Supporting Information

Electro-triggering and electrochemical monitoring of dopamine exocytosis from a single cell by ultrathin electrodes based on Au nanowire

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Instruments. Au nanowire (NW) electrodes-based cell research system consists of an optical microscope (Olympus IX71), a three-dimensional piezoelectric stage (Sigma Koki) to position a recording Au NW electrode, and a three-axis microstage to position a stimulating Au NW electrode. Each of a stimulating and a recording Au NW electrode was operated by its own electrochemical workstation (CHI 660D).

In the electrochemical recording part, 3-electrode configuration was constituted with a Au NW electrode, Ag/AgCl, and Pt wire as working, reference, and counter electrodes, respectively, to maximize the precision of electrochemical measurement by preventing the distortion of the applied potential caused by the solution resistance (iR drop). In the electrical stimulation part, 2-electrode configuration instead of 3-electrode one was used with another Au NW electrode and a saturated calomel electrode (SCE) as working and reference electrodes, respectively. When 3-electrode configuration was used in the electrical stimulation part, the amperometric trace measured by a recording Au NW electrode was severely interfered with a significantly large peak occurring right after the application of a voltage pulse to the stimulating electrode. Before employing the 2-electrode system, we examined if the electrode configuration affects the electrochemical behavior of a Au NW electrode by measuring the electrochemical oxidation and reduction of a Au NW with 2-electrode and 3-electrode systems (Fig. S1). We observed no noticeable difference between the cyclic voltammograms, probably due to the small surface area of a Au NW electrode and, consequently, the negligible iR drop.

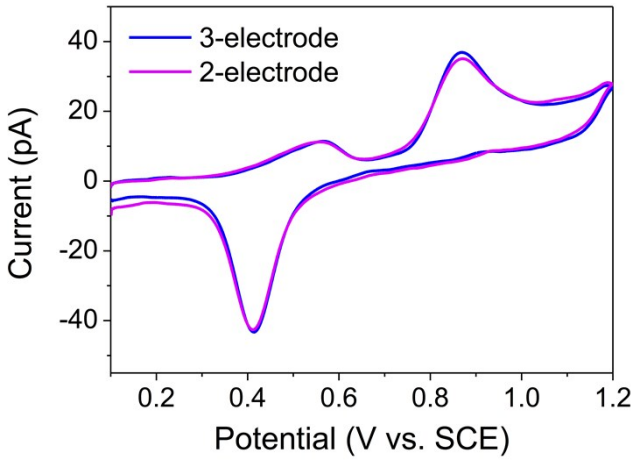

Fig. S1 Cyclic voltammograms of a Au NW electrode measured in a phosphate buffer solution (pH 7.4, scan rate: 100 mV/s) using 3-electrode (blue) and 2-electrode (magenta)

configuration.

Limit of detection for dopamine at Au nanowire electrodes. The limit of detection (LOD) is 12.9 μM as estimated from 3σ (σ : standard deviation of data of blank (buffer without dopamine)), which is similar to the previously reported LOD of bulk gold electrode although this is not the lowest LOD: 12 μM from *Electrochimica Acta*, 2010, **55**, 8953. The LOD can be improved by optimizing the detection method such as differential pulse voltammetry.

Determination of the voltage of electrical pulses. Since the extracellular solution contains O_2 dissolved, oxygen reduction reaction (ORR) occurs over ca. – 0.3 V as shown in Fig. S2. Once ORR occurs, not all the applied electrical potential would contribute to the electrical cell stimulation. In this reason, we set the negative limit of the stimulation voltage to – 0.3 V.

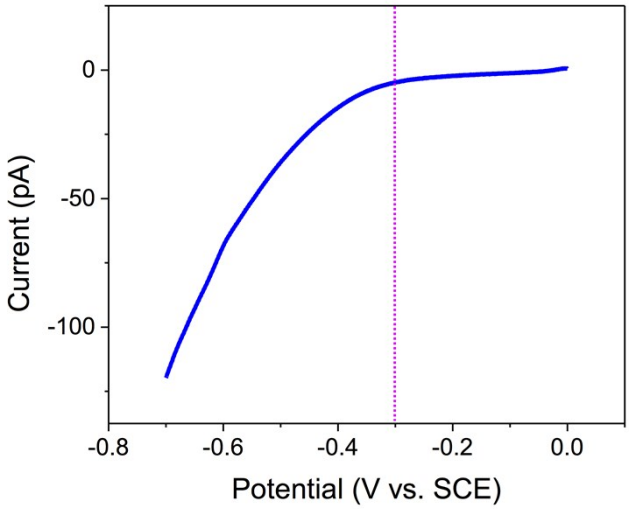

Fig. S2 Linear sweep voltammogram of a Au NW electrode measured in an extracellularsolution (100 mV/s) using 2-electrode configuration. Oxygen reduction reaction occurs from 0.3 V.

Cell Culture and Buffer Solution. PC-12 cells were grown in the RPMI-1640 medium (Life technologies Co.) supplementary with 10% horse serum, 5% fetal bovine serum, 100 U/mL of sodium penicillin G, and 100 μg/mL of streptomycin sulfate and maintained in a 5%

CO_2, water-saturated atmosphere at 37°C. The culture dishes were treated with 0.01% poly-L-lysine (Sigma Aldrich Co.).

The petri dish was washed twice with 2 ml of extracellular solution immediately before uses, and then filled with extracellular solution. The extracellular solution contains 140 mM NaCl, 2 mM $CaCl_2$, 4.2 mM KCl, 0.7 mM $MgCl_2$, 1 mM NaH_2PO_4, and 10 mM HEPES titrated to pH 7.4 with NaOH. All solutions were prepared with deionized water and all chemicals were obtained from Sigma and used without further purification.

Fabrication of Au nanowire electrodes. Au nanowires were grown by previously reported vapor transport method.[1] Au vapor was formed by heating Au slug at 1130 °C in a horizontal furnace system and carried to a c-plane sapphire substrate placed a few centimeters downstream by Ar gas with a flow rate of 100 sccm for ~ 30 min under chamber pressure of ~5 Torr. Au nanowires were grown vertically on the substrate from half-octahedral Au seeds on the substrate formed during the crystal growth.

A commercially available tungsten tip (GGB industries Inc., FL, USA) picked a single Au NW up from the sapphire substrate by softly touching it.[2,3] In order to stick the nanowire stably and tightly to the tungsten tip, the middle part of the tungsten tip was immersed in a drop of conducting carbon paste (CANS, Japan) and then the tip was moved back and forth up to the end of the tip, which re-aligned the Au nanowire. The tungsten part was insulated with UV-curable polymer (Norland Products Inc., NJ, USA) and nail varnish. Complete insulation of the tungsten part was confirmed by obtaining cyclic voltammogram of the assembled Au NW-tungsten tip electrode in a phosphate buffer solution under 3-electrode configuration. Anodic and cathodic currents for oxidation and reduction of Au, respectively, were clearly observed while no anodic current from tungsten as shown in Fig. S3 was observed. These oxidation peaks at 0.2 and 0.6 V during anodic scan and 0.4 and 0.2 V during cathodic scan can originate from the formation of tungsten oxide as indicated by Pourbaix diagram for tungsten.[4]

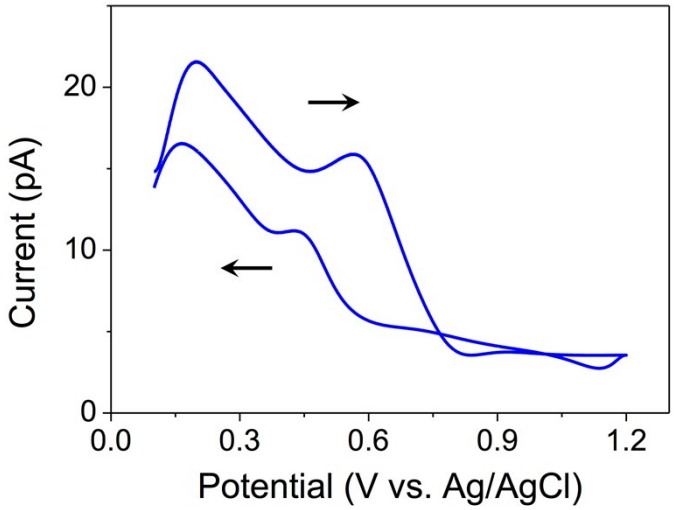

Fig. S3 Cyclic voltammogram of a tungsten tip measured in a phosphate buffer solution (pH 7.4, scan rate: 100 mV/s).

Statistical information of dopamine signals. We examined whether the height, half-width, and chage of dopamine signals (amperometric spikes) were affected by (1) the gap between a PC12 cell and a stimulating Au NW electrode, and (2) the magnitude of stimulating voltages.

When the stimulating Au NW electrode was positioned apart from a PC12 cell by ~20 to ~5 μm and – 0.3 V was applied to the stimulating electrode for 1 min, subsequently we observed the dopamine exocytosis. The information of the dopamine signals showed no significant tendency as a function of the cell-electrode gap (Fig. S4).

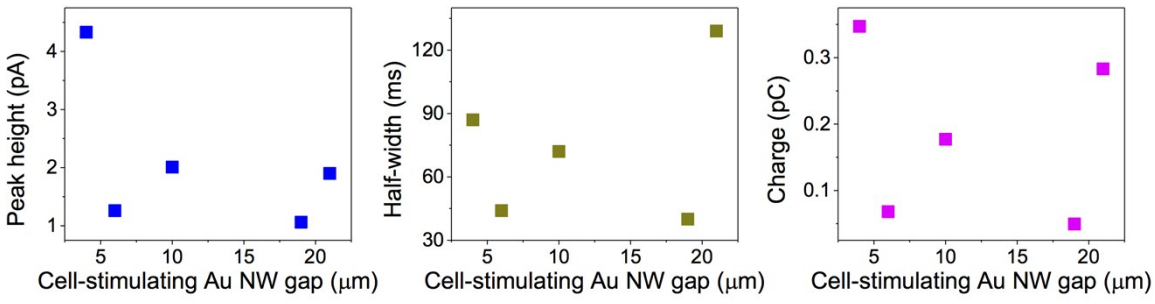

Fig. S4 Height (left), half-width (middle), and charge (right) of dopamine peaks as a function of the gap between a PC12 cell and a stimulating Au NW electrode (stimulating voltage : – 0.3 V).

The statistical information of the dopamine signals in Figure 3a-c was not significnatly affected by the stimulating voltage; the difference lies within the standard deviation (Fig. S5).

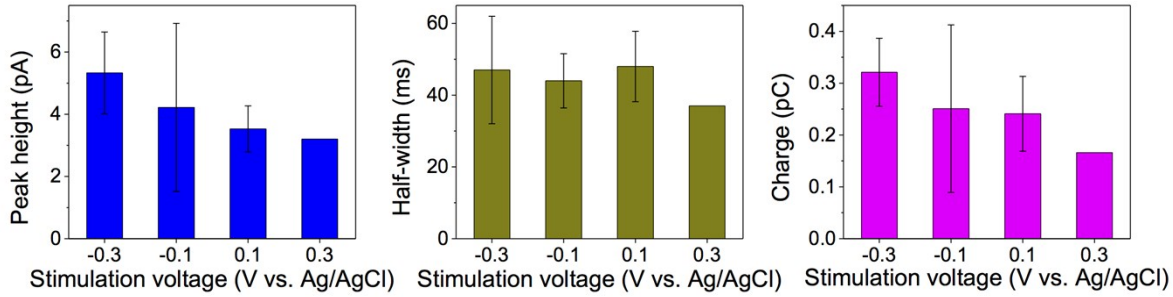

Fig. S5 Height (left), half-width (middle), and charge (right) of dopamine peaks as a function of stimulating voltage.

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