Supplemental Information for:

Carbon Nanotubes Grown on Metal Microelectrodes for the Detection of Dopamine

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Supplemental Methods

Solutions

Dopamine, ascorbic acid, DOPAC, adenosine, 5-hydroxytryptamine (serotonin), and histamine were purchased from Sigma−Aldrich (St. Louis, MO). Ten millimolar (10 mM) stock solutions of the analytes were prepared in $HClO_4$ and were diluted daily to the desired concentration in phosphate-buffered saline (PBS) (131.25mM NaCl, 3.00 mM KCl, 10 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.0 mM Na_2SO_4, and 1.2 mM $CaCl_2$ with the pH adjusted to 7.4).

Microelectrode preparation

A CNT-grown wire or CF, 1−2 cm long, was inserted into a polyimide coated fused-silica capillary (45 μm inner diameter (ID) × 90 μm outer diameter (OD), Polymicro Technologies, Phoenix, AZ) while submerged in 2-propanol, to reduce friction and ease insertion. The solvent was allowed to fully evaporate from inside of the capillary before the metal wire was sealed into the capillary with 5 min epoxy Loctite (Henkel Corporation, Westlake OH) and was allowed to fully cure for 24 h. The polyimide capillary was then inserted and epoxied in a 0.68 mm ID × 1.2 mm OD glass capillary that had previously been pulled into a glass pipet and cut to have an opening diameter of ∼100 μm.

Surface characterization

Scanning electron microscope (SEM) images were taken on Merlin field emission SEM (Zeiss, Thornwood, NY) with a secondary electron detector using an accelerating voltage of 2 kV and a working distance of 5.3 mm. Transmission electron microscope (TEM) images were taken on a Libra 120 (Zeiss, Thornwood, NY). Raman spectroscopy measurements were performed with a Renishaw 100 confocal micro-Raman system (Renishaw, Hoffman Estates, IL) with a 1800 lines/mm diffraction grating, 532 nm laser focused to a spot size of about 2 μm through a 100x objective, and a Peltier-cooled charge-coupled device detector.

Surface areas were estimated by either integrating the background current and multiplying by time or using the background current at 0.25 V. These currents were divided by specific capacitance (24 μF/cm^2, a standard capacitance for glassy carbon[1]) times scan rate to determine surface areas. Both methods gave similar areas. Since CNTs are only half coated on metal/carbon fiber substrates, the capacitive charging current used for surface area calculation was performed by the difference of background current of CNT-metal electrode minus half of the background current of bare metal electrodes. While the specific capacitance might vary by CNT material, it allows a rough estimation of surface area.

Electrochemistry

FSCV was performed with a ChemClamp potentiometer (Dagan, Minneapolis, MN, with 1 mega Ohm Chem Clamp Headstage). The waveform was generated and the data was collected using a High Definition Cyclic Voltammetry breakout box (UNC Chemistry Department, Electronics Design Facility) and PCIe-6363 computer interface cards (National Instruments, Austin, TX). Electrodes were backfilled with 1 M KCl and a silver wire was inserted to connect the electrode to the potentiostat probe. The typical triangular waveform swept the applied potential from −0.4 V to 1.3 V (except where noted) at 400 V/s versus an Ag/AgCl reference electrode, at a scan frequency of 10 Hz. Data collection was computer-controlled by the HDCV analysis software program (University of North Carolina, Chapel Hill).

Electrodes were tested using a flow-injection system, as previously described.[2] Analyte was injected for 5 seconds and current versus time traces were obtained by integrating the current in a 100 mV window centered at the oxidation peak for each cyclic voltammogram (CV). Background-subtracted CVs were calculated by subtracting the average of 10 background scans, taken before the compound was injected, from the average of five CVs recorded after the analyte bolus was injected.

In vivo measurements

Male Sprague-Dawley rats (250–350 g) purchased from Charles River were housed in a vivarium and given food and water *ab libitum*. All experiments were approved by the Animal Care and Use Committee of the University of Virginia. The rat was anesthetized with urethane (Sigma Aldrich) (1.5 mg/kg i.p.), the scalp shaved, and 0.25 mL bupivicaine (0.25% solution, Sensorcaine, MPF, APP Pharmaceuticals, LLC; Schaumburg, IL) given subcutaneously. The working electrode was implanted in the caudate putamen (in mm from bregma: AP + 1.2, ML + 2.0, and DV – 4.5 to –5.0), the stimulating electrode in the substantia nigra (AP −5.4, ML + 1.2, and DV – 8.0), and the Ag/AgCl reference electrode on the contralateral side of the brain. The DV placement of the stimulating electrode was adjusted downward until a robust dopamine signal was measured. After implantation in the brain, the FSCV waveform was applied to the CNT-Nb microelectrode for 30 min to allow the electrode to stabilize and the brain to recover. Stimulated release was electrically evoked using biphasic stimulation pulses (300 μA, 12–120 pulses, 60 Hz).

Statistics

All values are given as mean ± standard error of the mean (SEM) for n number of electrodes and all error bars are given as SEM. Unpaired t-tests were performed to compare properties between two groups. A one-way ANOVA with Bonferonni post-tests was used to compare effects among multiple groups. All statistics were performed in GraphPad Prism (GraphPad Software,Inc., La Jolla, CA). SEM and TEM images were processed using ImageJ (Rasband, W.S., ImageJ, National Institutes of Health, Bethesda, MD, U.S.A.).

Supplemental Figures

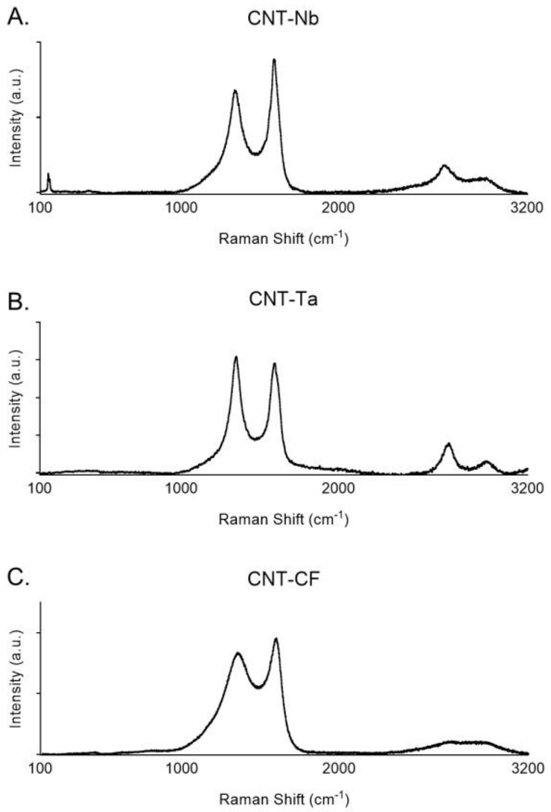

Figure S1. Raman spectrum of CNT grown on the surface of (A) Nb, (B) Ta and (C) CF wires.

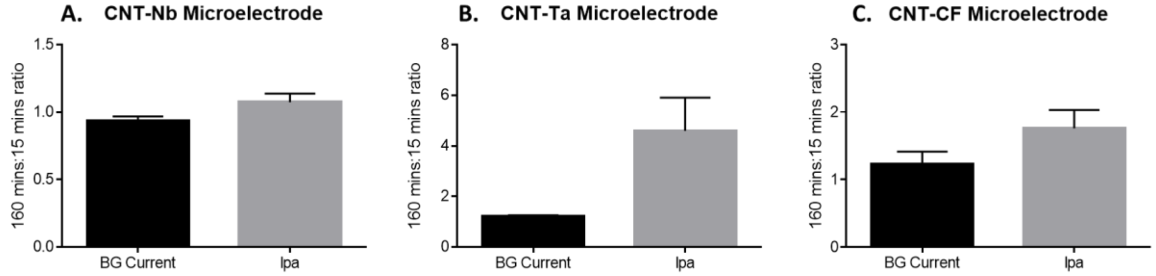

Figure S2. Effect of equilibration at (A) CNT-Nb, (B) CNT-Ta, and (C) CNT-CF microelectrodes (n = 4). Values given are the ratio of the current at 160 mins of equilibration vs 15 mins of equilibration. The black bar is the ratio of background current at 0.25V in PBS buffer, giving an indication of changes in background current. The gray bar is the ratio of oxidation currents for 1 μM dopamine. Ratios greater than 1 indicate that the peak increased in size during equilibration.

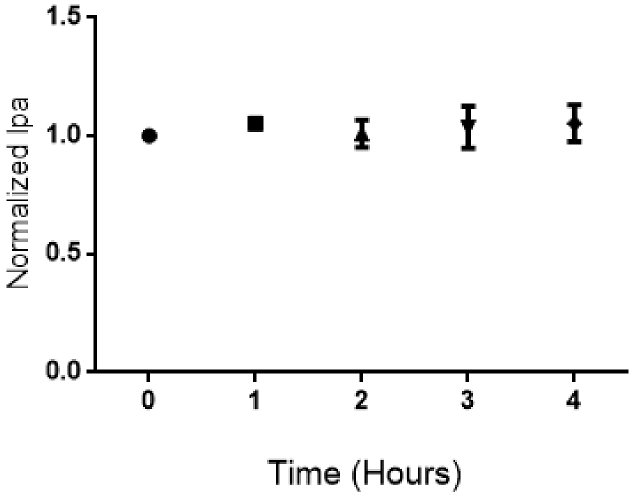

Figure S3. CNT-Nb microelectrodes stability experiment. Measurements of 1 µM dopamine were taken over a 4 h time period at CNT-Nb microelectrodes, with a scan rate of 400 V/s and sampling repetition rate of 10 Hz (n = 5, error bar indicates standard error of mean).

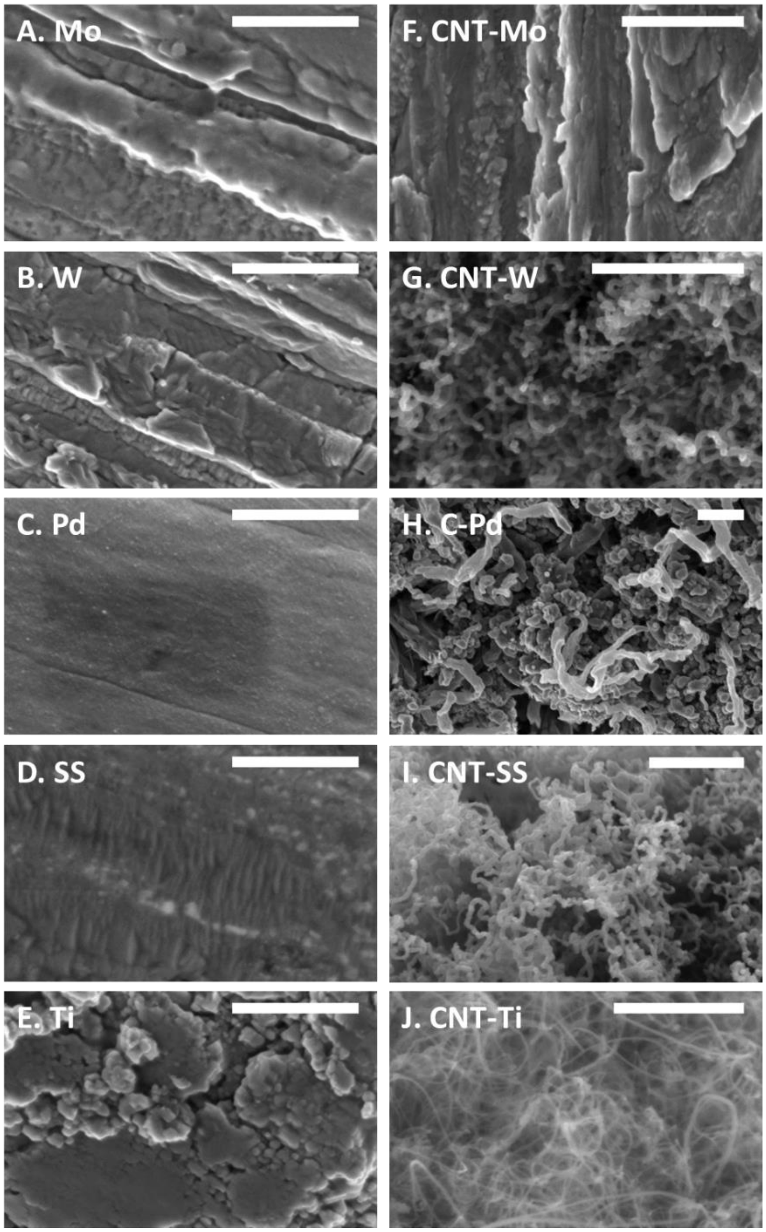

Figure S4. SEM images of (A) bare molybdenum, (B) bare tungsten, (C) bare palladium, (D) bare stainless steel, (E) bare titanium, (F) CNT-grown molybdenum, (G) CNT-grown tungsten, (H) carbon-grown palladium, (I) CNT-grown stainless steel, and (J) CNT-grown titanium. Scale bar: 500 nm.

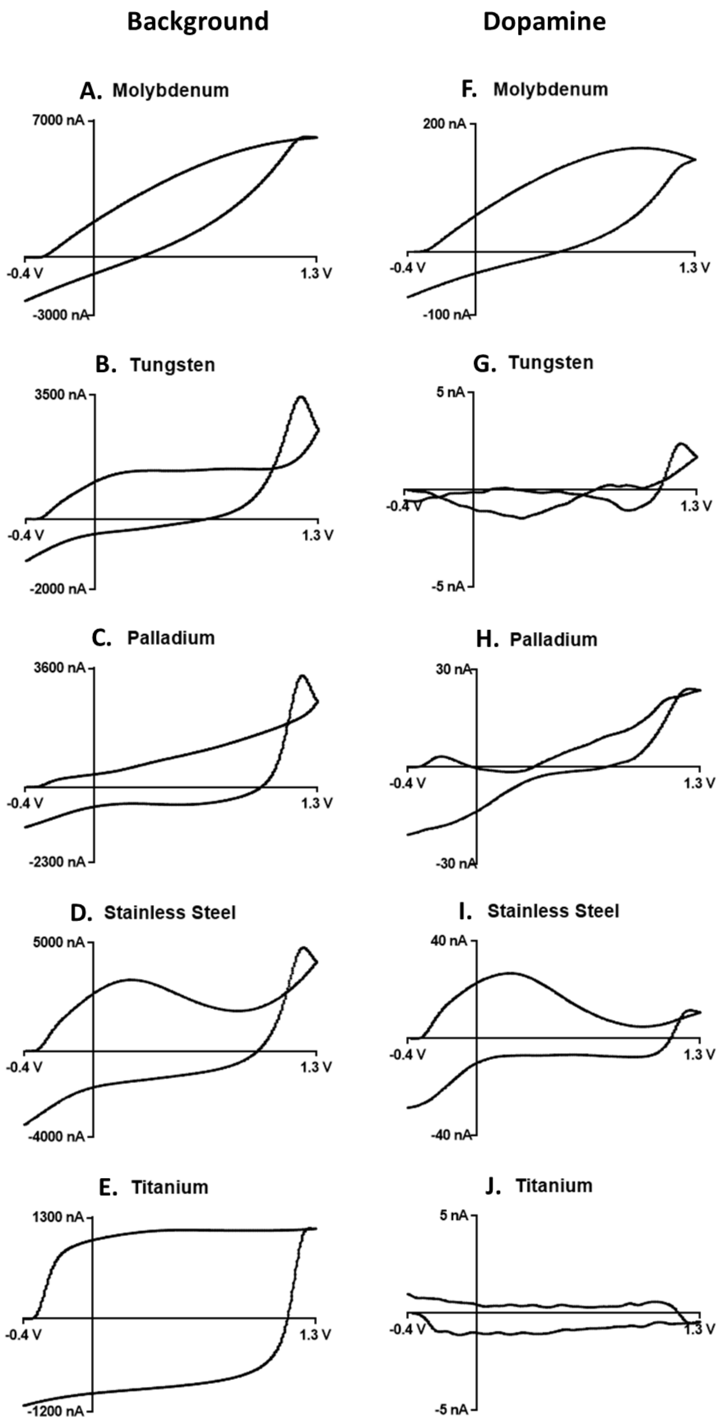

Figure S5. Electrochemical response of bare metals. Background current in PBS buffer solution for A. Molybdenum, B. Tungsten, C. Palladium, D. Stainless steel, and E. Titanium microelectrodes, and background subtracted cyclic voltammograms for 1 µM dopamine at bare F. Molybdenum, G. Tungsten, H. Palladium, I. Stainless steel, and J. Titanium microelectrodes.

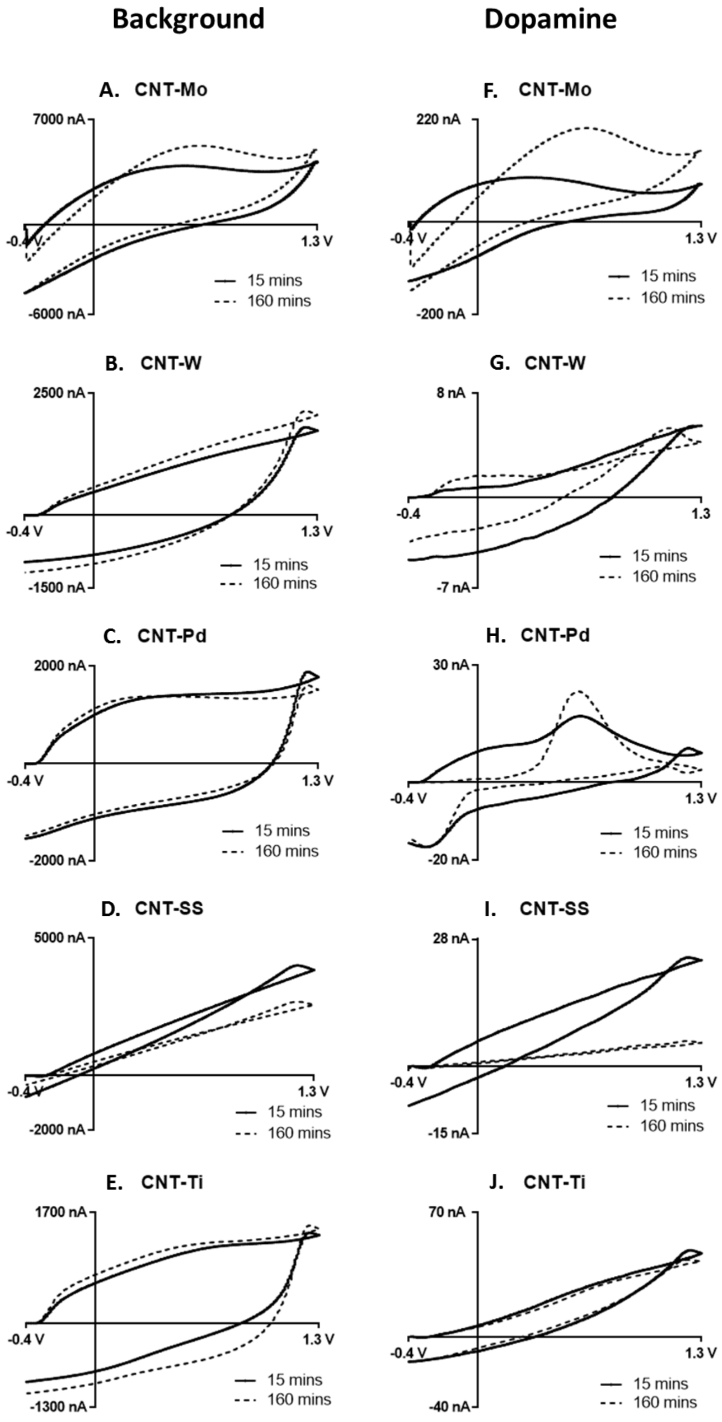

Figure S6. Comparison of the electrochemical response at CNT-grown metals microelectrodes. Background current at A. CNT-Molybdenum, B. CNT-Tungsten, C. CNT-Palladium, D. CNT-Stainless steel, and E. CNT-Titanium microelectrodes, and background-subtracted cyclic voltammogram for 1 µM dopamine at F. CNT-Molybdenum, G. CNT-Tungsten, H.CNT-

Palladium, I. CNT-Stainless steel, and J. CNT-Titanium microelectrodes. The solid line is the measurements taken after 15 min equilibration and the dashed line is after 160 min equilibration in PBS buffer solution using a waveform of -0.4 to 1.3 V and back at 400V/s, 10 Hz.

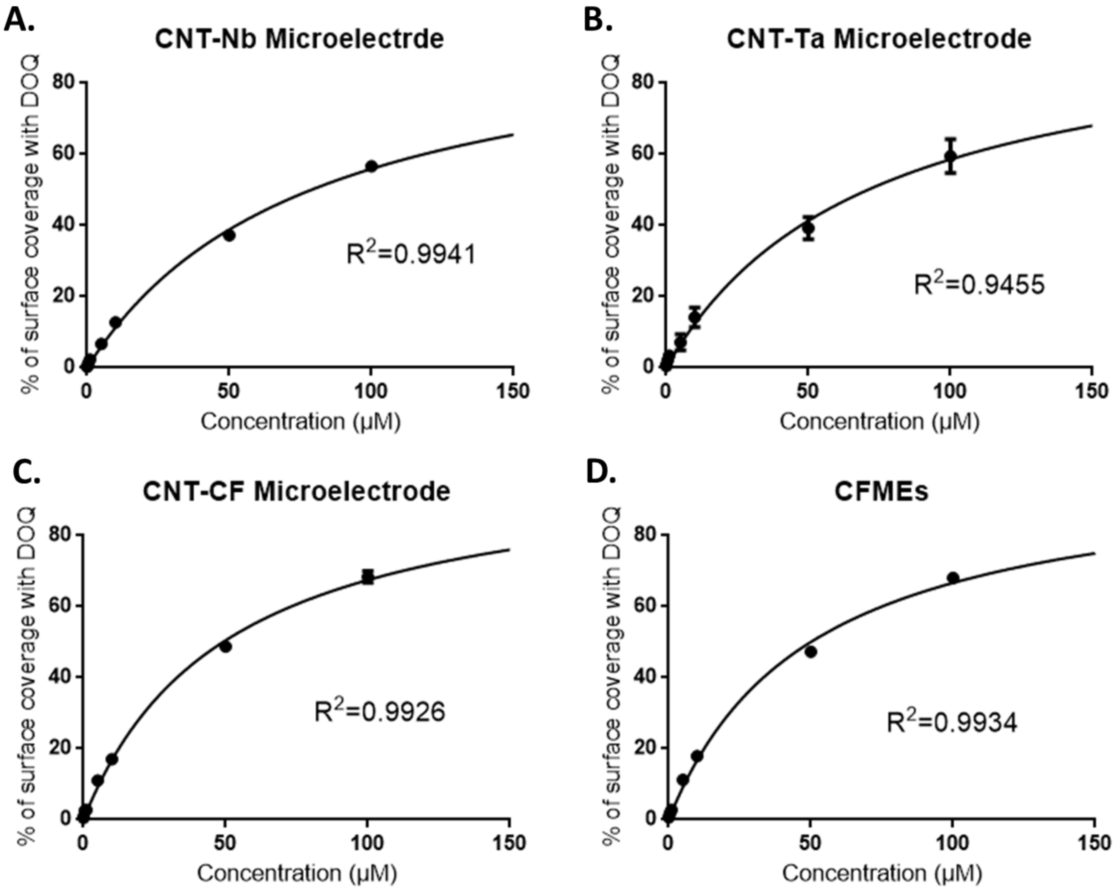

Figure S7. Plot of normalized cathodic current to corresponding dopamine concentration. The fitting curve is modeled based on Equation 3, where C_{DA} is the X axis and the fractional surface coverage is the Y axis. An equilibrium value, $\beta_{DOQ,}$ is fit for each curve. A. CNT coated Nb, B. CNT coated Ta, C. CNT coated CF microelectrodes, and D. CFMEs (n = 5 per electrode material, error bar is standard error of mean).

References

(1) McCreery, R. L. *Chem. Rev.* **2008**, *108* (7), 2646–2687.

(2) Strand, A. M.; Venton, B. J. *Anal. Chem.* **2008**, *80* (10), 3708–3715.

(3) Hennrich, F.; Krupke, R.; Lebedkin, S.; Arnold, K.; Fischer, R.; Resasco, D. E.; Kappes, M. M. *J. Phys. Chem. B* **2005**, *109* (21), 10567–10573.