Amperometric monitoring of vesicular dopamine release by gold nanocone electrode

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Table of contents

1. Experimental Section

Chemicals and reagents. Ferrocenemethanol ($FcCH_2OH$) and Nerve Growth Factor-β were purchased from Sigma-Aldrich. The lyophilized powder was dissolving by 0.2 mm filtered PBS containing 0.1% BSA to 1.0 μg/mL and frozen it at -20 °C, room temperature melting before used. F-12K media and fetal bovine serum were purchased from GIBCO (USA). Apiezon wax was provided by Apiezon (UK). Glass capillaries (1.5 mm outside diameter, 0.86 mm inside diameter) were obtained from Sutter Instrument Company (USA). NaCl, KCl, $MgCl_2$, $CaCl_2$, NaOH, NaH_2PO_4, HEPES, PBS (0.1 M, PH 7.4), penicillin and stretomycin were purchased from Sigma. All aqueous solutions were prepared with doubly distilled water produced by a Milli-Q (resistivity of 18.2 MΩ cm) system. All other chemical reagents used in this work were analytical grade without further purification.

Apparatus. The capillary was pulled by P-2000 (Sutter Instrument Co). JSM-7800F scanning electron microscope (JEOL Ltd., Japan) was applied to characterize the surface of nanocone electrode and sputter gold film. A combined energy dispersive X-ray spectroscopy (EDX) and elements mapping analysis system attached to the SEM were used for elemental analysis. Cyclic voltammograms (CVs) were recorded by an electrochemical workstation (CHI 750C, CH Instruments, Shanghai, China), Ag/AgCl and Pt electrodes were used as the reference and counter electrodes, respectively. The pipet was pulled by P-2000 (Sutter Instrument Co), and sputter coated (SCD 500 sputter coater). The single-cell experiment was completed with the help of an inverted optical microscope (IX 71), Multiclamp 200B patch-clamp amplifier (Axon, Instruments, CA, USA), Digidata 1550B digitizer (Molecular Devices), micromanipulator (Transferman 4, Eppendorf, Hamburg, Germany) and a manual injector (Eppendorf, Hamburg, Germany).

Fabrication of nanoelectrodes. The Au nanoelectrodes were fabricated by a three-step process. First, a nanopipette with a ~100 nm (o.d.) sharp tip was pulled from a piece of

borosilicate capillary (BF100-58-10) by a micropipette puller (P-2000, Sutter Instrument Co). And the parameters were: Heat = 330, Fil= 3, Vel = 30, Del= 200, Pull=null and Heat= 340, Fil= 2, Vel = 27, Del= 160, Pull = 230. To achieve larger tip size, the HEAT (Δ 5 unit) and VELOCITY (Δ 1-3 unit) were decreased. Then the nanopipette was treated by a plasma device (SY-DT01, Suzhou OPS Plasma Technology CO., Ltd) to remove the organics on the surface and make the pipet cleaner. Next, the outer wall of pulled nanopipette was sputter-coated (SCD 500 sputter coater) with a layer of Au with a thickness of ~ 40 nm. Finally, the nanopipette was insulated via a kind of apiezon wax (APIEZON, UK) to leave a small region of the Au layer at the tip as a nanoelectrode to detect electroactive molecules. To optimize the insulation process, the concentration of wax was optimized as 0.03 g/ml to achieve a good film-forming property. The viscous wax solution was prepared under the room temperature of 20 ^{o}C fast to limit the evaporation of CH_2Cl_2. Then the gold-coating tip was dipped into freshly prepared wax solution and pulled out immediately. This procedure was repeated until almost the whole nanopipette was insulated. At this time, only a very small region with golden yellow of the tip can be seen by naked eyes.

Characterization of gold nanocone electrodes. The morphology and the Aperture size of the Au nanoelectrode were characterized before and after insulating by SEM (JEOL Ltd., Japan). A combined energy dispersive X-ray spectroscopy (EDX) and elements mapping analysis system attached to the SEM were used for elemental analysis. Cyclic voltammetric measurements were carried out by a computer-controlled CHI 750 C electrochemical workstation (CH Instruments, Shanghai, China). In the homemade three-electrode electrochemical cell, the Ag/AgCl and Pt electrodes were used as the reference and counter electrodes, respectively. And the nanoelectrode was immerged into 0.1 M PBS (PH 7.4) containing 1.0 mM $FcCH_2OH$ and 0.1 M KCl to characterize the region of the Au layer exposed at the tip. The voltage scanning ranged from 0 to 0.5 V, and a scanning rate of 50 mV/s was applied to collect the current during the measurement.

Cell culture and buffer solution preparation. PC12 cells used in the experiments were obtained from the Shanghai Bogu Biotechnology Co., Ltd. The culture medium was F-12K supplemented with 10% fetal bovine serum and 100 units mL^{-1} penicillin/streptomycin in a 7% CO_2, 100% humidity atmosphere at 37 °C. The cells were grown on mouse collagen coated cell culture flasks (collagen type IV, BD Biosciences, Bedford, MA) and subcultured every 7-9 days. Part of the cells in flasks were transferred to the dish for single-cell experiment. The cell medium was replaced every 2-3 days throughout the lifetime of all cultures. In order to acquire neutrally differentiated PC12 cells, confluent undifferentiated PC12 cells were counted and resuspended in fresh F-12K Nutrient Mixture supplemented with 10% FBS, then 50 ng/ml NGF at certain volume was added to reach a concentration of 105 cells/mL, and maintained for 6 days.

Before experiment, the culture medium was replaced with a solution containing 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. Exocytotic responses were evoked by a high K^+ saline solution containing 50 mM NaCl, 2 mM $CaCl_2$, 80 mM KCl, 0.7 mM $MgCl_2$, 1 mM NaH_2PO_4, and 10 mM HEPES, this solution was also titrated to pH =7.4 with NaOH.

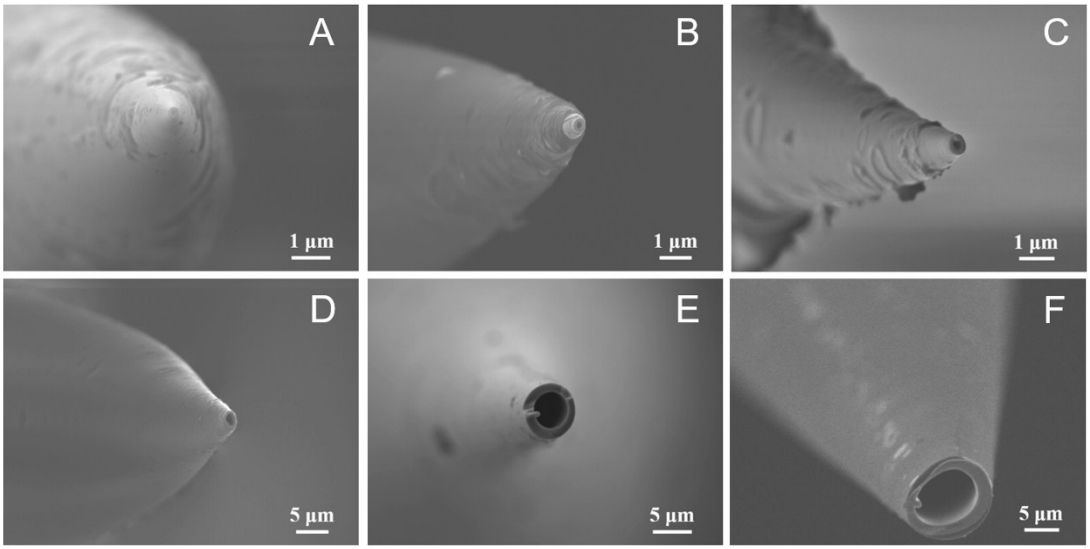
Single-Cell experiment. Electrochemical recordings at single cells were made on an inverted optical microscope (IX71, Olympus, Tokyo, Japan) inside a home-built Faraday cage. A silver wire was fixed on the surface of the Au electrodes to connect the electrochemical device. The recording of exocytotic events were carried out using an Axon Multiclamp 200B patch-clamp amplifier (Axon, Instruments, CA, USA) interfaced to a PC through a Digidata 1550B digitizer (Molecular Devices), and all apparatuses were grounded through a common ground. The working electrode was gently positioned onto a single cell using a micromanipulator (Transferman 4, Eppendorf, Hamburg, Germany). A slight deformation in the outline of the cell confirmed the close proximity of the electrode to the cell surface. Then the electrode was retracted slightly about 0.5 μm. When the tip of the Au nanoelectrode was

successfully positioned on the surface of a single cell and stable current baseline was achieved, chemical stimulants were delivered at ~50 μm away from the cell via a glass capillary containing high K^+ (100 mM) saline solution which connected to a micro automatic injector (Eppendorf, Hamburg, Germany) controlled by another micromanipulator. Exocytosis was stimulated with a 5-s, 30-psi pulse via the glass capillary. A constant potential (700 mV) was applied to the working electrode with respect to a single Ag/AgCl reference electrode placed in the cell bathing solution. Signals were sampled at 10 kHz, bessel filtered at 2 kHz, no events were recorded when the electrode was transiently withdrawn from an active recording site.

Data acquisition and analysis. Raw amperometric data were collected using "clampex" and then analyzed according to previously described methods.[1] The traces were carefully inspected after peak detection and false positives were manually rejected. Only traces with more than 10 peaks were used in the analysis.

2. SEM images of gold nanocone electrodes with different tip sizes

Fig. S1 displayed a series of nanoelectrodes with different tip size from tens of nm



to several μm successfully by adjusting the pulling parameters of glass capillary.

Fig. S1 SEM images of gold nanoelectrodes with different tip size from tens of nm to several μm

3. Comsol simulation of exposed gold surface area

For the quantitative characterization of the exposed area, the voltammetry of the nanocone was simulated using Comsol software as shown in Fig. S2. According to the value of limited current in Fig.2A, the length of gold film (segment c) was adjusted as 5.569 μm. The simulated surface area is 18.39 μm^2, which is 2.4 times larger than the geometry area measured in SEM image (7.77 μm^2). Since the sputtered gold film is not absolutely flat, the effective electrode area is relatively larger.

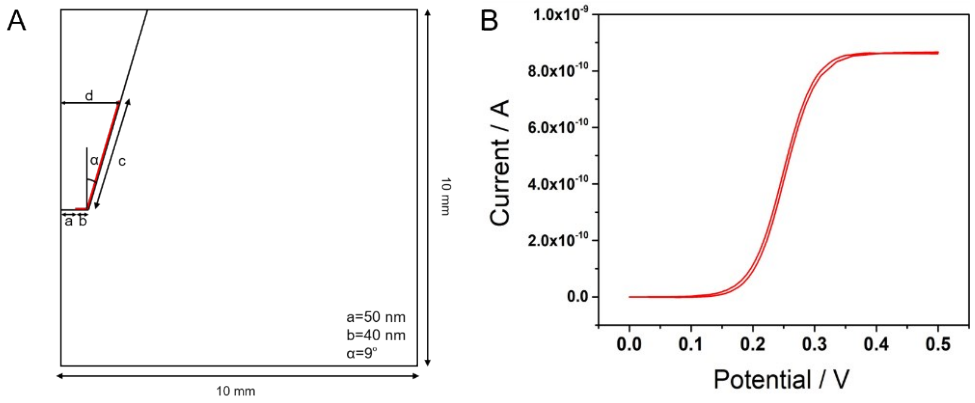


Fig. S2 (A) The model of the nanocone electrode used in finite element simulations. The region of the electrode (labeled in red) included the ring electrode at the tip surface (segment b) and an additional Au region at the capillary tip (segment c). (B) The voltammetry of the electrode obtained by simulation with the Au length (segment c) of 5.569 μm.

4. Electrochemical stability

As shown in Fig. S2, the cyclic voltammograms have good coincidence during five cycles of scanning, which exhibiting good electrochemical stability.

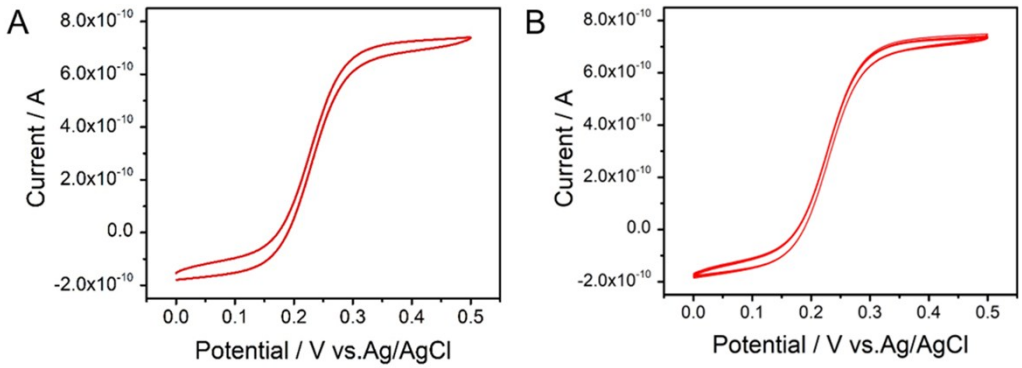


Fig. S3 Cyclic voltammograms at 50 mV s^{-1} with one cycle (A) and five cycles (B) for a nanoelectrode in 1 mM $FcCH_2OH$ solution containing 0.1 M KCl.

As shown in Fig. S3, the limiting current of the nanoelectrode in different days had almost no significant fluctuations, which demonstrated a good stability in long time and the potential for used into actual application.

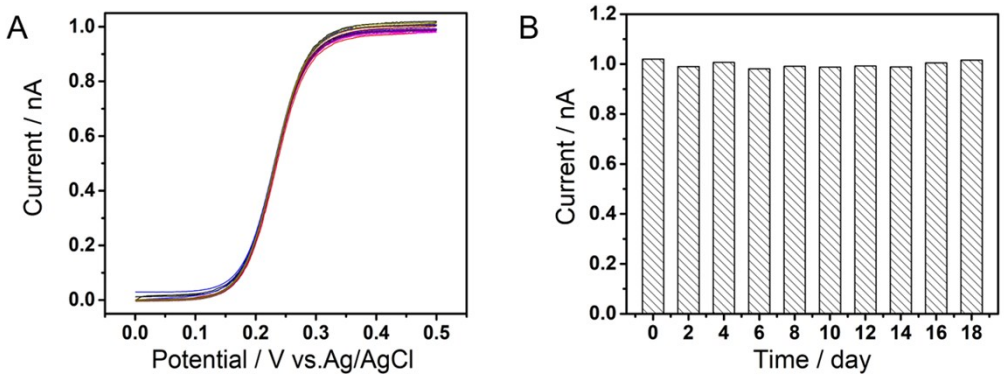


Fig. S4 (A) Voltammetric responses of the nanoelectrode in different days in a 1mM $FcCH_2OH$ solution containing 0.1 M KCl; (B) the limited current value of nanoelectrode in different days.

5. Voltammetric responses of the nanoelectrode to DA and its antifouling performance

As demonstrated in Fig. S4A, there is a sigmoidal shape of DA voltammetry response behavior which with a pair of redox peaks, indicating a fast radial-type diffusion model to the electrode accompanied by DA molecules adsorbed on the Au nanoelectrode surfaces. Fig. S4B shows that after dopamine oxidation experiment, the limited current was decreased slightly, which indicated that the nanoelectrode possess a good performance of antifouling.

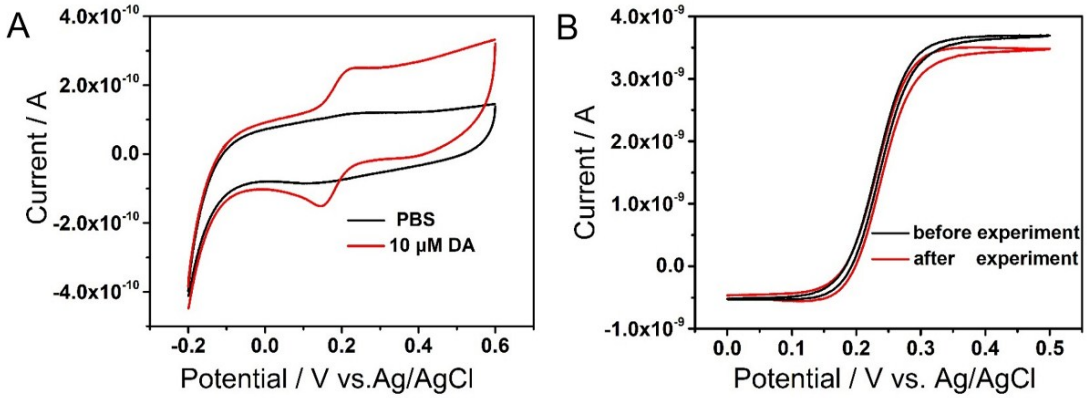


Fig. S5 (A) Voltammetric responses of a Au nanoelectrode in blank PBS (phosphate buffer solution, 0.01 M pH=7.4) (black line) and 10 μM dopamine solution (red line). (B) A nanoelectrode before and after experiment of dopamine oxidation in 1 mM $FcCH_2OH$ of 0.01 M PBS containing 0.1 M KCl.

6. Amperometric response of the nanoelectrode to DA and the linear fit curve

As shown in Fig. S5, the gold nanoelectrode was used as an electrochemical sensor to measure dopamine, the measurement result exhibited good linear relationship in the concentration ranges of 1.0 to 43.0 μM, with a detection limit of 184 nM (S/N =3) at an applied potential of 0.3 V.

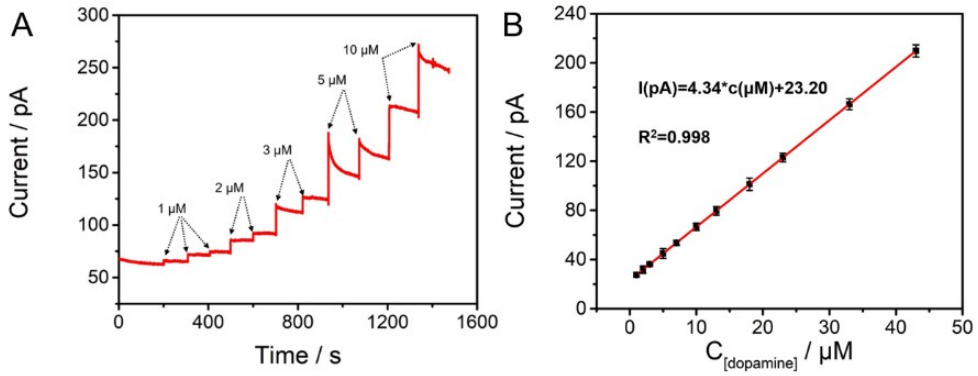


Fig. S6 (A) Amperometric i-t curves of gold nanoelectrode for the sequential addition of dopamine measured at 0.3 V (vs. Ag/AgCl); (B) Linear fit relationship between the peak current and the concentration of dopamine.

7. References

(1) E. V. Mosharov and D. Sulzer, *Nat Methods,* 2005, **2**, 651.