Supporting Information

Tracking Tonic Dopamine Levels *in vivo* using Multiple Cyclic Square Wave Voltammetry

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Electrode fabrication

The carbon-fiber microelectrode (CFM) design was modified from our previous studies[1,2]. Each CFM was fabricated by isolating and inserting a single carbon fiber (AS4, d = 7μm; Hexel, Dublin, CA) into a silica tubing (20μM ID, 90μM OD, 10μM coat with polyimide; Polymicro Technologies, Phoenix, AZ). The connection between the carbon fiber and the silica tubing was covered with polyamic acid (Sigma-Aldrich, St. Louis, MO) and heated to 200˚C to polymerize the polyamic acid into a polyimide film. The silica tubing was then attached to a nitinol (Nitinol #1, an alloy of nickel and titanium; Fort Wayne Metals, IN) extension wire with a silver-based conductive paste[1]. The carbon fiber attached nitinol wire was insulated with polyimide tubing (0.0089"ID, 0.0134"OD, 0.00225" WT; Vention Medical, Salem, NH) except the carbon fiber sensing part. The exposed carbon fiber was trimmed under a dissecting microscope to a length of approximately 50 μm. Teflon-coated silver wire (A-M systems, Inc., Sequim, WA) was prepared as an Ag/AgCl reference electrode and chlorinating the exposed tip in saline with a 9 V dry cell battery.

Preparation of brain slices for Ca^{2+} image

After rapid extraction of mouse brain, 300 μM horizontal striatum slices were prepared using a Vibratome 1000 (The Vibratome Company, St. Louis, MO). After a minimum period of 60 to 90 minutes, the slices were continuously perfused with oxygenated artificial cerebrospinal fluid solution (28–30°C) (in mM; NaCl 125, KCl 2.5, $CaCl_2$ 2, $NaHCO_3$ 26, NaH_2PO_4 1.25, $MgCl_2$ 1, glucose 25, pH 7.4 when bubbled with 95% O_2 and 5% CO_2) in a submersion-type recording chamber. In Ca^{2+} image analysis, Ca^{2+} response of striatal neurons was measured using the fluorescent Ca^{2+} probe (indicator) Fluo-3 AM (10 mM, Enzo Life Science, Farmingdale, NY) mixed with 1 ml pluronic acid (20% solution in DMSO, Life Technologies) in DMEM medium (with 10% FBS) for 40 min at 37˚C. Fluo-3 loaded slices on coverslip were mounted onto the chamber (12mm Chamlide AC, total volume of 500 ul, Live Cell Instrument, Korea), and placed onto an inverted microscope (Olympus IX70, Japan). Cells were excited with a LED

source pE-100 (CoolLED, UK) at 470 nm and emissions were recorded at 535 nm wavelengths, Fluorescent emission readings were recorded and stored on hard disk every 0.5 s. Intracellular concentrations ($[Ca^{2+}]_i$) were measured by digital video microfluorometry with an intensified charge-coupled-device (CCD) camera (QIClick, QImaging, Canada) coupled to the microscope and a computer with software (MetaMorph® NX, Molecular Devices). Ca^{2+} responses were presented as a pseudo ratio ($\Delta F/F$) to estimate comparison fluorescence intensity, because Fluo 3-AM is a non-ratiometric Ca^{2+} indicator. Single-wavelength values are different depending on the concentration of loaded dye in respective cells. Following formula indicates pseudo ratio. [3]

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

F_1 = measured intensity of the cell after stimulation

F_{base} = measured intensity of the cell before stimulation

Biological experiments protocol

Rats were housed with a 12:12 hr light and dark cycle (lights on at 0600 hr) with *ad libitum* access to food and water. The rats were anesthetized with an injection of urethane (1.6 g/kg, i.p.) and stabilized in a commercially available stereotaxic frame (David Kopf Instruments, Tujunga, CA) for the surgery. A longitudinal incision was made on the skin to expose the skull and three burr holes (0.5-1.0 mm diameter) were made in the skull of each rat for the implantation of a CFM, a bipolar electrical stimulating electrode (Plastic One, MS303/2, Roanoke, VA, USA) and an Ag/AgCl reference electrode. The reference electrode was positioned superficially in cortical tissue contralateral to the CFM and stimulating electrode. Electrode coordinates were referenced by a rat brain atlas[4] based on flat-skull position using bregma and dura as reference points with coordinates anteroposterior (AP), mediolateral (ML), and dorsoventral (DV). The CFM was placed in the right hemisphere in the dorsomedial striatum (AP +1.0 mm; ML +2.5 mm; DV -4.5

to -5.5 mm), and the stimulating electrode was inserted ipsilaterally just above the medial forebrain bundle (MFB, AP -4.8; ML +1.0; DV -8.0 to -9.0). A train of bipolar pulses (2 ms pulse width, 200 µA, 60 Hz) using WINCS Harmoni electrometer was delivered for 2 seconds to identify dopamine releasing sites in the striatum. FSCV signal was synchronized with electrical stimulation in order to interleave intervals of stimulation during FSCV scans[5]. Thus, electrical stimulation was not applied when the FSCV pulses (about 10ms) were delivered. The CFM and the electrical stimulating electrode were gradually adjusted until a robust phasic DA signal was detected at the CFM using FSCV.[6] Immediately thereafter, a M-CSWV waveform was applied at 0.1 Hz for ~10 minutes to allow stabilization of the recorded electrochemical signal. Striatal tonic DA levels were collected for one hour, then saline, 20mg/kg nomifensine, or 75mg/kg pargyline was injected i.p. and tonic DA levels were collected for additional 2 hours.

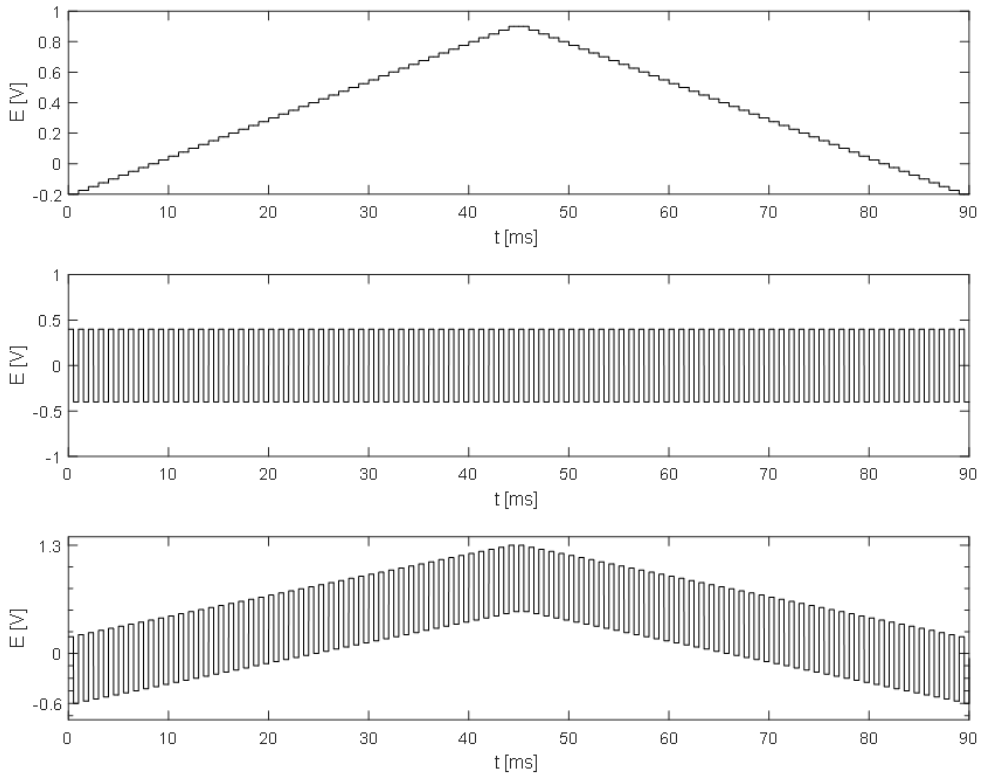

Figure S-1 Cyclic square waveform used throughout this study

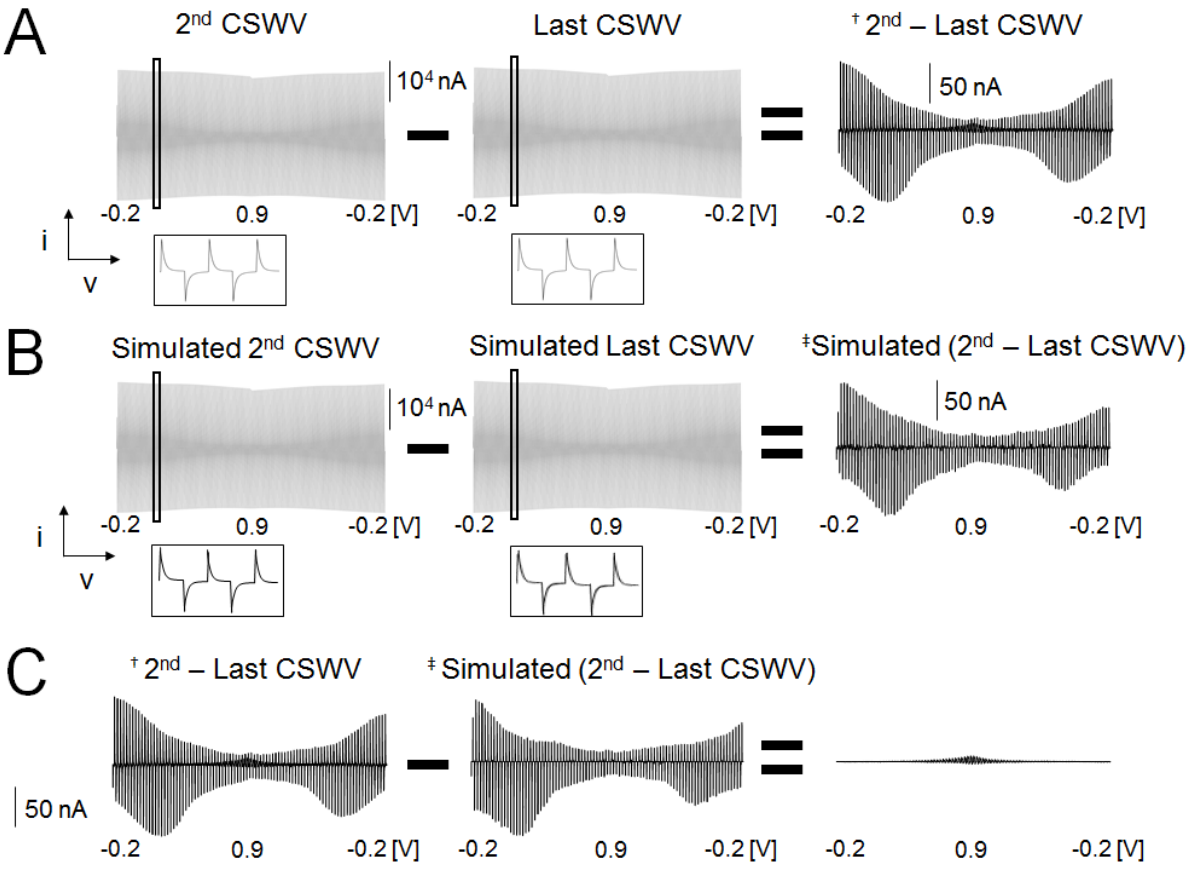

Figure S-2 Capacitive current response of M-CSWV and its modelling. (A) The background current and its difference between 2nd and last CSWV. (B) Modelled 2nd and last CSWV background current and its difference. (C) Capacitive current removal by subtracting modelled CSWV from raw data.

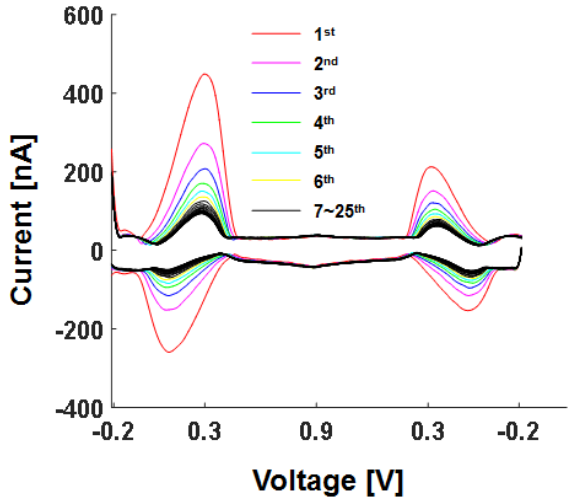

Figure S-3 Envelopes of DA responses. 25 cyclic square waveforms were used.

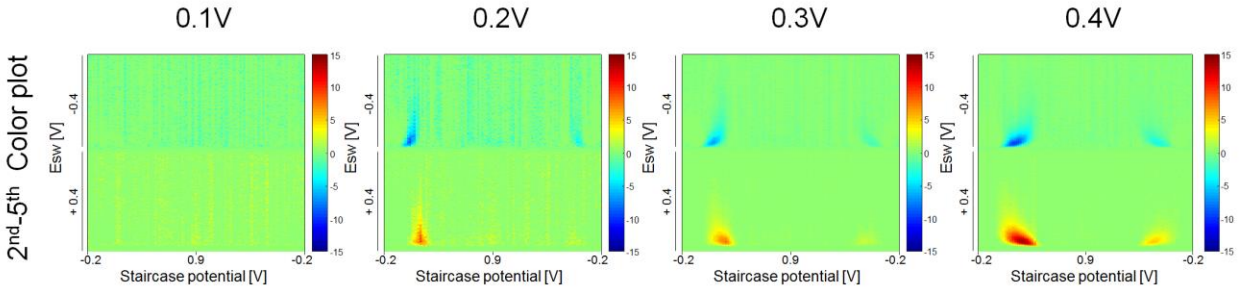

Figure S-4 Examples of E_{SW} effect on the DA response.

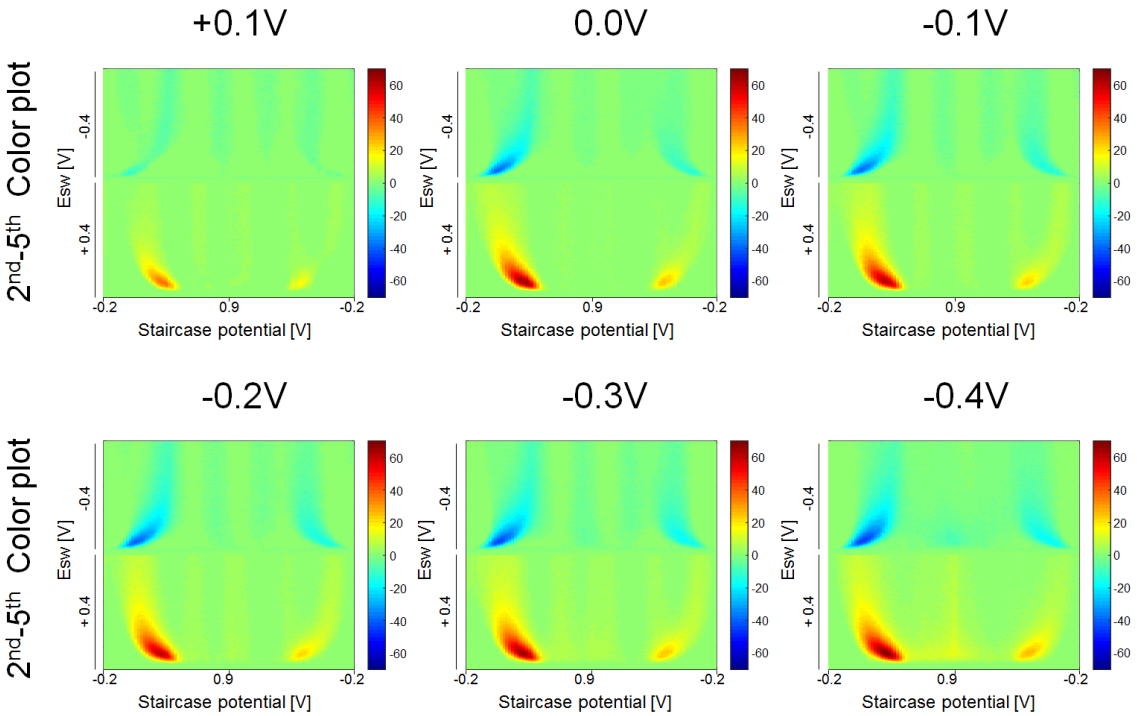

Figure S-5 Examples of $E_{Holding}$ effect on the DA response.

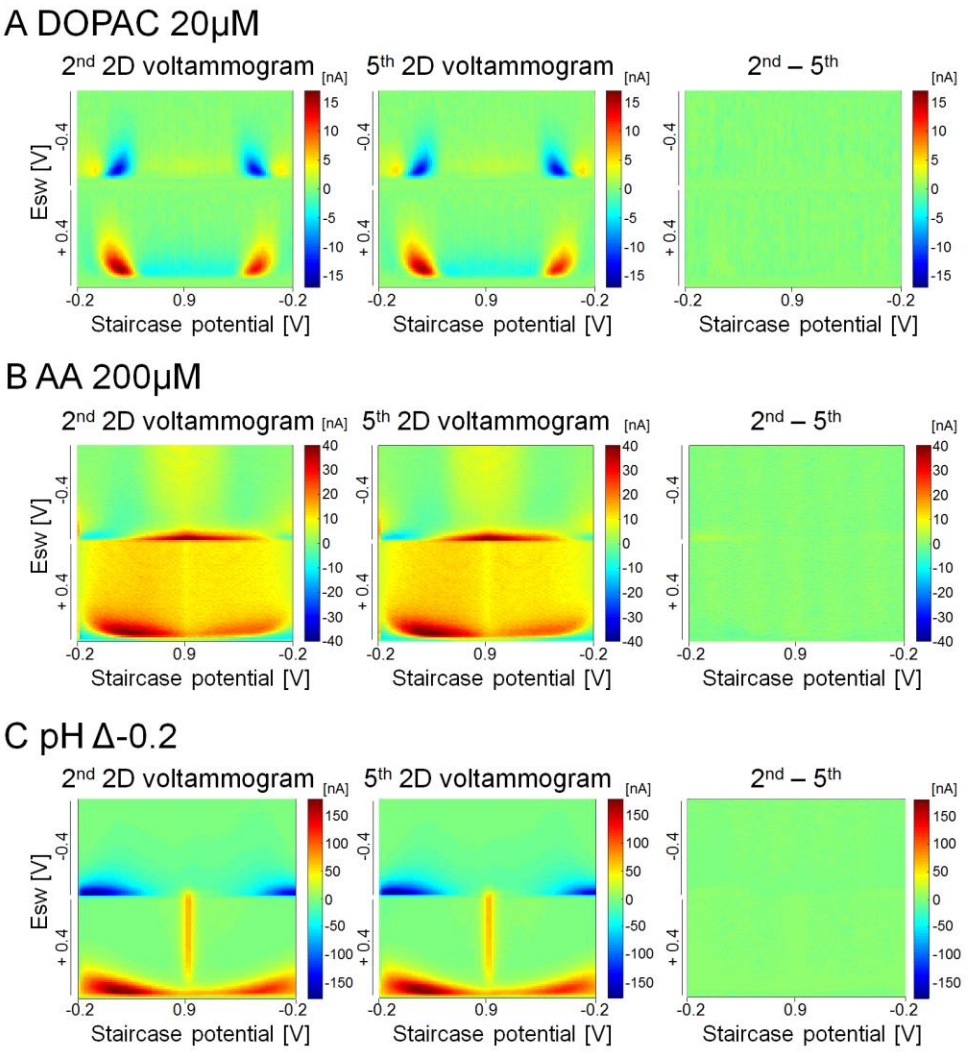

Figure S-6. Representative background-subtracted M-CSWV responses of DOPAC, AA, and pH change. (A) Representative background-subtracted 2D voltammograms of 20μM DOPAC. DOPAC can be offset by subtracting 2nd and 5th CSWV. (B) Representative background-subtracted 2D voltammograms of AA 200μM. (C) Representative background-subtracted 2D voltammograms of pH changes.

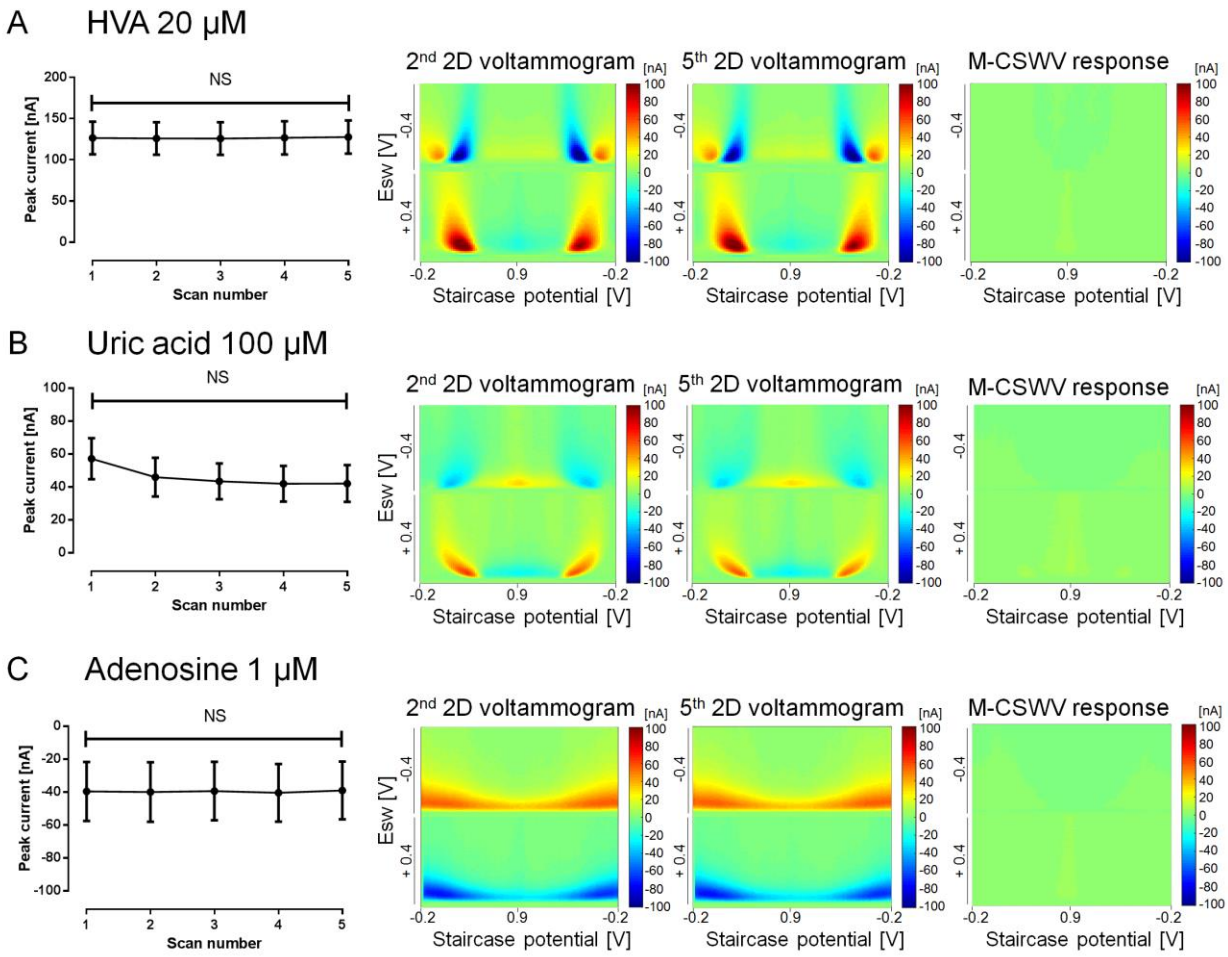

Figure S-7. Representative background-subtracted M-CSWV responses for homovanillic acid (HVA), uric acid (UA), and adenosine. (A) Peak oxidation currents of background-subtracted M-CSWV for HVA (20µM). HVA responses from 1st to 5th CSWV showed no significant differences (n=3, one-way ANOVA test, left panel). Representative background-subtracted 2D voltammograms of 20µM HVA (middle two pseudo color plots). HVA can be offset by subtracting 2nd and 5th CSWV (right panel). (B) Uric acid (100µM) responses from 1st to 5th CSWV showed no significant differences (n=3, one-way ANOVA test, left panel). Representative background-subtracted 2D voltammograms of Uric acid. (C) Adenosine (1µM) responses from 1st to 5th CSWV showed no significant differences (n=3, one-way ANOVA test, left panel). Representative background-subtracted 2D voltammograms of adenosine.

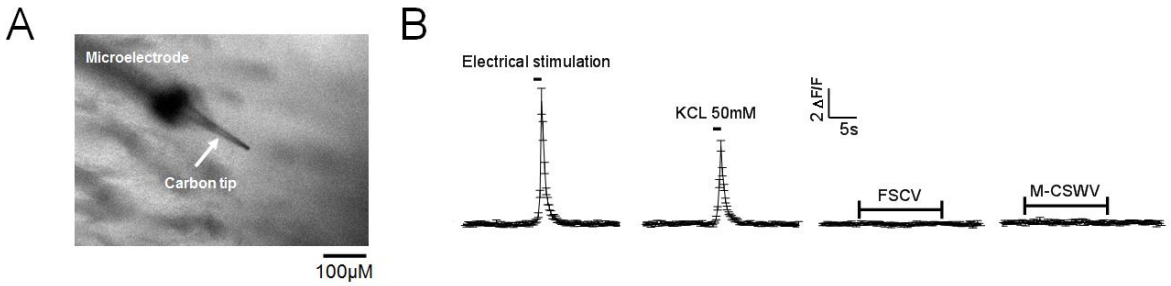

Figure S-8 *In vivo* feasibility test using brain slice of mouse. (A) CFM placement in the brain tissue. (B) Fluo-3 changes during experiments.

Method	Target location	Quantified tonic dopamine level	Refs
Amperometric monitoring with glass-sealed gold nanoelectrode	nucleus accumbens	41 ± 13 nM	7
Fast-scan controlled-adsorption voltammetry	nucleus accumbens	90 ± 9 nM	8
Microdialysis using online capillary liquid chromatography	striatum	10.7 ± 1.5 nM	9
Reverse microdialysis with stearate-graphite paste electrodes	striatum	86 ± 6 nM	10
Multiple cyclic square wave voltammetry	striatum	120 ± 18 nM	This work

Table S-1 Comparison of the different methods for the determination of tonic dopamine levels in rat brain

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