Supporting Information for

Novel, User-friendly Experimental and Analysis Strategies for Fast Voltammetry: 1. "The Analysis Kid" for FSCV

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1. Video tutorial of The Analysis Kid. A video tutorial of the web application was hosted in YouTube at the following link. The video tutorial intends to show users the main features of the web application and how to navigate between them.

2. GitHub code repository of the web application. The web application code can be accessed at the following link. The application utilizes the BSD-licenced KissFFT C++ library from Mark Borgerding.

3. Recommended format for reporting *The Analysis Kid.* The web application provides several distinct yet analogous methods to filter and model FSCV data. In this section we provide the authors' preferred ways to report the methods used.

Reporting can be preceded by "*The Analysis Kid*" followed by a parenthesis with the filtering method:

1. Gaussian smoothing
2. 2D low-pass filtering

And the Michaelis-Menten model used:

1. M-M, one reuptake kinetics
2. M-M, two reuptake kinetics

Additionally, users might also be interested in reporting the filtering parameters (e.g, number of repetitions and size of Gaussian kernel for Gaussian smoothing).

4. Computational speed of filtering methods. Measurements of time to perform 2D Gaussian convolution and 2D Butterworth low-pass were performed using JavaScript's "console.time()" and "console.timeEnd()" on Google Chrome v86. A Windows 10 Home, i7-5500U CPU, 12 GB RAM computer was used to carry out the test. Absolute values of execution times will strongly depend on specifications of the local machine and tasks load.

2D Gaussian convolution was set with a standard deviation of 3 pixels and 1 repetition. 2D Butterworth cut-off frequencies were set to be 15% of the maximum spectral frequency in each axis. Each filtering method was repeated 10000 times using a for loop, and the total and average time are presented in **Table C.1**. For the 2D Butterworth low pass filtering method, both the 2D FFT and 2D inverse FFT are considered as part of the filtering process and accounted in the measured time.

Table C.1. Computational Time of Filtering Methods

	2D Gaussian conv.	2D Butterworth low pass
Total time (ms)	1090124.23	2220078.67
Time per execution (ms)	109.01	222.01

5. Signal-to-noise ratio calculation. Measurements of SNR provided by the web app and reported in the manuscript were estimated as peak-signal-to-noise ratio of the color plots. Signal power, P_{signal}, was obtained from the absolute maximum peak in the color plot. Noise power, P_{noise}, was estimated as the standard deviation of a user-defined period of non-activity in the color plot. The SNR, expressed in decibels, was then calculated following **Equation C.1.**

$$SNR = 10 \cdot \log_{10}\left(\frac{P_{signal}}{P_{noise}}\right) \qquad\qquad (C.1)$$

6. Comparison of conventional 2D Gaussian smoothing and our fast implementation. Five *in vivo* evoked dopamine measurements were filtered using a conventional Gaussian smoothing algorithm and the approach with lower computational complexity described in the manuscript. **Table C.2** shows the average error found pixel-by-pixel between the two filtered color plots for each of the acquisitions. The low error validates the use of the fast approach as a very close approximation to a true Gaussian smoothing with a lower computational complexity.

Table C.2. Average Percent Error Between Conventional and Fast Gaussian Smoothing

Color plot	Average Pixel Error (%)
1	0.001747
2	7.67E-05
3	3.64E-04
4	8.94E-04
5	0.005982
Average	0.001813

7. Representative analysis of histamine (HA) color plot.

A.

B.

$$C(t) = 43.55 \cdot e^{-0.16 \cdot t}$$

$$t_{1/2} = 4.36 \text{ s}$$

$$SEE = 0.18 \ \mu M$$

C.
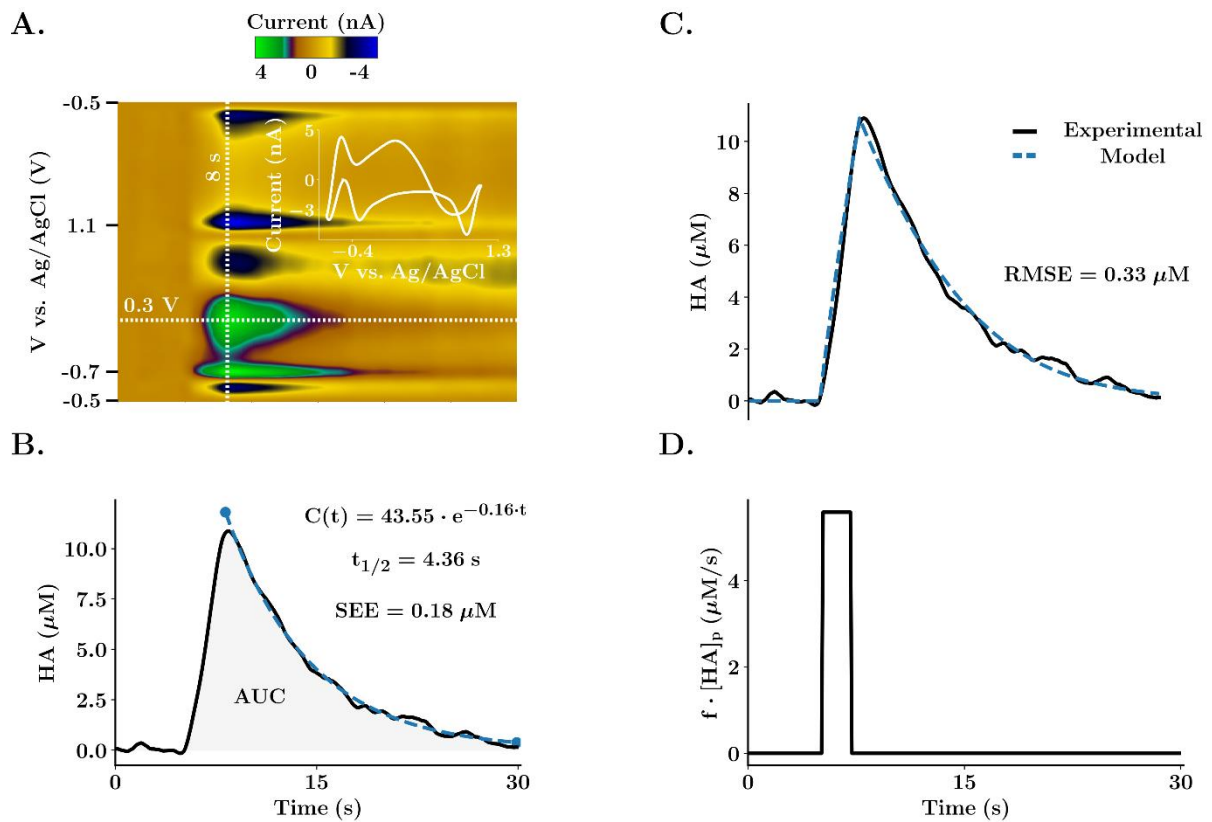

RMSE = 0.33 μM

D.

Figure C.1: Representative analysis of histamine evoked trace.
(**A**) FSCV color plot of HA evoked release in the premammillary nucleus of the posterior hypothalamus of the mouse brain. The horizontal dotted line illustrates the extracted current trace at the faradaic potential of interest. The vertical dotted line represents the extracted cyclic voltammogram, embedded in the CV. The inset white graph shows the extracted trace. (**B**) Histamine trace after calibration with a factor of 2.825 μM/nA. Blue dots represent the maximum and minimum amplitude points detected by the algorithm. Dashed blue line represents the exponential fit, also expressed as an equation together with the half-life of the reuptake and SEE. (C) Modelled one reuptake kinetics for the HA trace. The root mean squared error was used by the optimization algorithm to assess the goodness of fit. The model fitting yielded a $V_{max} = 3.33 \ \mu M/s$, $K_m = 1.37 \ \mu M$ and $[HA]_p = 0.07 \ \mu M$. (D) Release term of the modelled differential equation.
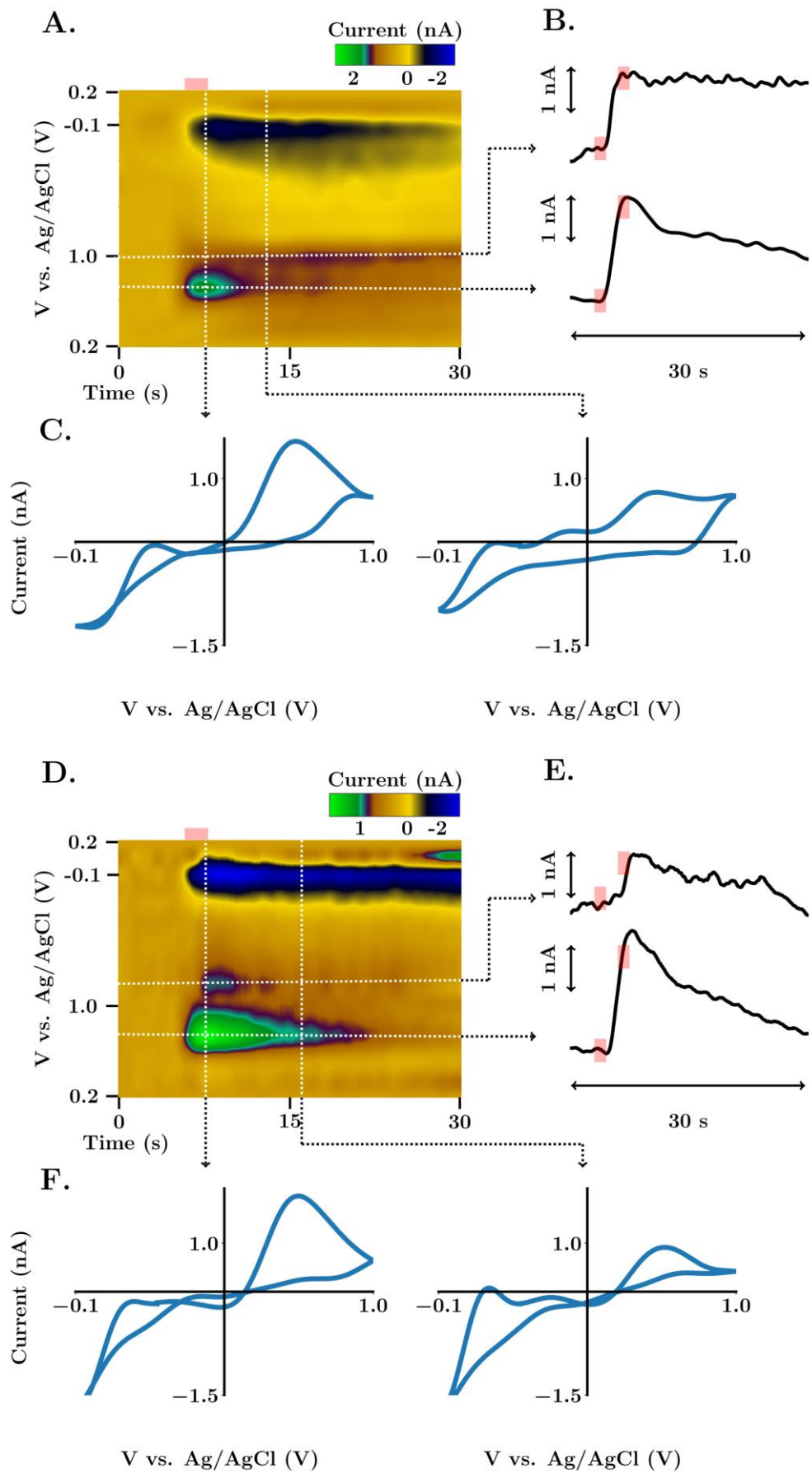
8. Evoked serotonin color plots.

Figure C.2: Examples of serotonin FSCV data collections in anesthetized mouse showing resolved serotonin signal throughout data collection.

(**A,D**) FSCV color plots of 5-HT evoked release in the CA2 region of the hippocampus of the mouse brain. The horizontal dotted lines illustrate the extracted current traces represented in **B** and **E** at potentials 0.85-1.0V (backwards scan) V (top) and approximately 0.7 V (bottom). The vertical dotted lines represent the cyclic voltammograms shown in **C and F**. In **C**, cyclic voltammograms are extracted at times 7.0 s (left) and 12.7 s (right). In **F,** cyclic voltammograms are extracted at times 7.0 s (left) and 16 s (right). Electrical stimulation is shown in **A, B, D** and **E** as red boxes. The cyclic voltammograms show a clear separation between the switching peak (centred around 0.85-1.0 V) and the serotonin oxidation peak at 0.7 V throughout the color plot for both acquisitions.
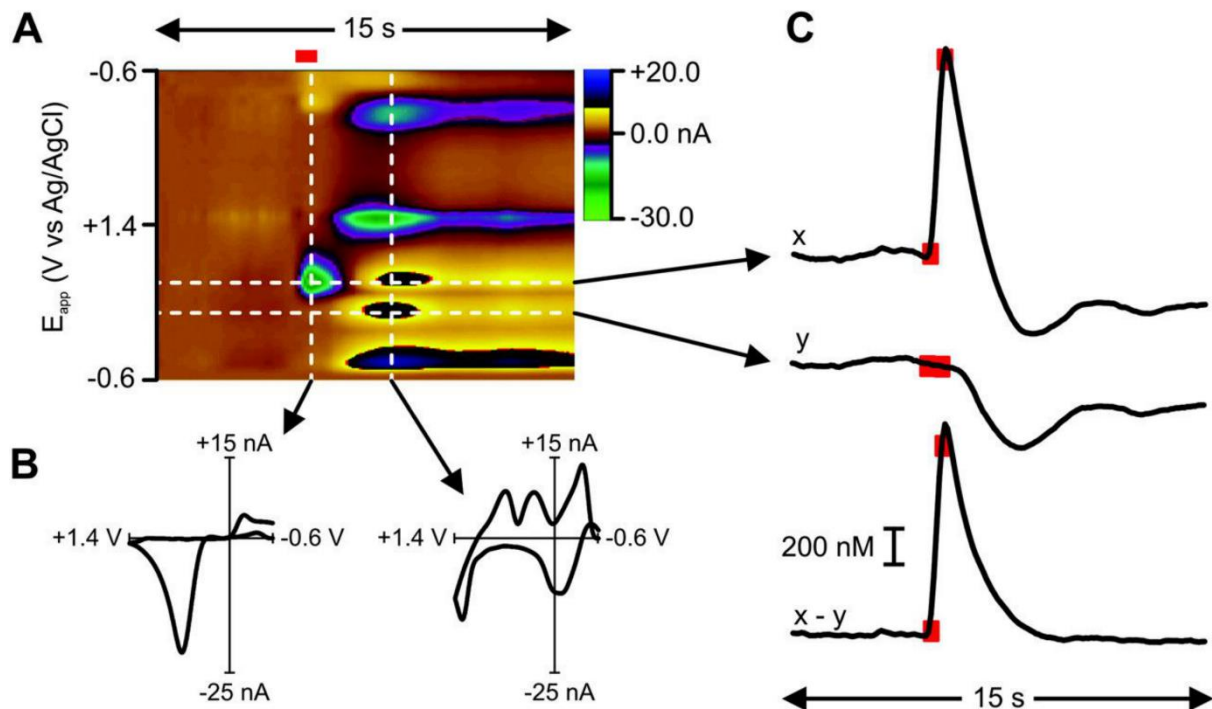

Figure C.3: An example dopamine FSCV data collection in freely-moving rats

Evoked dopamine release in the nucleus accumbens by stimulation of the ventral tegmental area in a freely moving rat. (**A**) Representative FSCV color plot of the acquisition. The horizontal dotted lines illustrate the extracted current traces represented in **C** at potentials 0.67 V (top) and 0.4 V (bottom). The vertical dotted lines represent the cyclic voltammograms shown in **C** at approximate times 6 s (left) and 8 s (right). Electrical stimulation is shown in **A** and **C** as a red square mark. Both the color plot and extracted cyclic voltammogram at 8 s show events that are clearly not changes in dopamine which overlap with the dopamine oxidation potential. In the original work, the authors subtracted the overlapping event from the current vs. time trace at the dopamine oxidation potential to remove the interference before conversion to dopamine concentration, shown in **C** (bottom). Reproduced from Roitman *et al.* with permission.[1]

References

(1)   Roitman, M. F.; Stuber, G. D.; Phillips, P. E. M.; Wightman, R. M.; Carelli, R. M. Dopamine Operates as a Subsecond Modulator of Food Seeking. *J. Neurosci.* **2004**, *24* (6), 1265–1271. https://doi.org/10.1523/JNEUROSCI.3823-03.2004.