Supporting Information

Sensitive and Selective Measurement of Serotonin *In Vivo* Using Fast Cyclic Square-Wave Voltammetry

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PEDOT:Nafion coated carbon fiber microelectrode fabrication

In this study, CFMs were fabricated as previously described[1]. The length of exposed carbon fiber was trimmed to approximately 50 µm in length under a stereomicroscope. PEDOT:Nafion coating was applied onto the exposed carbon fiber for all CFMs as described in a previous study[1]. PEDOT:Nafion deposition solutions consisted of 100 µL of a stock solution of 0.04 M EDOT (Sigma-Aldrich, St. Louis, MO, USA) in acetonitrile (prepared by the addition of 43 µL EDOT to 10 mL acetonitrile) and 200 µL of LQ-1105 Nafion (Ion Power Inc., DE, USA) in 20 mL acetonitrile (HPLC grade, EMD Chemicals Inc., Darmstadt, Germany).

The voltage for electrodeposition was controlled using a Gamry Instruments Reference 600 potentiostat (Warminster, PA, USA) in a three-electrode configuration. A tightly coiled silver wire was used as the counter electrode, and a straight silver-silver chloride (Ag/AgCl) wire was used as the reference electrode. Deposition was performed by applying a triangle waveform from +1.5 V to −0.8 V at 100 mV/s for 15 cycles, and using an open-circuit potential prior to waveform application. After electrodeposition, all electrodes dried in room temperature for 12 hours before use.

Physiological effects of N-FCSWV

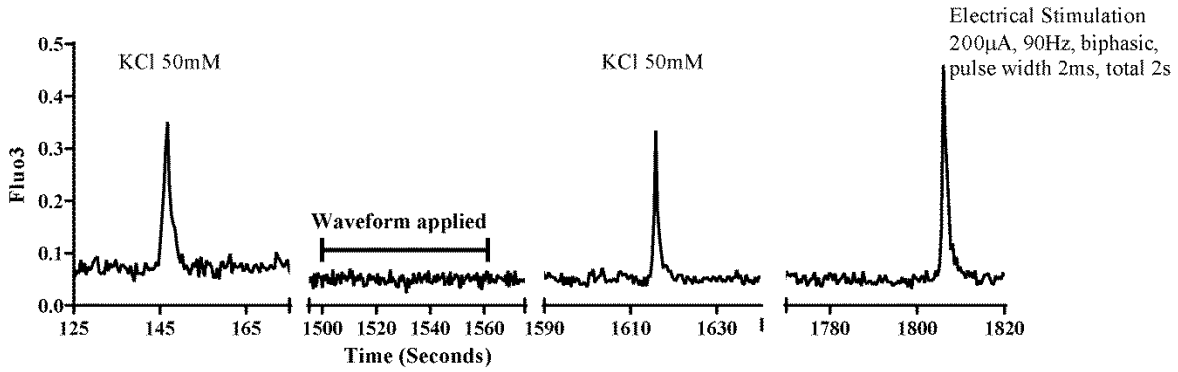

Figure S1. Physiological effects of N-FCSWV using Ca^{2+} imaging. Fluo-3 AM calcium indicator change in a representative neuron in response to a pulse infusion of KCl, followed by waveform alone, followed by pulse infusion of KCl, and finally local electrical stimulation.

Calcium imaging of a brain slice was performed to examine whether the large capacitive charging currents generated by the N-FCSWV waveform would affect local neuronal activity. Ca^{2+} responses were presented as a pseudo ratio (ΔF/F) to compare fluorescence intensities because Fluo-3 AM is a non-ratiometric Ca^{2+} indicator. Single-wavelength values differ depending on dye uptake in respective cells. The pseudo ratio was calculated with the following formula[2].

$$\Delta F/F = (F_1 - F_{base}) / F_{base}$$

F_1 = measured intensity after stimulation, F_{base} = measured intensity before stimulation

The applied square wave E_{sw} is relatively large in amplitude (0.4V), which can induce ~15μA of peak current. If this large current stimulates a neuron, it would undesirably influence neurotransmitter measurements *in vivo*. Application of KCl (50 mM) induced calcium changes, indicating neuronal activation (Fig. S1). However, calcium concentrations did not change during N-FCSWV recordings in the absence of stimulation. In addition, neurons were activated with 200 μA electrical stimulation (biphasic, 2 ms, 90 Hz, 2 s) as evidenced by changes in calcium concentrations. Although we did not show the responses of all 129 neurons in the slice, the same response pattern was observed for 127 neurons (2 neurons had artifacts). These results lend support to the notion that the N-FCSWV waveform does not activate neurons in the local recorded region despite their large amplitude.

Concentration versus time trace

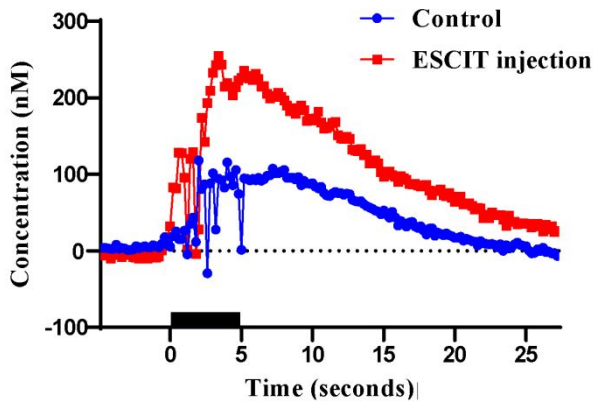

Figure S2. Concentration versus time. Representative concentration versus time trace after electrical stimulation of MFB. Blue circles indicate before ESCIT treatment and red squares indicates 30 minutes after ESCIT treatment.

Concentration versus time traces of electrical stimulation-evoked serotonin release. Serotonin changes after electrical stimulation applied to the MFB pre- and post-treatment of ESCIT. Stimulus artifacts were recorded together during the applied electrical stimulation.

Physiological effects of N-FCSWV

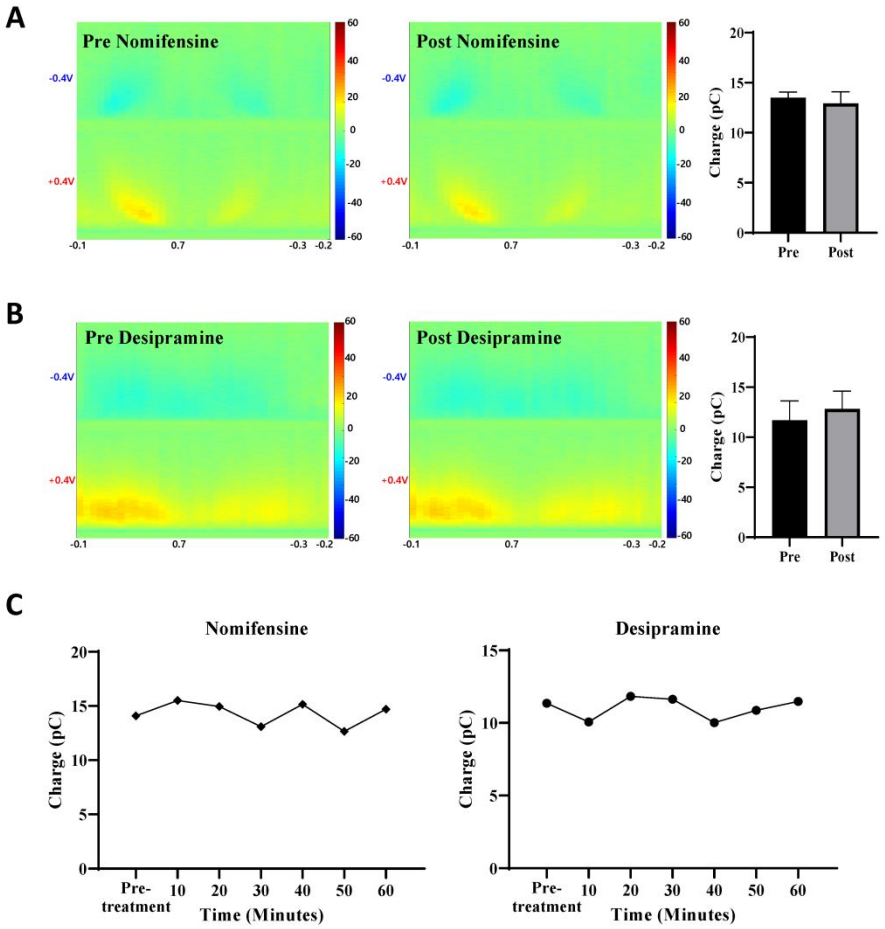

Figure S3. Pharmacological confirmation in vivo. Serotonin release in the rat SNr evoked by electrical stimulation of the rat MFB. (A) Representative 2D voltammogram of evoked serotonin release before (left, control) and 30 minutes after administration of nomifensine(20 mg/kg, i.p.), a selective dopamine reuptake inhibitor, (right). There were no significant changes in evoked responses between pre and post nomifensine treatment, (n=3). (B) Representative 2D voltammogram of evoked serotonin release before (left, control) and 30 minutes after administration of desipramine(20 mg/kg, i.p.), a selective norepinephrine reuptake inhibitor (right). There were no significant changes in evoked responses between pre and post desipramine treatment, (n=3). (C) Representative serotonin response changes by electrical stimulation every 10 minutes over 1 hour post-drug treatment.

Pharmacological confirmation was obtained using systemic (i.p.) injections of the selective dopamine reuptake inhibitor, nomifensine, and selective norepinephrine reuptake inhibitor, desipramine (n=3 for each). Neither nomifensine nor desipramine injection resulted in changes in the 2D voltammogram plot of serotonin, compared to the response recorded prior to each treatment. For statistical analysis (t-test), 10 points of each pre- and post-treatment data were captured. There were no significant pre- and post-treatment effects of both nomifensine and desipramine ($p=0.1714$ and $p=0.1882$, respectively).

Histamine response to N-FCSWV

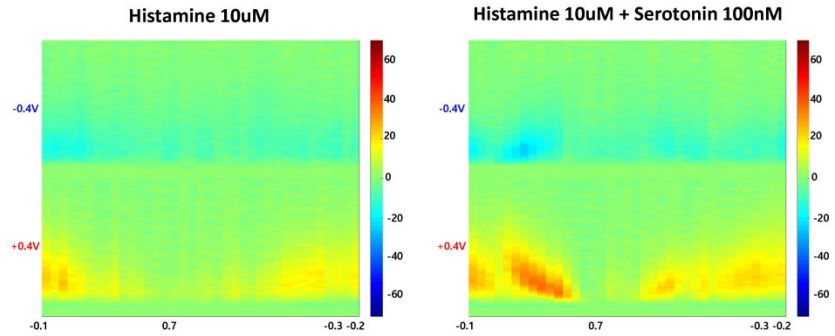

Figure S4. Histamine response to N-FCSWV. 2D pseudo color plot of histamine 10μM (left) and mixture of histamine 10μM and serotonin 100nM (right)

Compared to measurements of serotonin *in vitro* (beaker), *in vivo* serotonin responsses showed additional features. A recent study has reported that electrical stimulation-evoked serotonin and histamine release occur simultaneously in the same coordinates used in the current study[3]. *In vitro* beaker tests were performed to record histamine responses to N-FCSWV. 10 μM of histamine and a mixture of serotonin 100 nM and histamine (10 μM) was presented in the beaker with TRIS buffer and N-FCSWV responses were recorded.

Electrodes location confirmation

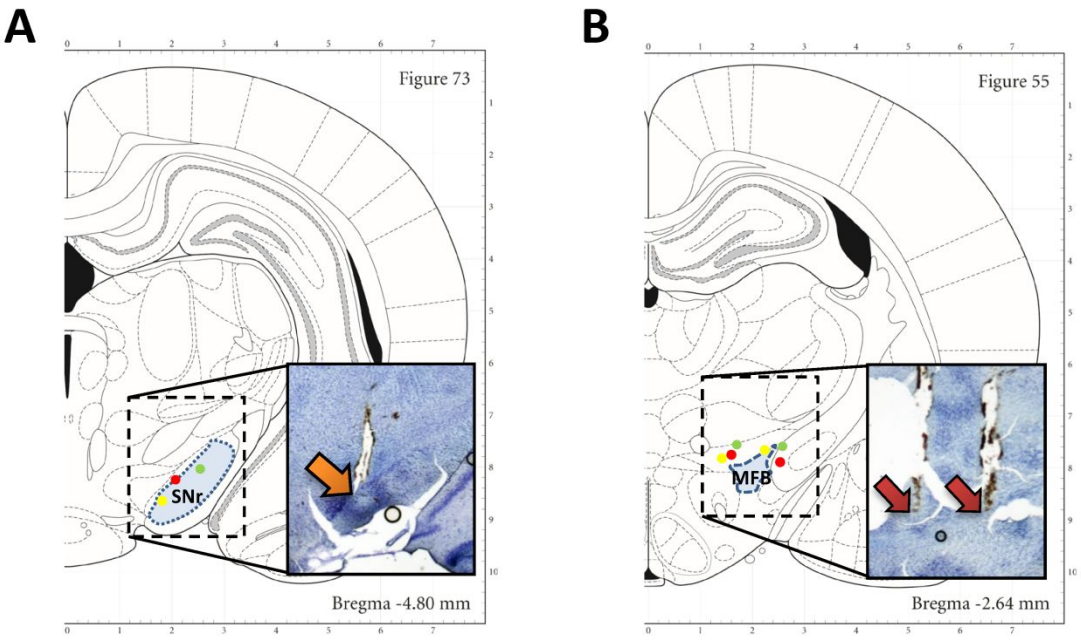

Figure S5. Diagram of electrode locations in rat brain slices (40 µm thickness). (A) Locations of carbon fiber microelectrodes implanted into the SNr (AP:-4.8mm, Paxinos and Watsons, 2007). Each colored circle marks the tip of CFM electrodes. Arrow in square image indicates where CFM tip is located in the brain histology slices. (B) Locations of stimulation electrodes implanted into MFB (AP:-2.7mm, Paxinos and Watsons, 2007). Each pair of colored circles marks the tips of the stimulation electrodes. Arrows in square image indicate where electrode tips were located in brain histology slices.

Histological staining was conducted to electrode target site confirmation (n=3). The histological analysis showed that the CFM had accurately been implanted close to the SNr for all three subjects. In targeting the MFB, parallel bipolar stainless steel electrodes were accurately implanted close to the MFB area.

References

1. Vreeland, R. F.; Atcherley, C. W.; Russell, W. S.; Xie, J. Y.; Lu, D.; Laude, N. D.; Porreca, F.; Heien, M. L., Biocompatible PEDOT:Nafion Composite Electrode Coatings for Selective Detection of Neurotransmitters in Vivo. *Analytical Chemistry* **2015,** *87* (5), 2600-2607.
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3. Hashemi, P.; Dankoski, E. C.; Wood, K. M.; Ambrose, R. E.; Wightman, R. M., In vivo electrochemical evidence for simultaneous 5-HT and histamine release in the rat substantia nigra pars reticulata following medial forebrain bundle stimulation. *J. Neurochem.* **2011,** *118*, 749-759.