Supporting Information

Glassy Carbon Microelectrode Arrays Enable Voltage-Peak Separated Simultaneous Detection of Dopamine and Serotonin Using Fast Scan Cyclic Voltammetry

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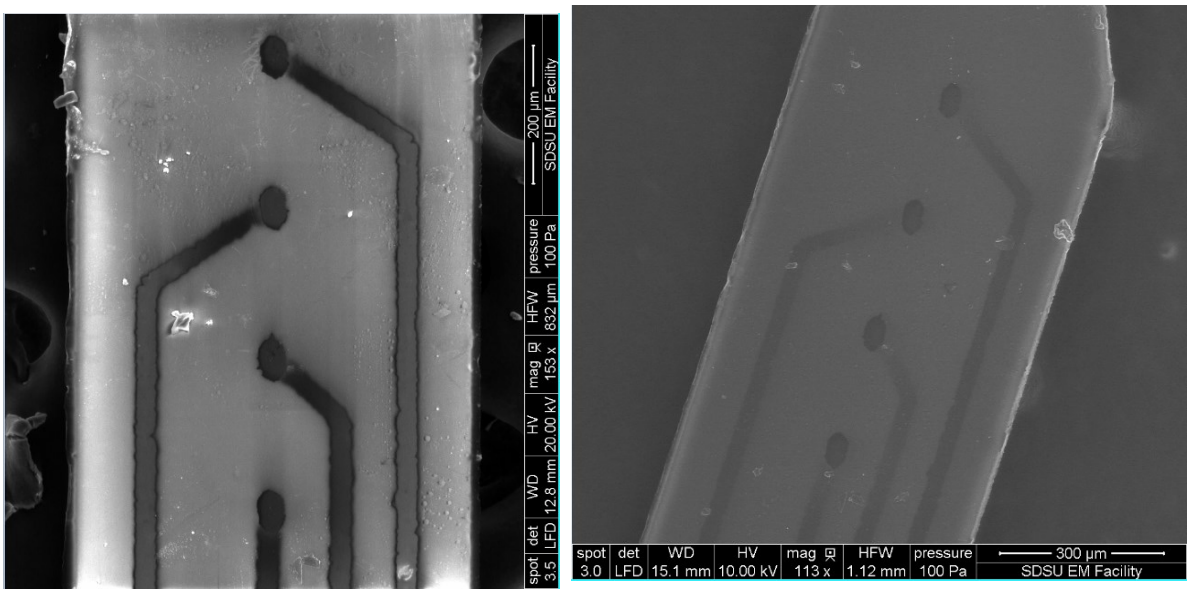
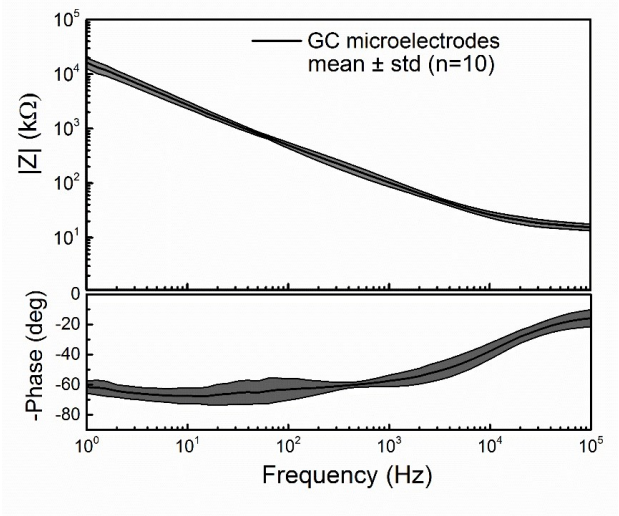
Glassy Carbon, Neural Probes, Microelectrode Arrays, C-MEMS, Fast Scan Cyclic Voltammetry, *in vivo* Neurotransmitter Detection, Dopamine, Serotonin, Simultaneous Detection



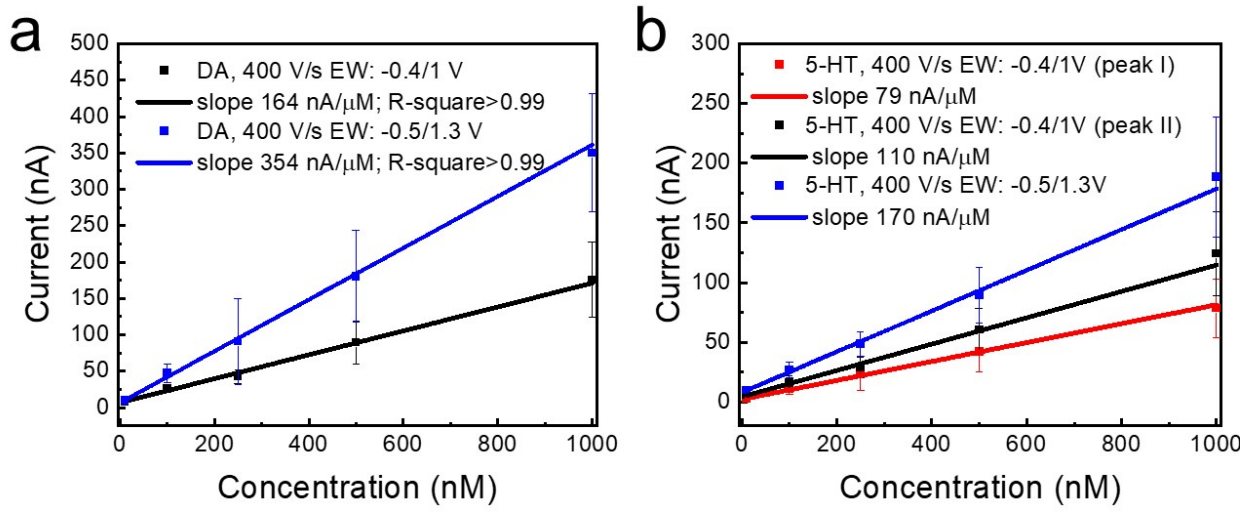
Supplementary Figure 1. SEM image of the glassy carbon microelectrodes.

Electrochemical Characterization through Electrical Impedance Spectroscopy

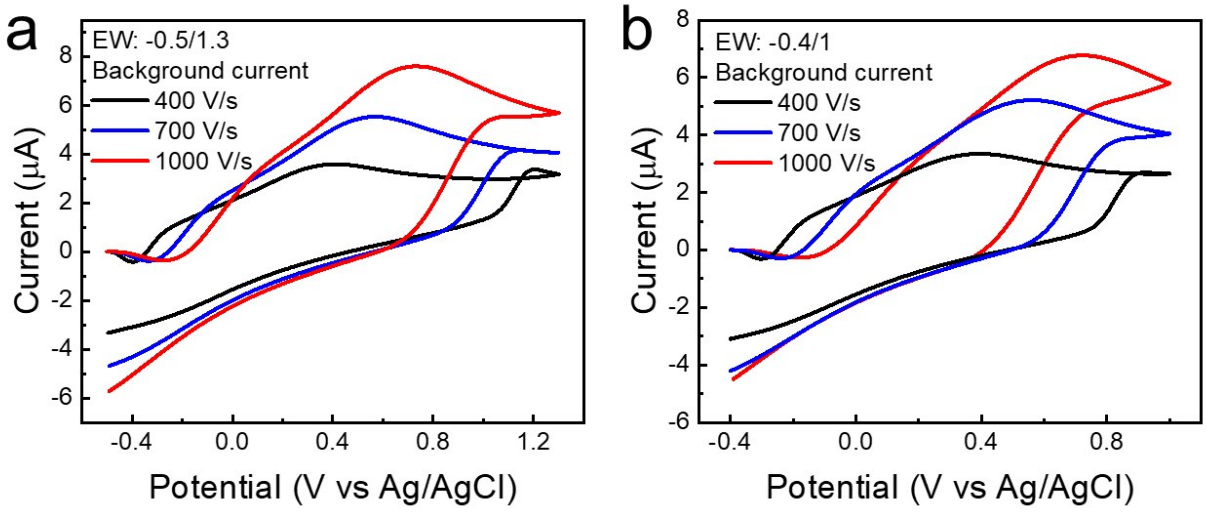
The electrochemical behavior of the microelectrodes was studied in phosphate-buffered saline solution (PBS; 0.01 M, pH 7.4; Sigma Aldrich, USA) using electrochemical impedance spectroscopy (EIS). During the EIS measurements, a sine wave (10 mV RMS amplitude) was superimposed on the open circuit potential while varying the frequency from 1 to 10^5 Hz. EIS was carried out using a potentiostat/galvanostat (Reference 600+, Gamry Instruments, USA) connected to a three-electrode electrochemical cell with a platinum counter electrode and Ag/AgCl reference electrode. EIS plots are shown in Supplementary Figure 2. The impedance values at 10 Hz, 100 Hz and 1 kHz are 2.7E6±424.9kΩ, 494.3±58.93 kΩ, and 103.1±16.8kΩ, respectively (n = 10).



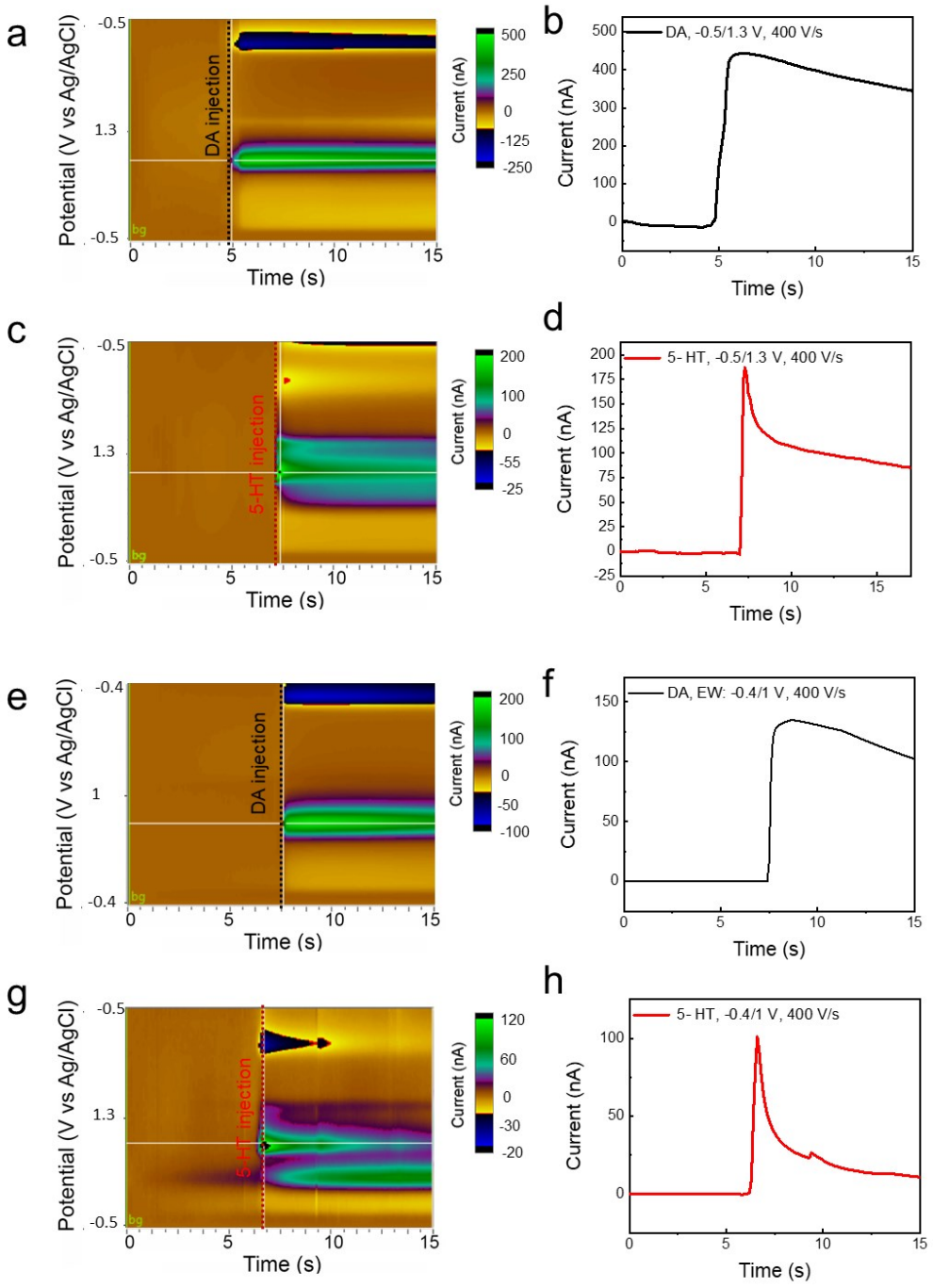
Supplementary Figure 2. EIS results for GC microelectrodes, (mean ± std, n = 10). The impedance values at 10 Hz, 100 Hz and 1 kHz are 2.7E6±424.9kΩ, 494.3±58.93 kΩ, and 103.1±16.8kΩ, respectively (mean and standard deviation, n = 10).



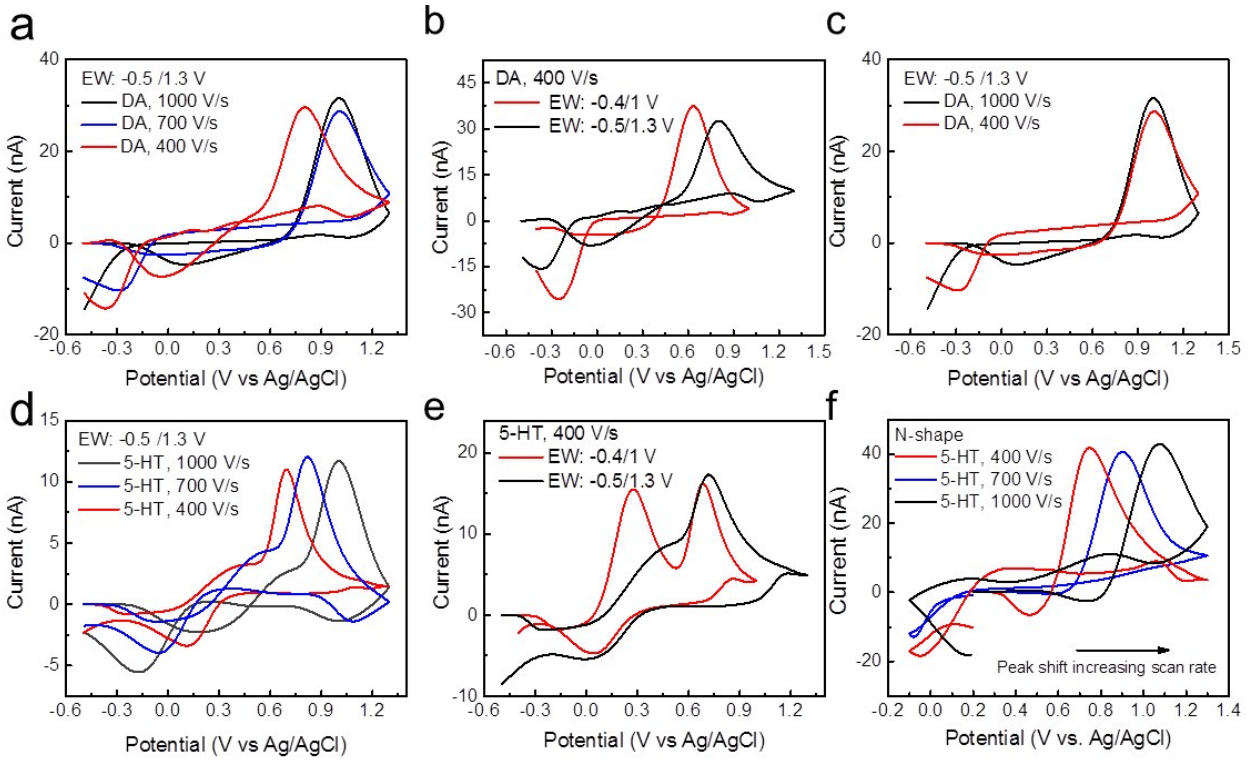
Supplementary Figure 3: *in vitro* FSCV calibration curves: (a) background subtracted oxidation current of DA versus DA concentration. Conditions: 400 V/s scan rate and EW: -0.5/ 1.3V in blue, and EW: -0.4/ 1V in black. In both cases**,** the average (n=10) sensitivity is linearly correlated. **(b)** background subtracted oxidation current of 5-HT versus 5-HT concentration. Conditions: 400 V/s scan rate and EW: -0.5/ 1.3V in blue, and EW: -0.4/ 1V in black for oxidation Peak II and in in red for oxidation Peak I. In all the cases**,** the average (n=10) sensitivity is linearly correlated. **(c)** background subtracted oxidation current of 50 %DA: 50%5-HT mixture versus concentration. Conditions: 400 V/s scan rate and EW: -0.4/ 1V in red for oxidation Peak II and in in black for oxidation Peak I. The average (n=8) sensitivity is linearly correlated.



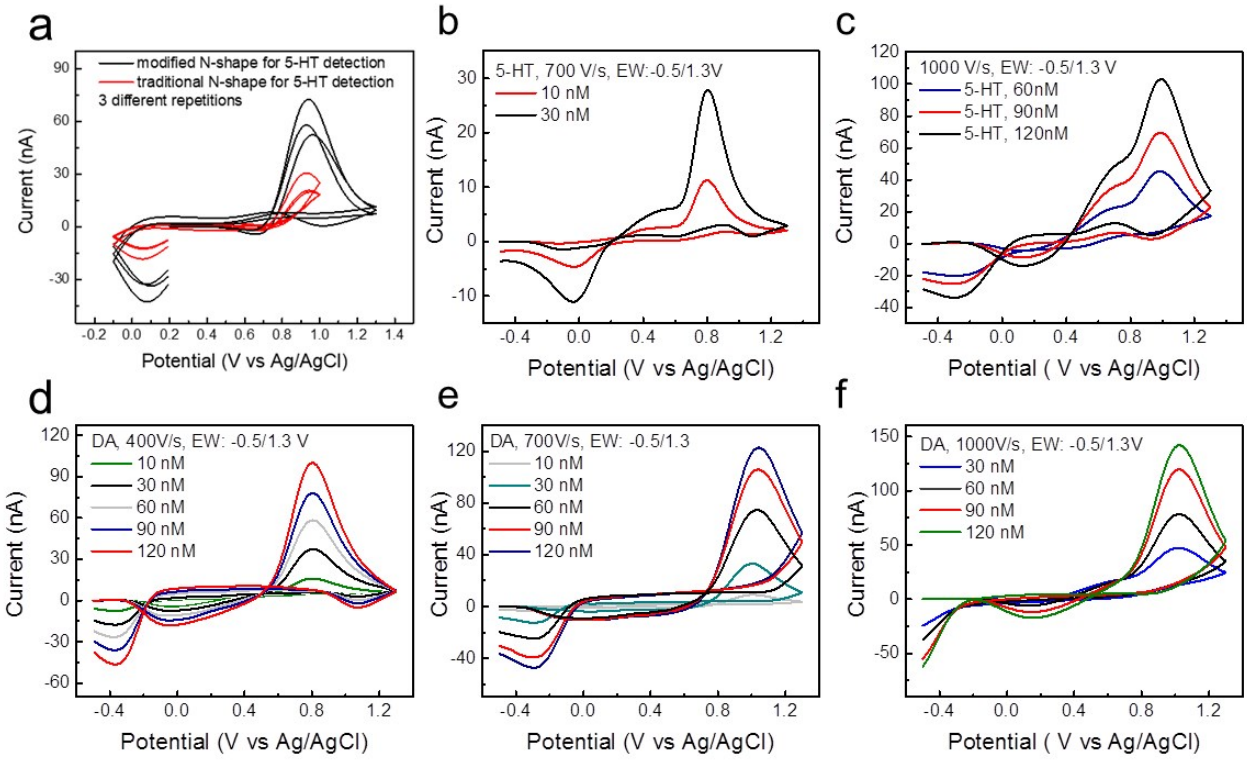
Supplementary Figure 4a: Representative capacitive background CV plots at 400,700 and 1000 V/s for EW: -0.5/1.3V (a), and EW: -0.4/1 V (b).



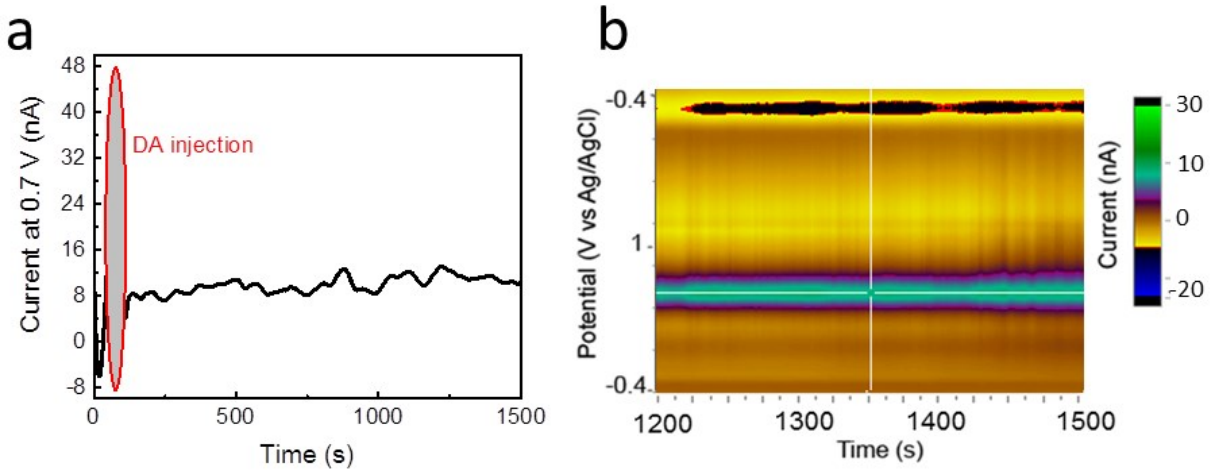
Supplementary Figure 4b: Representative color plots (a, c, e, g) with the corresponding current/time (b, d, f, h) plots in response of bolus injection of DA (a, b, e, f) and 5-HT (c, d, c, h) at 400 V/s for EW: -0.5/1.3V (a-d), and EW: -0.4/1 V (e-h).



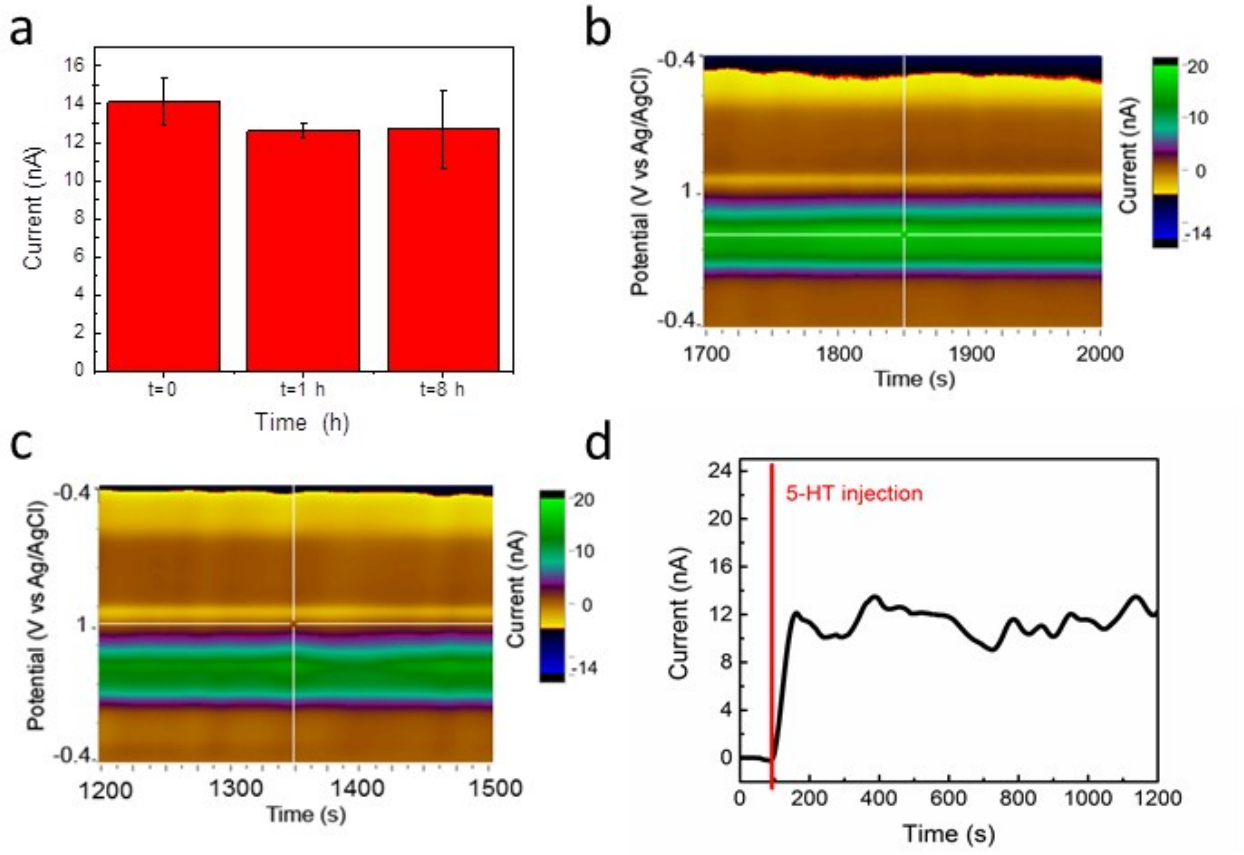
Supplementary Figure 5. Effect of Electrochemical Window (EW) and scan rates on DA (a, b, c) and 5-HT (d, e, f) kinetics. (a) Effect of scan rates on the response of DA. Peak shift is observed for wider EW (-0.5/1.3): for 1000 V/s, Ox = 1.01±0.02V V, Redx < -0.5V (black line),while for 700V/s, Ox = 1.02±0.05V, Redx = -0.29±0.06V (blue line), and for 400V/s, Ox = 0.79±0.01V, Redx = -0.35±0.01V (blue line), (b) Effect of EW and scan rates on the response of DA at 400V/s and (c) 1000V/s. Wider EW at 400V/s scan rate shifts Ox peaks to the right and Redox to the left. Due to the slow DA kinetics at GC electrodes using high scan rates (1000V/s), the wider EW (-0.5/1.3) allows for discrimination of Ox peak but not Redox peak. The -0.4/1V EW does not allow for DA peak discriminations. (d) Effect of scan rates on the response of 5-HT (400, 700 and 1000 V/s): at 400 V/s and -0.4/1V EW 5-HT present 2 oxidation peaks, while using wider EW (-0.5/1.3) and scan rates ≥ 700 V/s, primary and secondary peak seem to converge. (e) Effect of scan rates on 5-HT detection using modified *N*-shaped waveform.



Supplementary Figure 6 (a) Modified *N*-shape Waveform (black) versus Jackson waveform [41] (red), 3 different repetitions. It is important to note that in order to detect the positive oxidation peak (at 1.08V), we had to modify the traditional *N*-shaped waveform used for 5-TH detection at CFEs by extending the switching potential to 1.2V. (b, c) 5-HT detection (different concentrations) using the FSCV triangular waveform with -0.5 V holding and 1.3 switching potential (10 Hz), at 700V/s (b) and (c) 1000V/s. (d, e, f) DA detection (different concentrations) using the FSCV triangular waveform with -0.5 V holding and 1.3 switching potential (10 Hz), at 400V/s (d), 700 V/s (e), and 1000V/s (f).



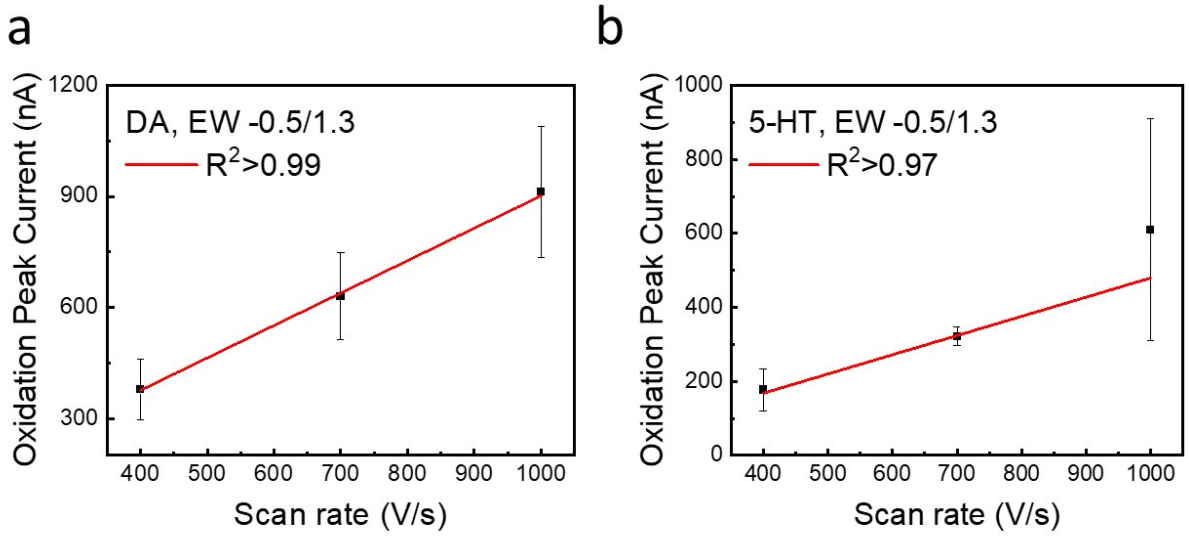
Supplementary Figure 7 DA fouling test. GC microelectrodes were continuously scanned in presence of 50 nM of DA using the triangular FSCV at 400 V/s. Due to our experimental set-up conditions, the potentiostat can record maximum 40 minutes of FSCV recordings at the time. (a) Representative time/current plot (corresponds to current at 0.7 V) for a recording session of 25 minutes. The current peak amplitudes in response to 50 nM of DA showed small oscillation over the entire recording session, both for oxidation and reduction peaks. Additionally, only small variations were observed in the FSCV capacitive background current demonstrating the electrochemical stability of the GC surface and enabling the continuous detection for the entire recording session. (b) Example of a color plot corresponding to 25 minutes of continuous DA detection.



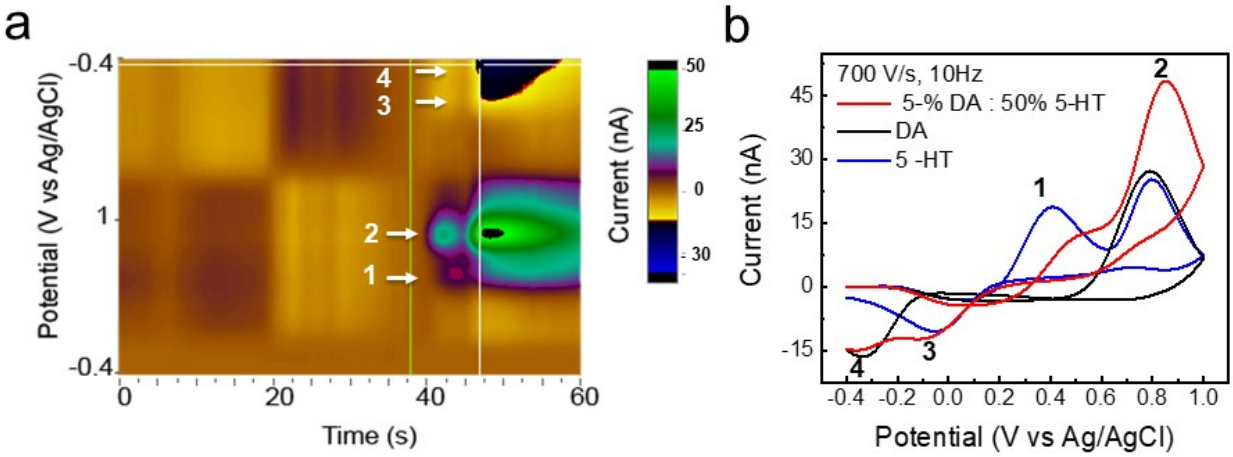
Supplementary Figure 8: 5-HT fouling test. GC microelectrodes were continuously scanned in presence of 50 nM of 5-HT and the 5-TH detection was monitored for 8 h using the triangular FSCV at 400 V/s. Due to our experimental set-up conditions, the potentiostat can record maximum 40 minutes of FSCV recordings at the time, thus every 20-40 minutes the PBS solution was changed, and a new injection performed. (a) The current peak amplitudes in response to 50 nM of 5-HT were stable with no significant (One-way Anova, $p>0.05$) drop in detection sensitivity after 1h or 8 h. (b, c). Examples of a color plot corresponding to 25 minutes of continuous 5-HT detection in two different recording sessions. (d) Representative time/current plot for a recording

In this study, both for DA and 5-HT, we considered physiologically relevant concentrations to make a data-supported case for determining biofouling of these electrodes in a realistic *in vivo* environment, where the FSCV waveform is constantly scanning. Indeed, the electrodes will be subject to high concentration only for a very short time (for fractions of seconds) during a fast release, while continuously scanning at the basal levels. Thus, our decision to continuously scan for 8h in constant presence of DA/5-HT. In vivo basal/tonic levels of 5-HT/DA have been reported to be in the nanomolar range (<100nM). *Aya Abdalla el al*. reported *in vivo* ambient serotonin level of 64.9 ± 2.3 nM in the CA2 region of the hippocampus, using fast-scan controlled adsorption voltammetry (FSCAV) at carbon fiber microelectrodes. Ian M. Taylor et. al measured a basal DA concentration of 82 ± 6 nM in the rat dorsal striatum using square wave voltammetry at

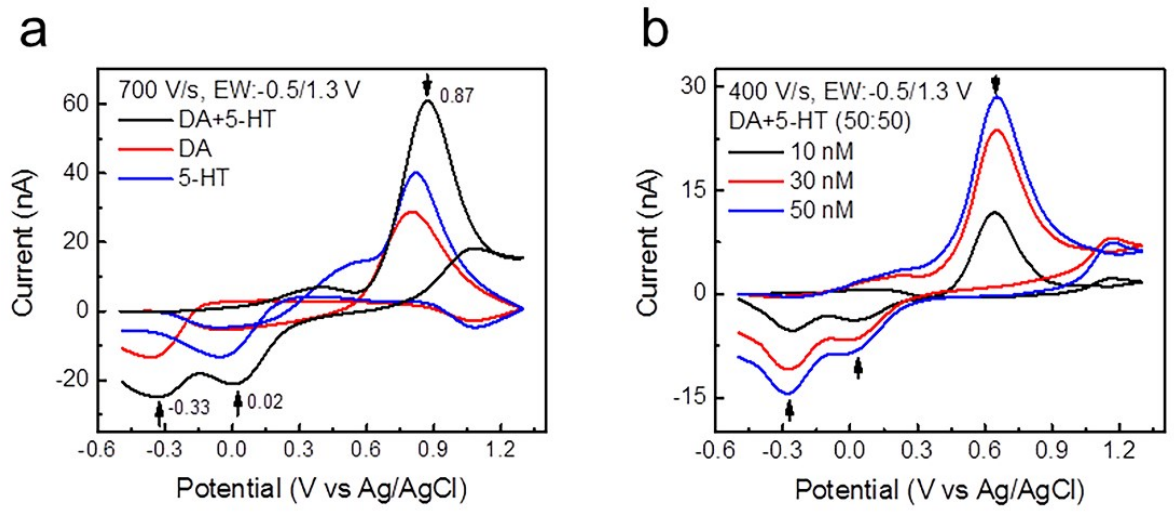
PEDOT/CNT coated carbon fibers[11]; C. W. Atcherley et al. measured 90 ± 9 nM in the nucleus accumbens of mice using by FSCAV12, J. A. Johnson et al. detected 41 ± 13 nM in the nucleus accumbes of rats convolution-based FSCV.[13]



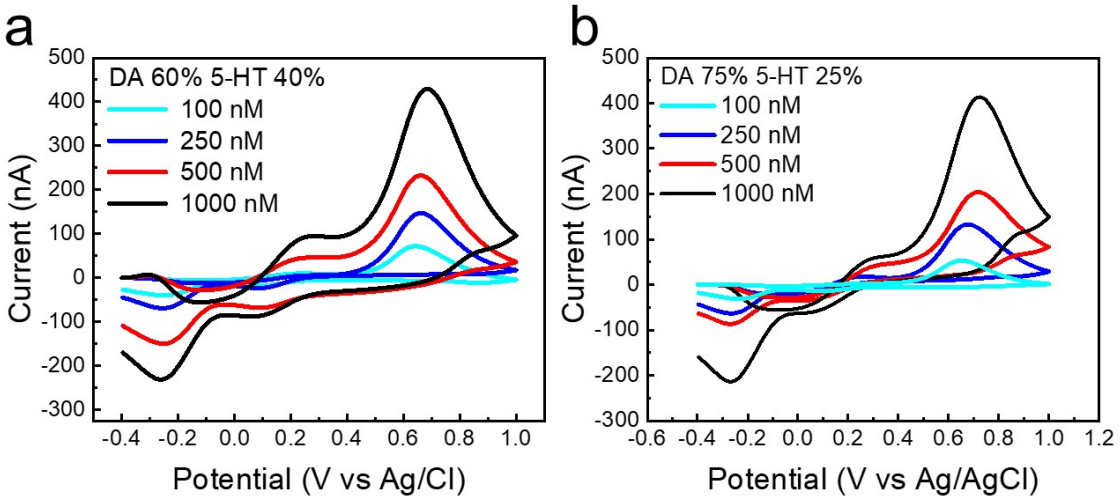
Supplementary Figure 9: Adsorption Studies: effect of scan rate. Oxidation peak currents versus the scan rate for both 1 µM DA (a) and 1 µM 5-HT (b). We observed that the current increases linearly as a function of the scan rate, denoting adsorption control.



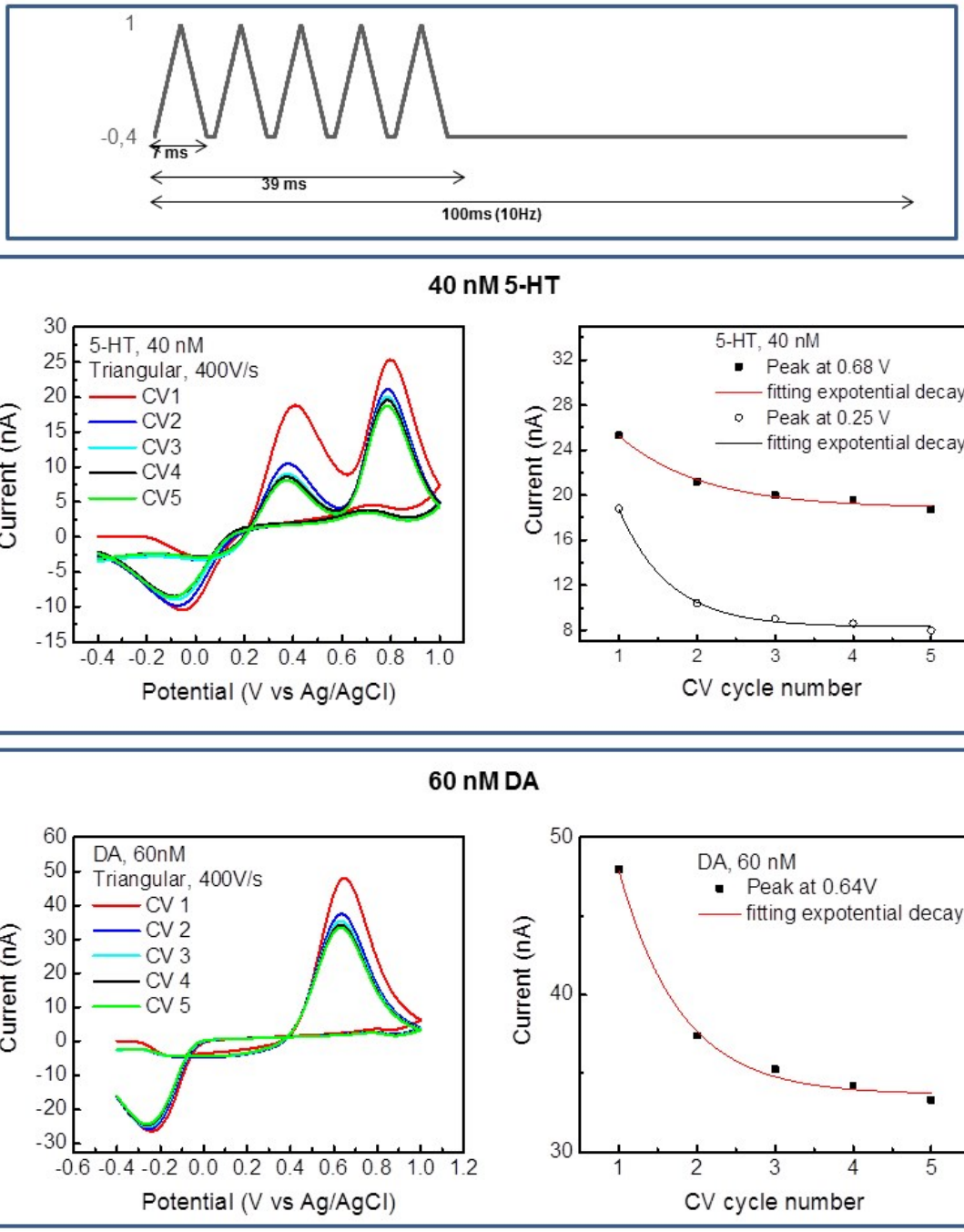
Supplementary Figure 10: Simultaneous detection of DA and 5-HT using -0.4/1 V EW at 700 V/s: color plot and (a) background subtracted CV (b) for 50 nM DA and 5-HT mixture (red) versus DA (black), 5-HT (blue), respectively.



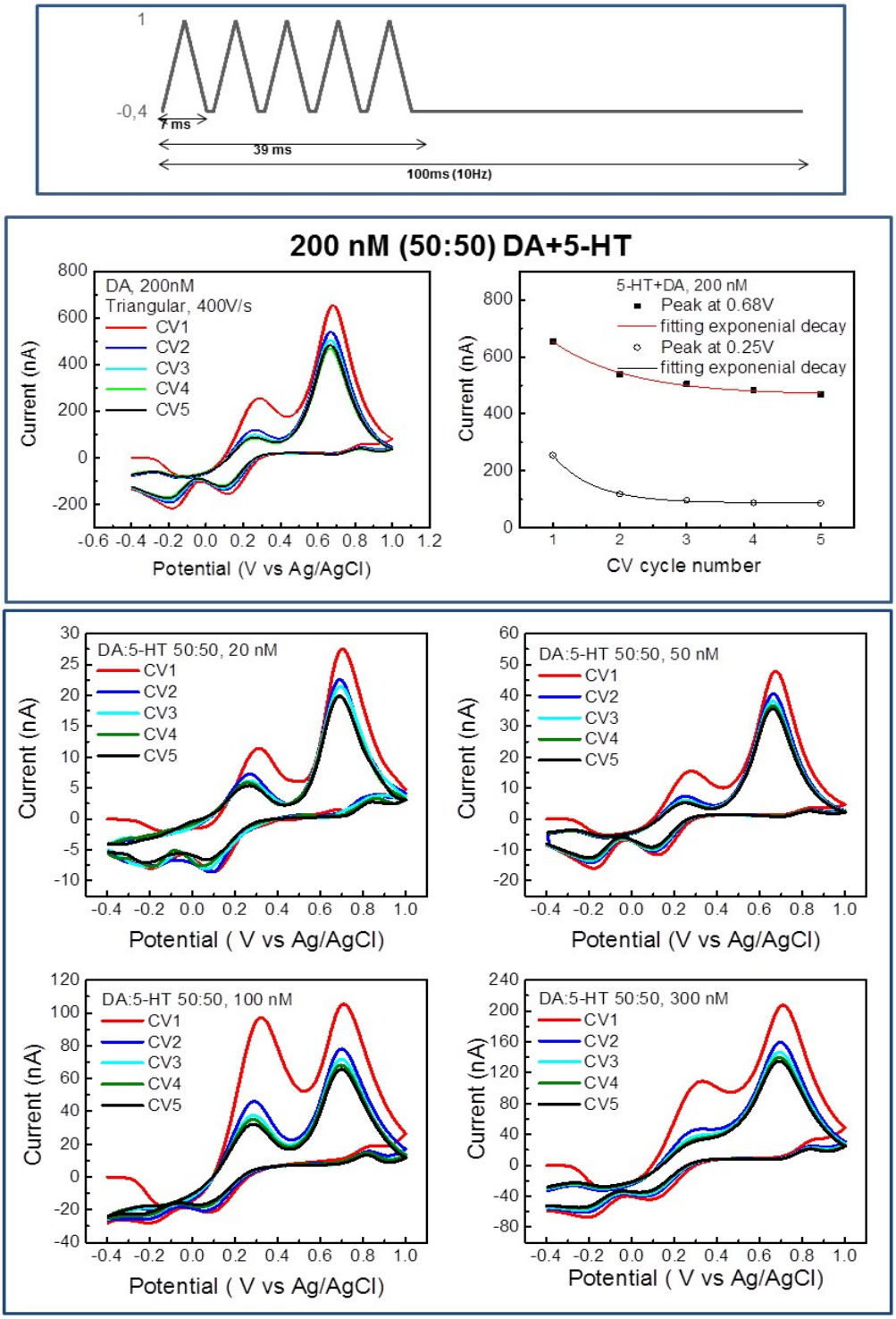
Supplementary Figure 11. **Simultaneous detection of DA and 5-HT using large EW: -0.5/1.3 V** at different scan rates (a) 400 V/s: 10nM (black), 30nM (red) and 50nM (blue) of DA and 5-HT mixture, (b) 700 V/s: 100 nM DA (red), 5-HT (blue) and their mixture (black) respectively.



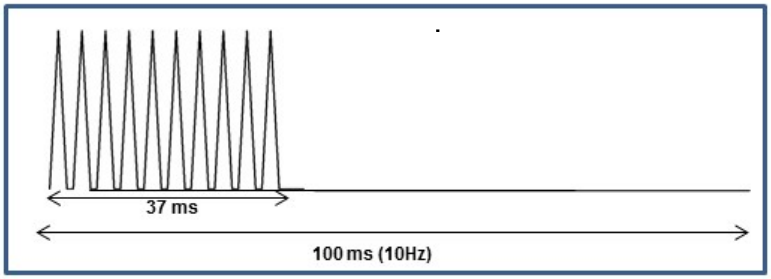
Supplementary Figure 12. Simultaneous detection of DA and 5-HT when using DA:5HT mixture at different ratios, i.e. (60% DA and 40% 5-HT) and (75% DA and 25% 5-HT).

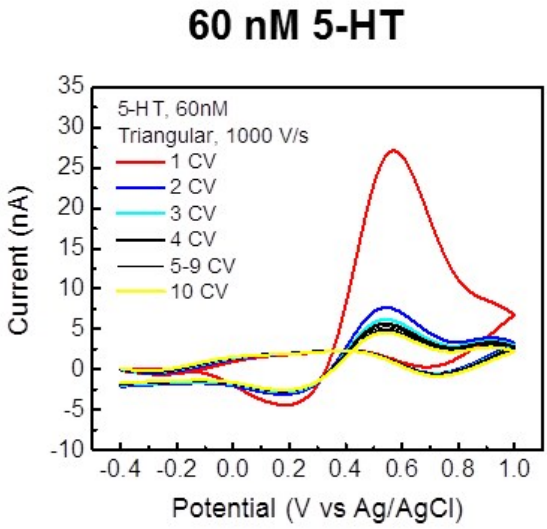


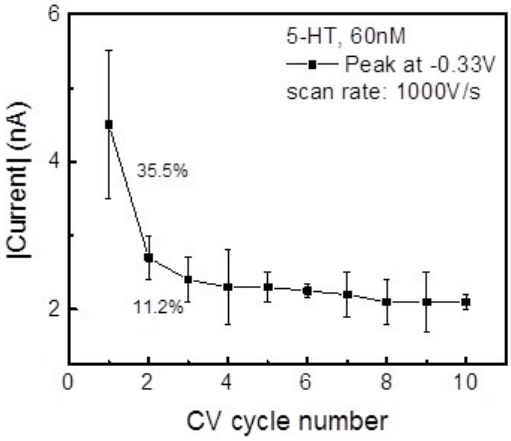
Supplementary Figure 13. Other examples of oxidation peaks decay of 5-HT and DA under M-FSCV at 10 Hz **(400V/s, triangular waveform, 5 scans with 1 ms of pause, EW: -0.4/1V).**

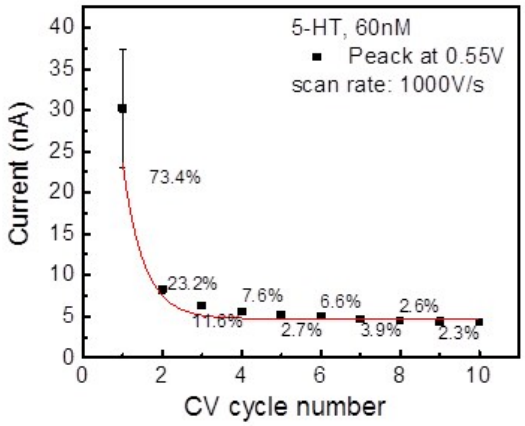


Supplementary Figure 14. Other examples of oxidation peaks decay of mixture of 5-HT and DA (different concentrations) under M-FSCV at 10 Hz (400V/s, triangular waveform, 5 scans with 1 ms of pause, EW: -0.4/1V).

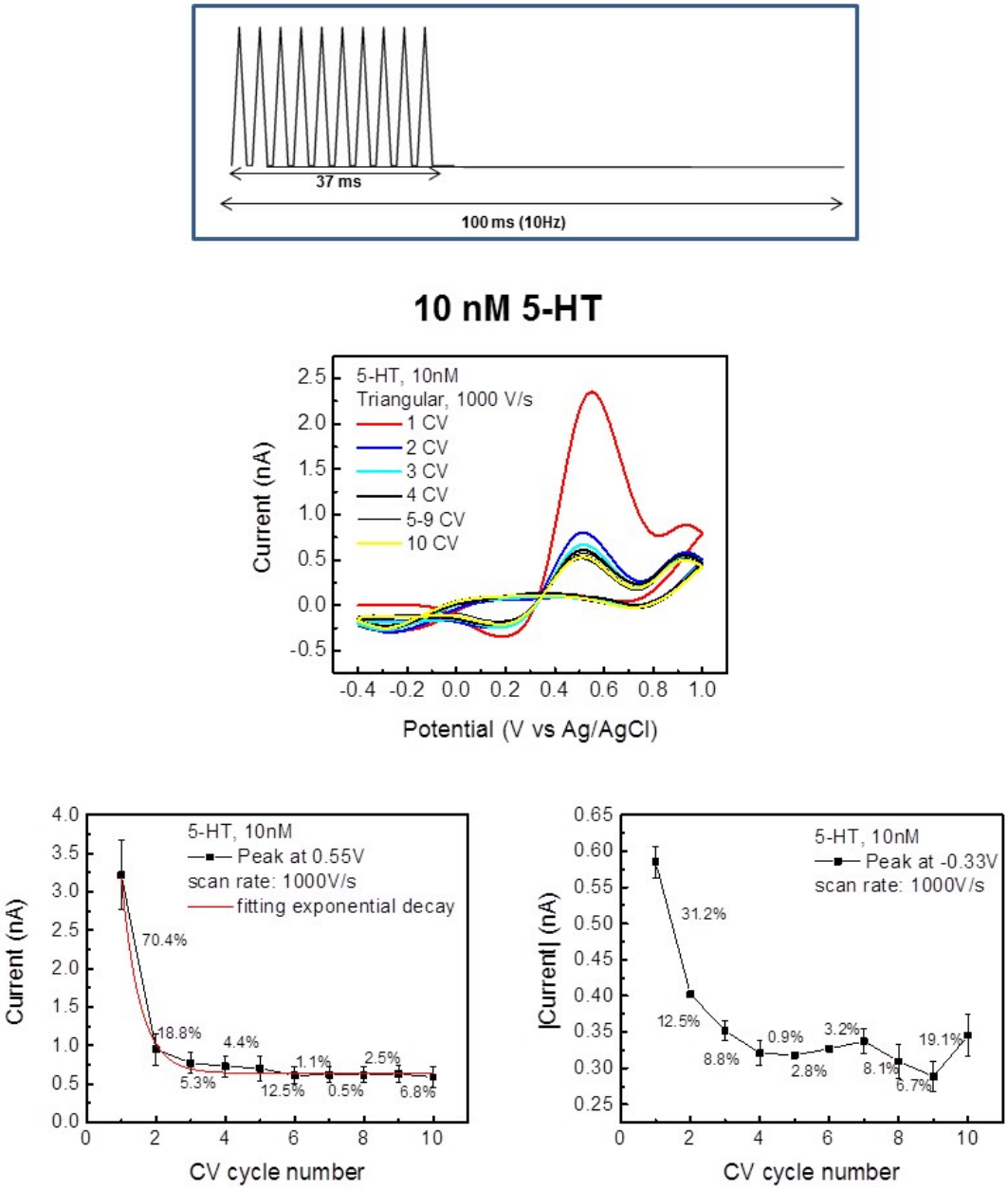




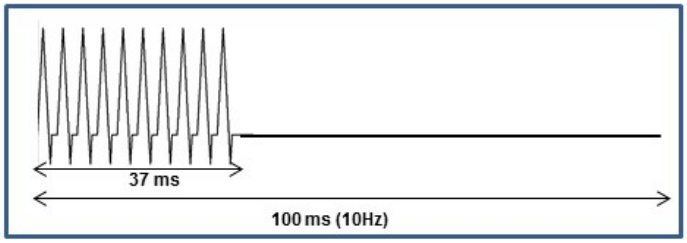


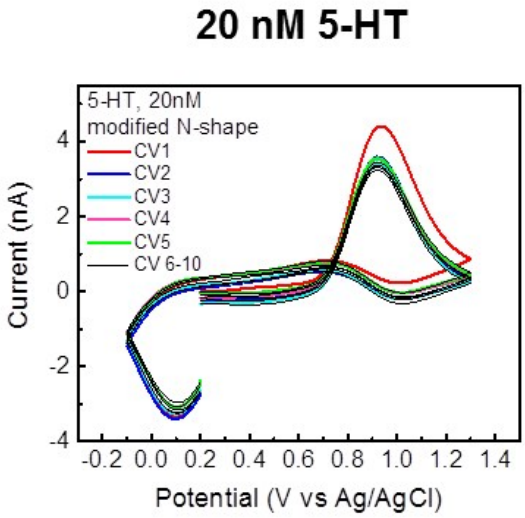


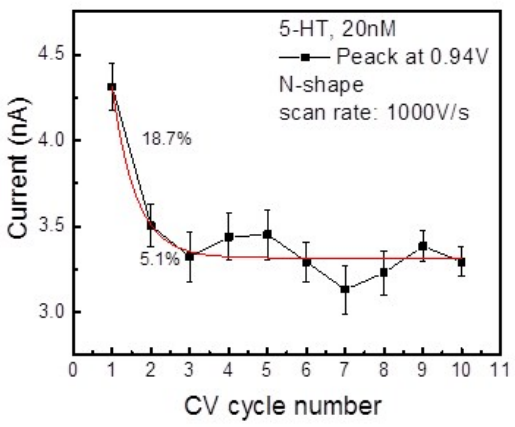
Supplementary Figure 15 a. Examples of oxidation and reduction peaks decay of serotonin (60 nM) under M-FSCV at 10 **Hz (1000V/s triangular waveform, 10 scans, with 1 ms of pause, EW: -0.4/1V).**

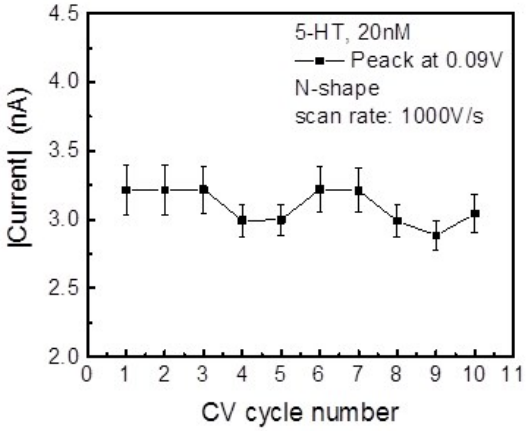


Supplementary Figure 15 b. Examples of oxidation and reduction peaks decay of serotonin (10 nM) under M-FSCV at 10 Hz **(1000V/s triangular waveform, 10 scans, with 1 ms of pause, EW: -0.4/1V).**









Supplementary Figure 16. Example of oxidation and reduction peaks decay of serotonin under *M*-FSCV at 10 Hz (1000V/s, 10 scans with 1 ms of pause, modified *N*-shape waveform).

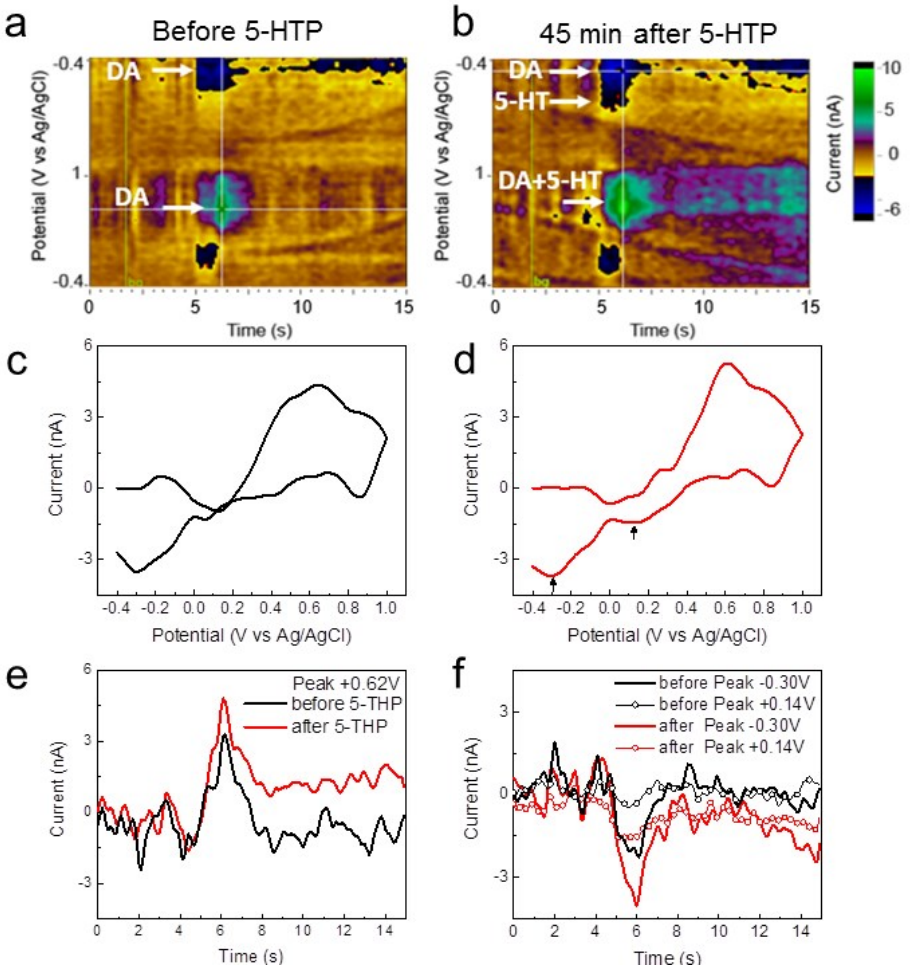
***Supplementary Section 2: In vivo* Co-detection of Dopamine and Serotonin: a proof-of-principle**

The proof-of-principle of *in vivo* electrochemical sensing performance of the GC microelectrodes for simultaneous FSCV detection of DA and 5-HT in the rat striatum. The procedure for recording DA and 5-HT simultaneously was adopted from the experiments by Swamy and Venton [19]. The GC MEA was implanted in the caudate putamen where DA terminals are located for measuring the DA release evoked by electrical stimulation of the cell bodies in substantia nigra. *In vivo* evoked 5-HT concentrations are expected to be drastically lower than DA concentrations in the striatum [19, 76]. Thus, to simultaneously detect DA and 5-HT evoked release, a pharmacologically manipulation of the 5-HT level is needed. After the MEAs were properly targeted and five consistent releases of DA were detected, carbidopa was administered 30 minutes before the injection of a synthetic precursor of 5-HT, 5-hydroxytrptophan (5-HTP), to prevent 5-HTP from being converted to 5-HT before it reaches the brain [19, 77]. 5-hydroxytryptophan (5-HTP) has been shown to drastically increase 5-HT release in the striatum [19, 77]. The color plot representations shown in Figure 7 a, b illustrate the current response following the *in vivo* electrical stimulation (60Hz, 250μA) at the substantia nigra region, before (a) and 45 minutes after the administration of carbidopa and 5-HT. The y-axis shows the voltage applied using a triangular waveform, starting from a starting potential of -0.4 V, ramped up to a switching potential of 1 V and back to -0.4 V holding potential. The x-axis indicates the recording time in second where the stimulation of 1 second.

Before carbidopa and 5-HTP were administered, DA current responses were recorded and a representative case of them is shown in Figure 7 a. The background subtracted voltammetry curve in Figure 7 c shows that the oxidation peak around 0.62 V and the reduction peak at ca, -0.3 V

correspond to the oxidation peak and reduction peak of DA previously observed in vitro using the same waveform. 45 minutes after the 5-HTP administration (Figure 7 b, d), the color plot and background subtracted CV curve showed the characteristic 5-HT reduction peak around 0.14V, simultaneously with the DA reduction peak at ca, -0.3 V, as carbidopa does not affect the dopamine release [19, 77]. The oxidation peak presents a 23% current increase (Figure 7 c, e) and, in addition to the DA peak at ca. -0.3 V, the reduction peak corresponding to 5-HT around 0.14 V showed up, with a 4-times increased amplitude compared to the baseline before 5-HTP injection (Figure 7 b, d, e). We observed distorted *in vivo* CVs compared very well with the *in vitro* data. This can be attributed to the unique and dynamic chemical and biological neuro-environment in the brain with its more complex and resistive nature as compared to experiments in PBS solution in a controlled environment [78-80]. Indeed, CV peaks have been observed to shift *in vivo* due to changing electrode impedance and, more importantly, the *in vivo* reduction peaks have been observed to be smaller in magnitude [60].

However, both from the color plot (b) and the time/current plots (Figure 7 e, f), it is possible to observe that 5-HT demonstrated a slower clearance, i.e. the oxidation peak is longer and does not come back to the baseline in the 15 second of recording session. This is likely due to the fact that pharmacological manipulation performed to elevate the basal level of 5-HT can overwhelm the capacity of serotonin uptake, thus slowing down the 5-HT re-uptake in the region. We think the results obtained here validate the proof-of-concept that 5-HT and DA can be simultaneously discriminated at GC microelectrodes using FSCV. This preliminary result is encouraging and will serve as steppingstone for further extensive *in vivo* evaluation.



Supplementary Figure 17. *In vivo* co-detection of dopamine and serotonin using GC microelectrodes and a triangular waveform with EW: -0.4 /1 V, 400 V/s scan rate, recording at 10 Hz. The color plot representation of the DA (a) and DA and 5-HT (b) release by electrical stimulation (60Hz, 250µA) at substantia nigra region, before (a) and after (b) the administration of 5-hydroxytrptophan (5-HTP) to pharmacologically manipulate the 5-HT level. (a, c) The oxidation peak of DA release characteristics before 5-HTP injection was observed at 0.64 V and reduction peak at -0.3 V. b, d) 45 mins after 5-HTP injection, color plot and background subtracted FSCV presented a serotonin reduction peak around 0.2 V, together with the DA reduction peak at -0.3 V, indicating that co-detection of dopamine and serotonin can be observed in vivo using GC

microelectrodes. (e, f) comparison of the current/time plots before and after 5-HTP administration corresponding to the 0.62 V positive potential peak (e) and the 0.14 and -0.3V reduction potential peaks (f).