studies employ various waveforms optimized for different target analytes. This particular waveform (Fig. 1A) allowed simultaneous dopamine and oxygen measurements.[4] (See supporting information for a video file of these data.) During the experiment, the color chart scrolls to the left as each CV is collected. Controls allow the user to choose an input channel, select the most recent CV as the basis for background subtraction, and adjust the color scale. Figure 1B–D display screen shots captured during three sequential 20 s periods. In Figure 1B (where the waveform is applied but data storage was not initiated) transient decreases in oxygen resulting from natural activity appear as positive currents centered on −1.4 V (data point 750). In the next interval, file collection was initiated and an electrical stimulation occurs (Fig 1C). Stimulated dopamine release is evidenced by positive peaks (oxidation) at 0.6 V (data point 160) and negative peaks (reduction) at −0.2 V (data point 450). Uncharacterized extracellular changes induced by the stimulation result in a positive, non-faradaic current artifact at the cathodic switching potential. A biphasic increase in oxygen

follows generating negative (reduction) current fluctuations at −1.4 V, which peak at 124 s (Fig 1C) and 144 s (Fig 1D). In Figure 1D, features at 0.2 V (data point 50) and 0 V (data point 400) show a prolonged alkaline pH shift accompanying the oxygen fluctuations. These data enable evaluation of neurotransmitters in the regulation of cerebral blood flow.[4]

Simultaneous Detection of Neurotransmitter Release and Single Unit Activity

Neuronal communication involves electrical impulses and chemical messengers. The combined echem/ephys technique provides a unique opportunity to study these interdependent processes simultaneously with high spatial and temporal resolution. The combined echem/ephys technique has seen significant advancement since Millar and coworkers initiated its development over thirty years ago.[20] In the last decade, a miniaturized switching headstage enabled experiments in freely-moving animals,[17] allowing combined echem/ephys measurements for delineating the neuronal circuitry involved in behavior.[21][22][23] This technique has been paired to iontophoretic drug delivery[8] to identify the receptors that locally gate dopamine neurotransmission.[24]

As described in the Experimental Section, combined echem/ephys experiments are complex and require coordination between several instruments (Fig S-1). During combined measurements, a digital relay output from the PCIe-6363 triggers a solid-state switch in the headstage that controls acquisition modes. The timing scheme during an experiment with behavioral DIOs is shown in Figure 2A. When the digital output from HDCV goes low, the headstage current-to-voltage amplifier circuit connects to the carbon-fiber electrode and the waveform is applied. When the digital output goes high, the current-to-voltage amplifier disconnects and a voltage-follower amplifier connects to the carbon-fiber electrode to measure the spiking activity of proximal neurons. Typically these modes alternate at 5 Hz to allow adequate time to detect single unit activity. HDCV concurrently records any digital inputs, and these time stamps can be used to align data to the appropriate behavioral event.

Figure 2B shows an example of simultaneous echem/ephys data collected during intracranial self-stimulation (ICSS) with the waveform scanning from −0.4 V to +1.3 V at 400 V/s for the detection of dopamine.[25] ICSS is a paradigm in which an animal depresses a lever to deliver an electrical stimulation to its own brain. With the stimulating electrode implanted in select brain regions, animals find this rewarding. In our design, lever extension into the behavioral chamber is preceded by a tone and light cue.[18] The data here are aligned to the onset of this cue and reveal that extracellular dopamine and cell firing increase in this location following the cue.

Undesirable current artifacts can occur during the transitions between free-floating and applied potentials. Although the headstage amplifier uses a state-of-the-art CMOS switch between the voltage and current amplifiers, a small amount of charge injection and input capacitance occurs when switching, and artifacts appear in the data (Fig 2C, right). In older approaches, this was minimized by adding extra data points preceding the voltage ramp that produced a buffer for current stabilization. Extra data points were then removed during post-collection analysis. In HDCV the change of state of the digital relay line does not have to be synchronized to the CV frequency clock. This allows for a wider voltage application window without changing the time frame of echem collection. The current artifact occurs at a set time before and after voltammetry acquisition, and no post collection correction is needed during analysis.

Two Dimensional Data Filtering

FSCV is useful for the detection of rapid chemical fluctuations of nanomolar concentrations.[26] To resolve these small signals, digital filtering strategies are employed to

remove high-frequency noise. Previously, TarheelCV filtered each cyclic voltammogram with a 4th order low-pass Bessel filter (cutoff frequency of 2 kHz for a 400 V/s scan rate),[27] an option that is also available in HDCV. For further noise removal, users previously employed an 8-point nearest neighbor smoothing kernel. This filter generates a two-dimensional moving-point average that smooths differences within the CV domain and across consecutive CV scans and can be repeated multiple times. The disadvantages of this nearest-neighbor smoothing kernel are that it executes slowly and that the frequency effects are not intuitive. In contrast, HDCV offers a more rational two-dimensional Fast-Fourier transform (2D-FFT) filter for noise reduction. With a 2D-FFT filter the color plot is transformed into the frequency domain before a filtering mask is applied. The retained frequencies are then transformed back into the spatial domain by the inverse Fourier transform. The effects of a low-pass 2D-FFT filter are analogous to a smoothing kernel, removing high frequency-noise components in the CV and time dimensions. However, the 2D-FFT approach is preferred because it is rapidly calculated while maintaining the temporal resolution in the CV and the CV frequency domains.

In the 2D-FFT filtering menu, the transformed signal and noise components of the data are viewed (Fig 3A). Cutoff frequencies are defined by an ellipse representing the −3dB point across both domains (inner orange ellipse). The outer ellipse (shown in black) indicates the −50 dB boundaries where increased signal attenuation occurs. The clipped transform displays the intensities of the retained frequencies.

Figure 3B shows the results of different filtering methods on data collected during electrically stimulated norepinephrine release in an anesthetized rat.[19] The original unfiltered data are shown on the left. The oxidation of norepinephrine is visible in the color plot at 0.6 V on the forward scan. An alkaline shift in pH produces the features around −0.2 V on the forward and backward scans. The trace above the color plot represents norepinephrine concentration extracted with PCR and the dotted line in the color plot represents the time of the CV shown below. The middle panels show these data after filtering with a 2 kHz Bessel function in the cyclic voltammetry domain and an 8-point smoothing kernel. Noise is significantly reduced but the oxidation peak potential for norepinephrine is offset by 100 mV. The right panes illustrate the same data after use of the 2D-FFT filter in HDCV. The 2D-FFT filter removes noise similarly, while retaining the peak position of the CV.

Analysis of Continuous Data

FSCV is well suited to capture subsecond changes in neurotransmitter concentrations that naturally occur. However, FSCV also has the ability to measure slower concentration changes, although this feature has been less frequently used.[628] Traditional approaches collected data in 15, 30, or 60 second epochs so the data could be easily read. Data sets were then reorganized after the experiment into several more files that were time-locked around specific behavioral events. This approach was both tedious and created redundancies.

HDCV facilitates the collection and analysis of large amounts of data. Unlike TarheelCV, 'continuous' data ranging from minutes to hours can be stored within an HDCV file. Experimental progress is viewed with live displays while the file is written. Afterwards, the file can be reviewed in the analysis program. Memory limitations are avoided by recalling a portion of the binary file at a time. CVs used for digital background subtraction can be taken from any point in the file, while zoom and pan controls allow the user to navigate through time in the file.

The utility of HDCV's extended time views was demonstrated while analyzing dopamine release following intravenous drug administration.[29] Here we show a prolonged dopamine

response triggered by presentation of audio noise to an awake rat. Figure 4 shows data from a multi-minute experiment, where data were collected in separate 30 s files using TarheelCV. Noise begins shortly into the first file and continues for the remaining time. When viewing this data in TarheelCV, each 30 s period must be individually background subtracted (yellow line). This can lead to discontinuous data sets.[30] A concatenated version of the data viewed in HDCV is shown below (Fig 4B). One set of CVs from the beginning of the file is used for background subtraction. Both representations of the data reveal a decrease in dopamine (negative current at 0.6 V) when the white noise begins. A CV captured at 75 s in Panel 4A shows little change because of the background subtraction method. However, a CV taken at the same time point in the continuous version of the data reveals continuation of the negative dopamine transition (Fig 4C).

Continuous analysis of FSCV experiments is limited by the signal-to-drift ratio, with the drift arising from the background current. Principal component regression (PCR) is one method of assessing this limitation. In Figure 4D, the dopamine concentrations from the continuous file were extracted with PCR utilizing dopamine and pH training sets. In this example analysis, an extraordinary large decrease in extracellular dopamine is apparent after the onset of the noise. Positive concentration spikes at the end of the trace are due to electrical stimulations. However, the residual, which represents signals not accounted for by PCR analysis, surpasses the expected noise levels (shown as Q_a, Fig 4D inset) at 135 s. This indicates that the components of the training set do not accurately describe all significant sources contributing to the current signal, most likely due to drift in this example. Therefore, concentrations determined by PCR past this time point are statistically unreliable.[16] If the unknown current source is identified and added as a component of the training set even longer analysis is possible.[6]

Time Bin Generation and Signal Averaging

The application of FSCV in behavioral neuroscience has provided a greater understanding of dopamine in reward, learning and addiction.[22,31] Behavioral paradigms often involve a series of events that are repeated at random intervals. In these experiments data are continuously recorded while events of interest, such as a cue or lever press, are marked by digital transitions produced by an operant conditioning system. Both electrochemical and digital data can be viewed simultaneously in HDCV Analysis (Fig 5A).

The analysis of such data requires breakdown into smaller time bins, usually aligned to a given digital event. Each time bin is separately viewed, electrical artifacts are removed, and concentrations of different analytes are extracted with PCR. Finally, signal-to-noise is improved by averaging multiple time bins from similar events. The analysis routine in TarheelCV was disjointed and time-consuming, requiring an individual file per time bin, and externally generated training sets for PCR. Now, HDCV streamlines this analysis by incorporating the entire process and providing a greater degree of feedback. After opening a continuous file, the user indicates the digital line of interest, the direction of the transition and the time frame surrounding the event. HDCV allows visualization of each time bin. An option on the main screen allows removal of electrical artifacts by replacing them with an average of CVs taken before and after the artifact. All of this work can be saved in a file that is separate from the original data, which avoids storage of redundant information.

An example time bin recorded during an ICSS experiment is shown in Figure 5B. The data in Figure 5A was recorded during multiple ICSS trials, each one beginning with the audiovisual cue (digital line 4). The time bin shown in Figure 5B was aligned to this cue. The user can select the set of voltammograms for digital background subtraction for each time bin.

HDCV supports each step of PCR by making it easy to capture and save standards from many files. Tools that aid in the construction of a valid training set include a principal component score plot used to evaluate linearity of training set cyclic voltammograms, a K-matrix plot which evaluates the predictive ability of the calibration model for each analyte, and a Cook's Distance calculation which is used to identify improper training set samples. All of these approaches are described elsewhere.[30] HDCV integrates these tools into the user interface, whereas before users were forced to use multiple programs for calculations including Microsoft Excel and MATLAB.

The user-constructed training set is then applied to a data file or set of time bins. A new window displays concentration and residual information for each time bin, and the user can further refine which time bins to keep for averaging. Figure 5D shows results obtained from this process. The dopamine concentration for the time bin aligned to the ICSS cue shown in 5D is displayed on the left. An initial increase in dopamine concentration is seen in response to the cue at time 0. A second increase appears approximately 3 s later when the animal receives an electrical stimulation for pressing the extended lever. The central panel in Figure 5D shows the average from 40 similar time bins revealing the same features but with an increased signal-to-noise ratio.

Proper time bin alignment is crucial when interpreting data from such paradigms. In the averaged data (Fig 5D, middle), the alignment provides an attenuated view of the stimulation response because the animal pressed the lever at variable times after the cue. Averaged time bins are easily realigned to another digital line in HDCV, a process that required reanalysis of the entire data set in TarheelCV. In Figure 5D (right) the same time bins are averaged to the stimulus onset to provide an accurate response profile. All of these analysis features reduce the amount of time required to analyze the results of such experiments (from days to hours).

Conclusion

HDCV is a reliable, established program for FSCV. It has multiple features that enable flexible data acquisition and facilitate data analysis. The program retains features of previous programs while adding improvements in signal processing, data interpretation, and alignment with external events. The program was designed to enable future experiments that enhance the information obtained concerning neuronal processing during behavioral activities. The software is freely available for non-profit use on the website of the UNC Chemistry Electronics Facility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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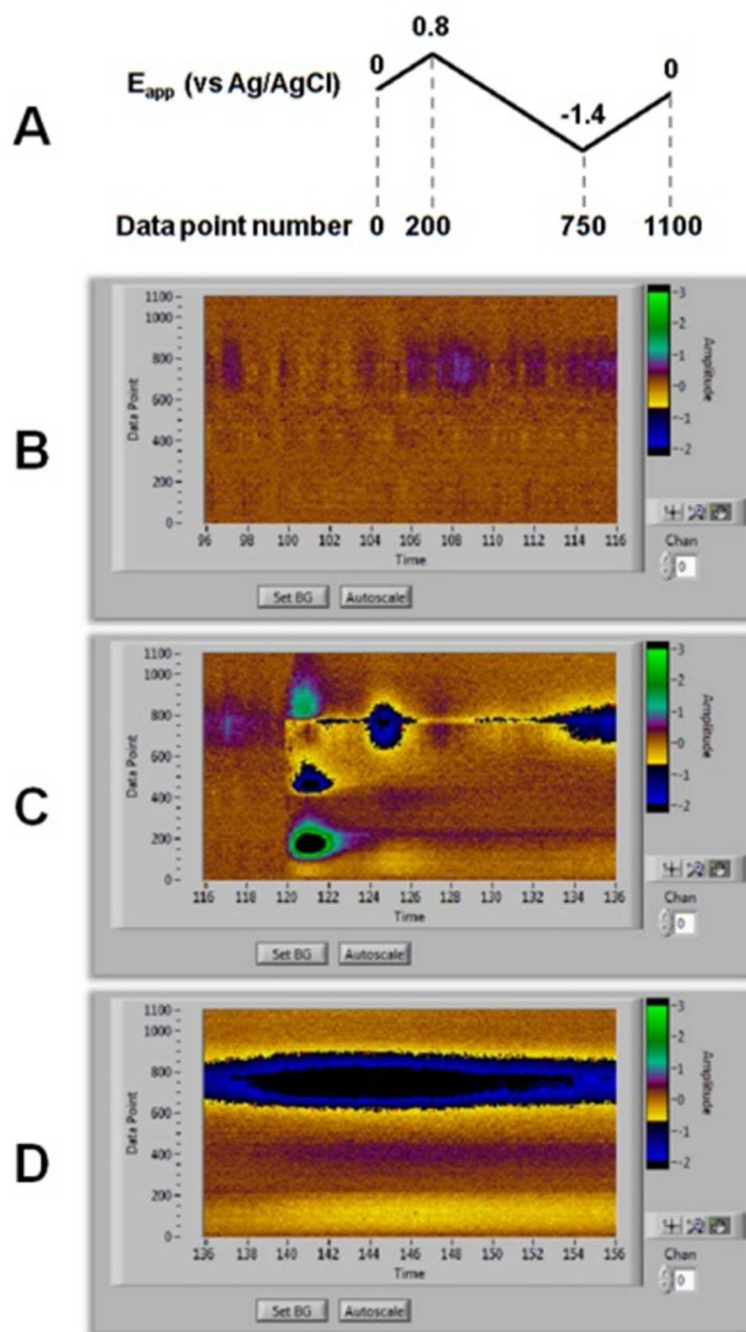
Figure 1.
Results from *in vivo* recordings with a carbon-fiber electrode implanted in the nucleus accumbens of an anesthetized rat using a waveform designed for simultaneous detection of O_2 and dopamine. (A) Voltage waveform and data points used to produce the voltammograms. This scan is applied at 400 V/s every 100 ms. (B, C, D) Screen shots of the live, active color chart taken in three consecutive time intervals. Waveform data point number appears on the ordinate and time (s) is shown on the abscissa. Digital background subtraction has been set just before the present view. (B) Oxygen decreases are visible around data point 750. (C) An electrical stimulation evokes dopamine release (data points

160 and 450) and increases in oxygen. The oxygen change continues into the next 20 s time window (D).
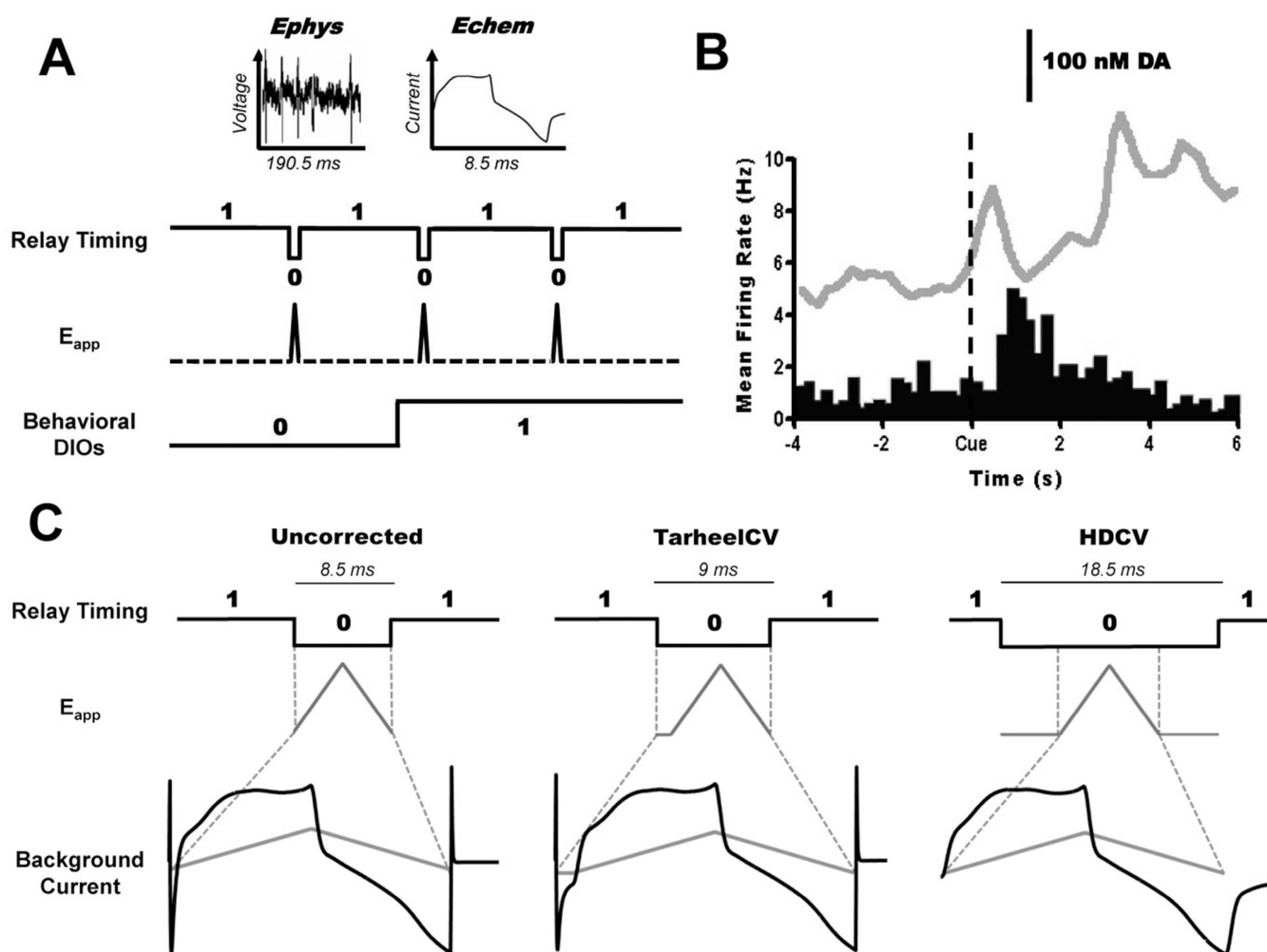
Figure 2.
Simultaneous electrochemistry/electrophysiology (echem/ephys) measurements. (A) Basic timing diagram during behavioral experiments. The state of the digital relay timing line controls when the potential of the electrodes floats (E_{app} dotted) and the waveform is applied (E_{app} solid). Behavioral events are recorded as digital transitions. (B) Example average (n = 30 trials) of dopamine concentration (grey trace) and average single unit firing rate (histogram) during ICSS recorded in the nucleus accumbens of an awake rat. The stimulating electrode in this study was implanted in the dopaminergic neurons of the ventral tegmental area. Data are aligned to the audiovisual cue. (C) Current response during waveform application. Voltage is applied to the electrode when the relay state is low while the recording window, defined by the CV application frequency, is denoted by the grey dashed lines. When the relay is in sync with the CV frequency clock (uncorrected, left) current spikes occur at the beginning and end of the voltage ramp. The CV frequency clock also triggers the relay in TarheelCV (middle), but the recording window is increased to add holding time before the voltage sweep. In HDCV (right) a line distinct from the CV frequency clock is configured to hold low 5 ms before and after the recording window.
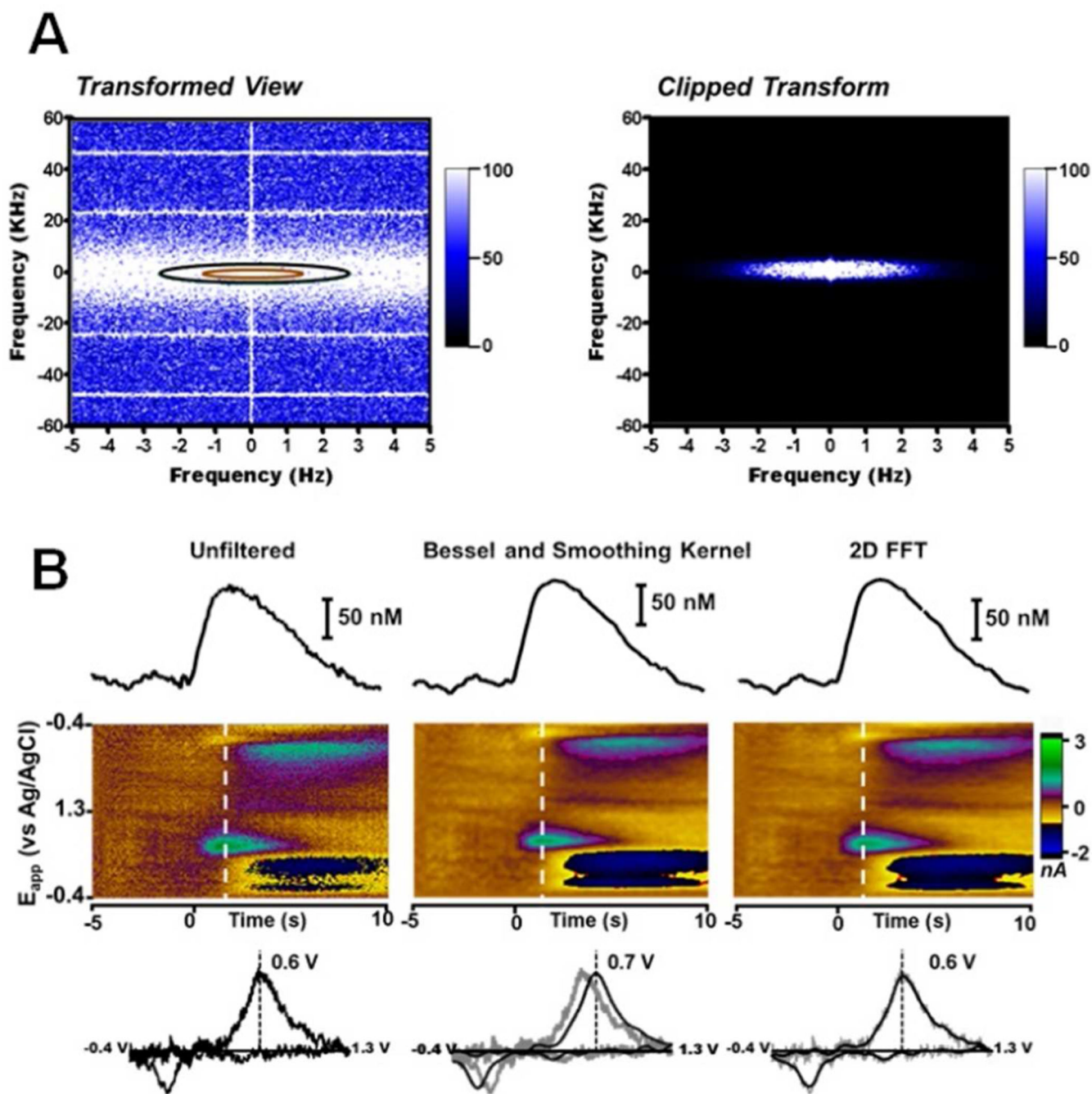
Figure 3.
Use of the 2D FFT filter. (A) Transformed view of the original and filtered (clipped) data. Signal intensity at each frequency is shown for the CV domain (ordinate) and the time domain (abscissa). Both axes are centered at 0 Hz and extend from $-f_s/2$ to $f_s/2$, where f_s is the sampling frequency. The -3 dB and -50 dB cutoffs are shown by the orange and black ellipses respectively. A Bessel roll-off is applied to smooth the transition between the -3 dB point and -50 dB point. Cutoff frequencies (time domain: 1.35 Hz, CV domain: 2 kHz) were chosen to resemble application of a 2 kHz low pass Bessel filter and an 8 point nearest neighbor smoothing kernel. (B) Comparison of filtering methodologies on data taken during *in vivo* norepinephrine release in the bed nucleus of the stria terminalis of an anesthetized

rat. Release was evoked by electrical stimulation of the ventral noradrenergic bundle. The original unfiltered data is shown to the right. Stimulation onset is denoted at time 0. Norepinephrine concentration extracted by PCR is shown as a trace above the color plot. The CV below is taken from the time point indicated by the white dotted line. The middle panel shows this data after filtering with the 2 KHz low pass Bessel filter and smoothing kernel. The right panel shows the data after the 2D FFT filter with the parameters shown in part (A).
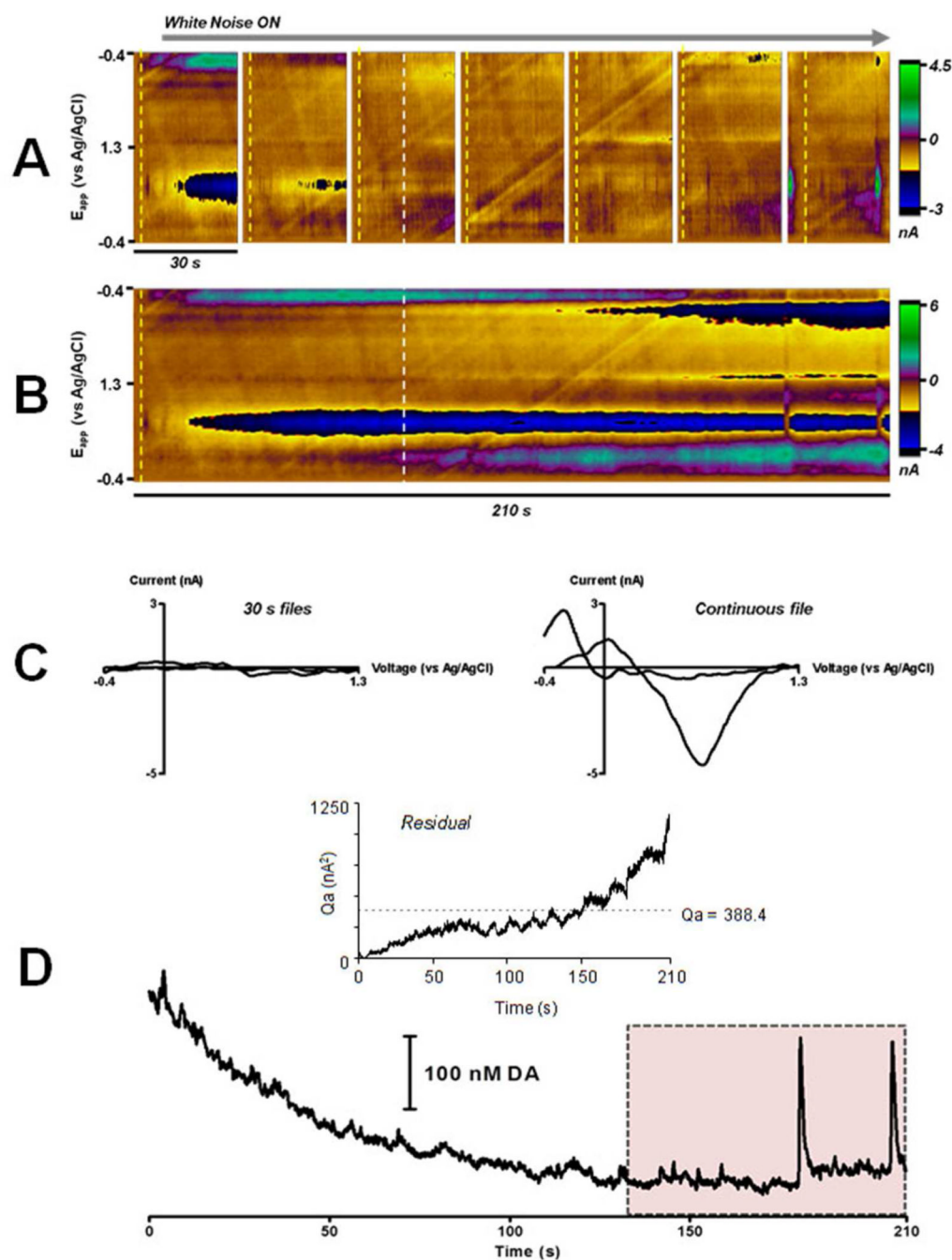
Figure 4.
Continuous data analysis in HDCV. (A) Electrochemical recording of dopamine with the carbon-fiber electrode implanted in the nucleus accumbens of a freely-moving animal. Data is shown in 30 s file segments, as originally collected with the TarheelCV acquisition program. White audio noise begins at the bar above the color plots. Each file is background subtracted at the time indicated by the yellow dotted line. (B) Data from part (A) concatenated into a continuous HDCV file through a data convertor program. Unlike TarheelCV, HDCV reads only the requested portion of the data file at a single time permitting long periods of data to be analyzed with a single background subtraction time point (yellow dotted line). (C) Background-subtracted CVs taken from the 30 s and

continuous files at the same time point (white dotted lines in A and B). (D) PCR of the continuous data reveals a decrease in dopamine concentration over time. Based on the residual analysis (inset) the concentrations in the shaded box are statistically unreliable.
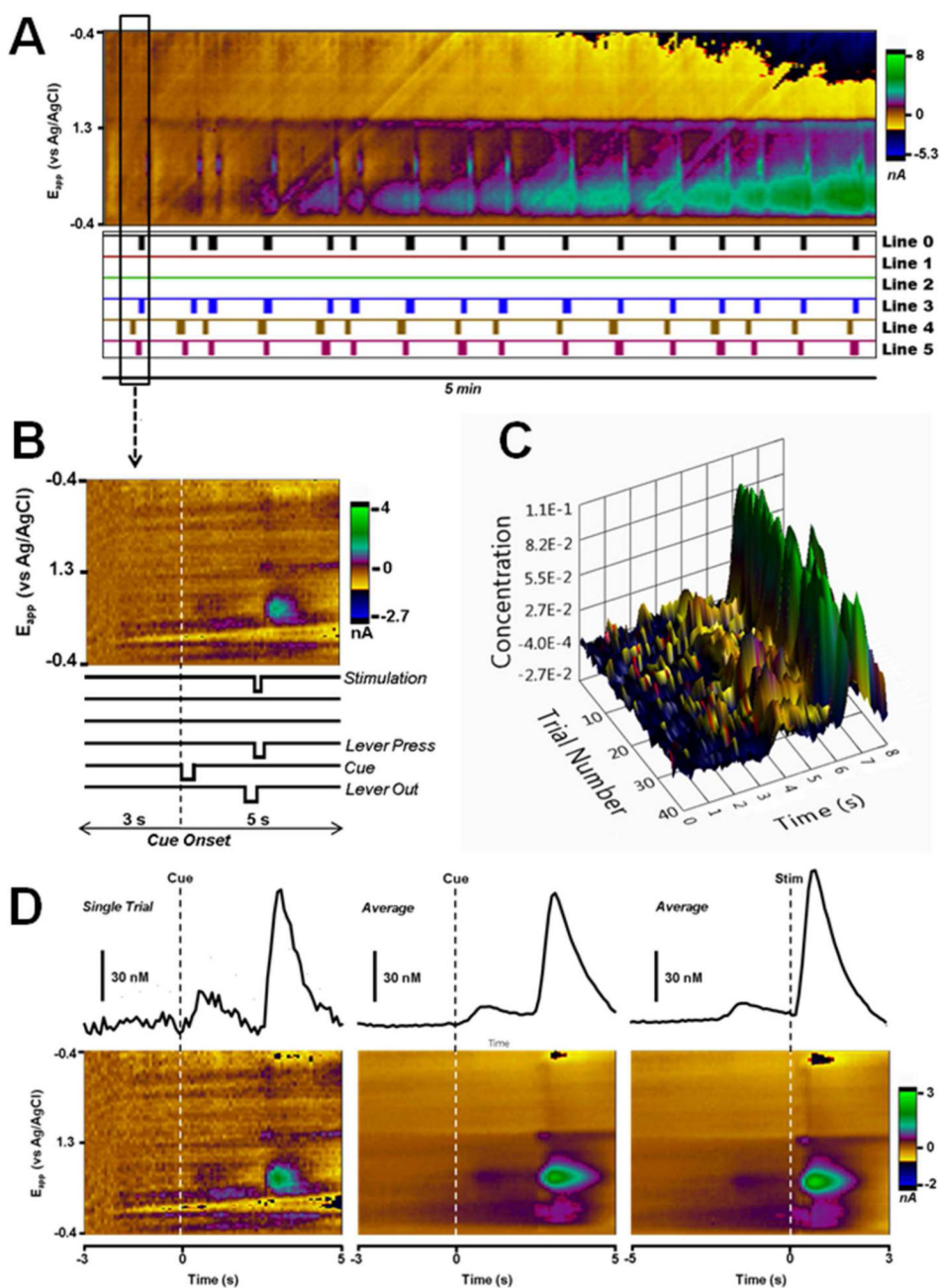
Figure 5.
Time bin analysis procedure. (A) A five minute view of continuous data collected during ICSS. HDCV displays both electrochemical (color plot) and digital input data simultaneously on its main screen. (B) A time bin extracted from the data in (A). The time bin is aligned to the falling edge of line four, which indicates the presentation of an audiovisual cue. Digital transitions corresponding to lever out, lever press and stimulation events also occur within this time frame. (C) Progression plot showing dopamine concentration (MM) versus time for 40 consecutive ICSS trials aligned to the cue. (D) Single and average (n = 40 trials) time bin data extracted with the training set shown in (C).

Dopamine concentration is shown as a trace above each color plot. The identity and timing of the alignment event is indicated by the dotted vertical lines.