

# Vertically Aligned Carbon Nanotube-Sheathed Carbon Fibers as Pristine Microelectrodes for Selective Monitoring of Ascorbate in Vivo

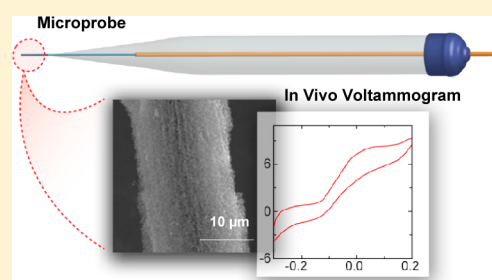
Ling Xiang,<sup>†</sup> Ping Yu,<sup>†</sup> Jie Hao,<sup>†</sup> Meining Zhang,<sup>\*,‡</sup> Lin Zhu,<sup>§</sup> Liming Dai,<sup>\*,§</sup> and Lanqun Mao<sup>\*,†</sup>

<sup>†</sup>Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

<sup>‡</sup>Department of Chemistry, Renmin University of China, Beijing 100872, China

<sup>§</sup>Department of Macromolecular Science and Engineering, Case Western Reserve University, Cleveland, Ohio 44106, United States

**ABSTRACT:** Using as-synthesized vertically aligned carbon nanotube-sheathed carbon fibers (VACNT-CFs) as microelectrodes without any postsynthesis functionalization, we have developed in this study a new method for in vivo monitoring of ascorbate with high selectivity and reproducibility. The VACNT-CFs are formed via pyrolysis of iron phthalocyanine (FePc) on the carbon fiber support. After electrochemical pretreatment in 1.0 M NaOH solution, the pristine VACNT-CF microelectrodes exhibit typical microelectrode behavior with fast electron transfer kinetics for electrochemical oxidation of ascorbate and are useful for selective ascorbate monitoring even with other electroactive species (e.g., dopamine, uric acid, and 5-hydroxytryptamine) coexisting in rat brain. Pristine VACNT-CFs are further demonstrated to be a reliable and stable microelectrode for in vivo recording of the dynamic increase of ascorbate evoked by intracerebral infusion of glutamate. Use of a pristine VACNT-CF microelectrode can effectively avoid any manual electrode modification and is free from person-to-person and/or electrode-to-electrode deviations intrinsically associated with conventional CF electrode fabrication, which often involves electrode surface modification with randomly distributed CNTs or other pretreatments, and hence allows easy fabrication of highly selective, reproducible, and stable microelectrodes even by nonelectrochemists. Thus, this study offers a new and reliable platform for in vivo monitoring of neurochemicals (e.g., ascorbate) to largely facilitate future studies on the neurochemical processes involved in various physiological events.



Development of highly selective microelectrodes with excellent analytical properties (i.e., without electrode surface modification) is of great importance for in vivo monitoring of neurochemicals in real time, because these pristine microelectrodes could be reproducibly fabricated free from person-to-person and electrode-to-electrode deviations.<sup>1</sup> Almost all in vivo voltammetric methods reported so far use carbon fibers (CFs) as microelectrodes.<sup>2</sup> In these cases, CF microelectrodes have to be subjected to manual surface modification, such as dip-coating and drop-coating, for selective transduction of (bio)recognition events into physically readable electronic signals unless some special electrochemical techniques, such as fast-scan cyclic voltammetry, are employed.<sup>2c-h</sup> The involvement of these additional manual modification procedures inevitably makes it difficult, if not impossible, to reproducibly prepare in vivo electrodes without person-to-person or electrode-to-electrode deviation.

Herein, we report a new strategy to use vertically aligned carbon nanotube-sheathed carbon fibers (VACNT-CFs) as pristine microelectrodes for in vivo monitoring of ascorbate. As one of the most important species in the central nervous system, ascorbate plays various important physiological roles.<sup>3</sup> For example, it functions not only as an antioxidant and free radical scavenger in the intracellular antioxidant network but

also as a neuromodulator for both dopamine- and glutamate-mediated neurotransmission.<sup>3c-f</sup> Although ascorbate itself has good electrochemical properties, its large oxidation overpotential (ca. 300 mV) makes selective electrochemical measurements of ascorbate in vivo a longstanding challenge.<sup>4</sup> Early attempts using carefully prepared CFs as pristine microelectrodes have made some progress in selective in vivo detection of ascorbate.<sup>5</sup> However, it remains very difficult to reproducibly pretreat CFs because their surface properties depend strongly on the sources of CFs.<sup>6</sup> Recently, we found that the use of carbon nanotubes (CNTs) could greatly facilitate the oxidation of ascorbate at low potential (ca. -50 mV),<sup>7</sup> opening a new avenue for selective ascorbate detection.<sup>7</sup> Compared with the use of CFs randomly modified with nonaligned CNTs as microelectrodes for in vivo monitoring of ascorbate,<sup>8</sup> the use of as-synthesized VACNT-CFs as pristine microelectrodes can effectively avoid the person-to-person and/or electrode-to-electrode deviations associated with manual modification procedures, leading to rapid and real-time in vivo

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monitoring of ascorbate with good reproducibility and high stability. After electrochemical pretreatment in 1.0 M NaOH solution, VACNT-CF pristine microelectrodes were demonstrated to facilitate electron transfer with ascorbate for efficient ascorbate detection in a highly reproducible manner. Therefore, this study provides a new platform of practical significance for *in vivo* measurements of ascorbate important to various physiological and pathological processes.

## EXPERIMENTAL SECTION

**Reagents and Solutions.** Dopamine (DA), iron(II) phthalocyanine (FePc), silicon tetrachloride ( $\text{SiCl}_4$ ), sodium ascorbate, uric acid (UA), 5-hydroxytryptamine (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC), ascorbate oxidase (*Cucurbita* species, EC 1.10.3.3), and L-glutamate were all purchased from Sigma and used as supplied, and the solutions were prepared just before use. Artificial cerebrospinal fluid (aCSF) was prepared by mixing NaCl (126 mM), KCl (2.4 mM),  $\text{KH}_2\text{PO}_4$  (0.5 mM),  $\text{MgCl}_2$  (0.85 mM),  $\text{NaHCO}_3$  (27.5 mM),  $\text{Na}_2\text{SO}_4$  (0.5 mM), and  $\text{CaCl}_2$  (1.1 mM) into Milli-Q water, and the solution pH was adjusted to pH 7.4. Other chemicals were at least analytical reagent grade and were used without further purification.

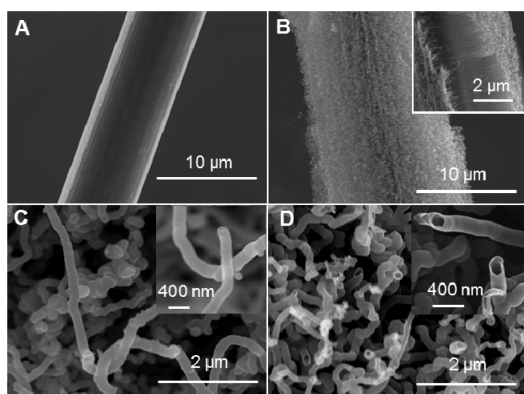
**Preparation of VACNT-CF Microelectrodes.** The growth of VACNTs on CFs (T650-35, Fabric Development Inc.) was performed as reported previously.<sup>9</sup> Briefly, the CFs were first treated in a high-temperature furnace at 1100 °C with a flow mixture of Ar ( $300 \text{ mL}\cdot\text{min}^{-1}$ ) and  $\text{H}_2$  ( $40 \text{ mL}\cdot\text{min}^{-1}$ ) carrying  $\text{SiCl}_4$  for 20 min in the presence of a trace of  $\text{O}_2$  under atmospheric pressure to form a thin layer of  $\text{SiO}_2$  on the CFs. VACNTs were then grown on these preactivated CFs by pyrolysis of FePc under Ar/ $\text{H}_2$  atmosphere at 800–1100 °C. The fabrication of VACNT-CF microelectrodes was quite similar to that for CF microelectrodes reported previously.<sup>8,10</sup> Briefly, a single VACNT-CF was cut to 1 cm in length and attached onto a copper wire with silver conducting paste. A glass capillary (o.d. 1.5 mm, length 100 mm) was pulled on a microelectrode puller (WD-1, Sichuan, China) into two capillaries, of which the fine tip was broken into 30–50  $\mu\text{m}$  in diameter. The pulled capillary was used as the sheath of the VACNT-CFs thus prepared. Then the VACNT-CF-attached copper wire was carefully inserted into the capillary, with the VACNT-CF exposed to the fine open end of the capillary and Cu wire exposed to the other end of the capillary. Both open ends of the capillary were sealed with epoxy resin with 1:1 ethylenediamine as the hardener. Excess epoxy on the VACNT-CF was carefully removed with acetone to form microelectrodes with pristine VACNT-CF electrode material. Thereafter, the VACNT-CF microelectrodes were dried at 100 °C for 2 h and the exposed VACNT-CF was carefully trimmed to 0.5–1.0 mm length under microscopy. The fabricated VACNT-CF and CF microelectrodes were first sequentially sonicated in acetone, 3 M  $\text{HNO}_3$ , 1.0 M NaOH, and water, each for 3 min, and then subjected to electrochemical activation. The VACNT-CF microelectrodes were electrochemically pretreated in 1.0 M KOH with +1.5 V for 80 s. For the control, the CF and VACNT-CF microelectrodes were electrochemically pretreated in 0.5 M  $\text{H}_2\text{SO}_4$ , first with potential-controlled amperometry at +2.0 V for 30 s, at –1.0 V for 10 s, and then with cyclic voltammetry within a potential range from 0 to +1.0 V at a scan rate of  $0.1 \text{ V}\cdot\text{s}^{-1}$  until a stable cyclic voltammogram was obtained.

**Apparatus and Measurements.** Electrochemical measurements were performed on a computer-controlled electrochemical analyzer (CHI 650D, Shanghai, China). The VACNT-CF and CF microelectrodes were used as working electrode and a platinum wire served as counter electrode. For both *in vitro* and *in vivo* electrochemical measurements, a tissue-implantable micro-sized Ag/AgCl electrode was used as reference electrode. The reference electrode was prepared by first polarizing Ag wire (1 mm diameter) at +0.6 V in 0.1 M hydrochloric acid for ca. 30 min to produce an Ag/AgCl wire and then inserting the as-prepared Ag/AgCl wire into a pulled glass capillary, in which aCSF was sucked from the fine end of the capillary (o.d. ca. 30  $\mu\text{m}$ ) and used as the inner solution. The other end of the capillary with the Ag wire exposed was sealed with epoxy. Scanning electron microscopy (SEM) was performed on a Hitachi S4300-F microscope (Japan).

**In Vivo Experiments.** Adult male Sprague-Dawley rats (300–350 g) were purchased from Center for Health Science, Peking University. The animals were housed on a 12/12 h light–dark schedule with food and water *ad libitum*. Animal experiments were performed as reported previously.<sup>7,8</sup> Briefly, the animals were anesthetized with chloral hydrate (345 mg/kg, ip) and positioned onto a stereotaxic frame. The VACNT-CF microelectrode was implanted into striatum (AP = 0 mm, L = 3 mm from bregma, V = 4.5 mm from dura) by standard stereotaxic procedures. The prepared micro-sized Ag/AgCl reference electrode was positioned into the dura of brain and secured with dental acrylic. Stainless platinum wire embedded in subcutaneous tissue on the brain was used as counter electrode. Local drug delivery by exogenous microinfusion of glutamate was constructed with silica capillary tubes (4 cm length, 50  $\mu\text{m}$  i.d., 375  $\mu\text{m}$  o.d.) bound with the VACNT-CF microelectrodes in parallel. The outlet of the capillary was ca. 400  $\mu\text{m}$  higher than the tip of microelectrode, with 50–100  $\mu\text{m}$  distance between the capillary and the microelectrode. Infusion solutions were delivered from gastight syringes and pumped through tetrafluoroethylene hexafluoropropene (FEP) tubing by a microinjection pump (CMA 100, CMA Microdialysis AB, Stockholm, Sweden). All local microinfusions were performed in the striatum of the rat brain at  $1.0 \mu\text{L}\cdot\text{min}^{-1}$ . Cyclic voltammetry (CV) and amperometric mode at a constant potential of +50 mV versus Ag/AgCl (aCSF) reference electrode were employed for *in vivo* measurements of ascorbate in rat brain.

## RESULTS AND DISCUSSION

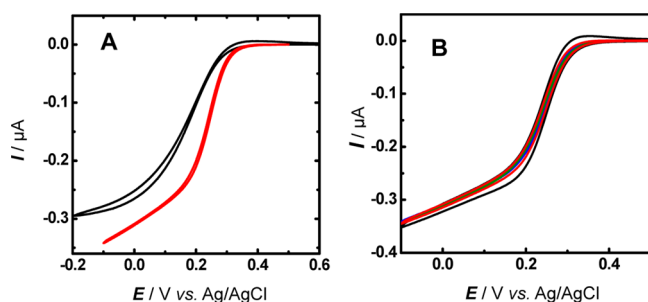
**Electrochemical Properties of the VACNT-CF Pristine Microelectrode.** Figure 1 displays scanning electron microscopy (SEM) images of CF (Figure 1A) and VACNT-CF (Figure 1B). As can be seen, the CF possesses a very smooth surface with a diameter of about 7  $\mu\text{m}$ . Figure 1B shows that the FePc-generated CNTs vertically aligned well around the  $\text{SiCl}_4$ -treated CF. A cross-section SEM image of VACNT-CF, taken after deliberately removing some CNTs from one side of the CF along its length (Figure 1B, inset), shows that VACNTs of ca. 3  $\mu\text{m}$  length were densely packed around the CF surface to form the coaxial VACNT-CF microelectrode. The VACNTs well-aligned around a CF can maintain good porosity with a large surface area and excellent electrochemical properties characteristic of CNTs, while the CF support could provide mechanical stability and efficient electrical conduction to and from the VACNTs. As we shall see later, therefore, VACNT-



**Figure 1.** SEM images of (A) CF, (B) VACNT-CF, and the tips of VACNT-CF (C) before and (D) after electrochemical treatment in 1.0 M NaOH.

CFs are ideal materials as pristine microelectrodes for in vivo monitoring of ascorbate.

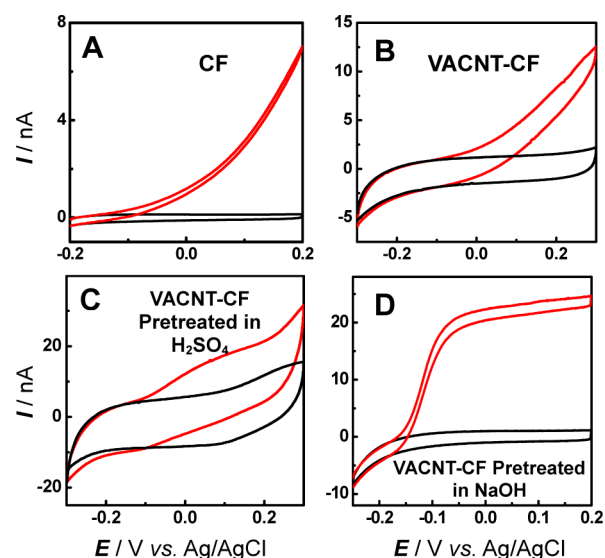
To investigate electrochemical properties for the VACNT-CF microelectrode, we performed cyclic voltammetry with  $K_3Fe(CN)_6$  as a redox probe. Figure 2A depicts typical cyclic



**Figure 2.** (A) Typical cyclic voltammograms (CVs) obtained at bare CF (black curve) and VACNT-CF (red curve) microelectrodes in aCSF containing 5 mM  $K_3Fe(CN)_6$ . Scan rate was  $10\text{ mV}\cdot\text{s}^{-1}$ . (B) Typical CVs obtained at VACNT-CF microelectrode in aCSF containing 5 mM  $K_3Fe(CN)_6$ . Scan rate was 5, 10, 20, or  $50\text{ mV}\cdot\text{s}^{-1}$  (from inner to outer).

voltammograms (CVs) at bare CF (black line) and VACNT-CF (red line) microelectrodes in aCSF containing 5 mM  $K_3Fe(CN)_6$ . The observed CVs at both electrodes exhibited a sigmoid-shaped voltammogram, and at the VACNT-CF microelectrode, the steady-state current remained almost unchanged when the scan rate was increased up to  $50\text{ mV}\cdot\text{s}^{-1}$  (Figure 2B). These features indicated nonlinear diffusion behavior for the electrochemical process on the VACNT-CF electrode with a steady-state current response characteristic of a micro-sized electrode. The smaller value of  $|E_{3/4} - E_{1/4}|$  at the VACNT-CF pristine microelectrode (76 mV) than that at the bare CF microelectrode (130 mV) revealed better reversibility for the  $K_3Fe(CN)_6$  redox probe at the VACNT-CF microelectrode, indicating fast electron transfer for CF-supported VACNTs, presumably due to the high electrocatalytic activity of CNTs and intimate contact between the VACNTs and CF support.<sup>9a,11</sup>

Figure 3 compares the oxidation processes of ascorbate at bare CF and VACNT-CF microelectrodes. At the bare CF microelectrode (Figure 3A), ascorbate is oxidized electrochemically with an ill-defined voltammetric response, indicating a sluggish electron-transfer process. It was interesting to find



**Figure 3.** CVs at different microelectrodes in aCSF (pH 7.4) in the absence (black lines) and presence (red lines) of 0.10 mM ascorbate. Scan rate was  $10\text{ mV}\cdot\text{s}^{-1}$ .

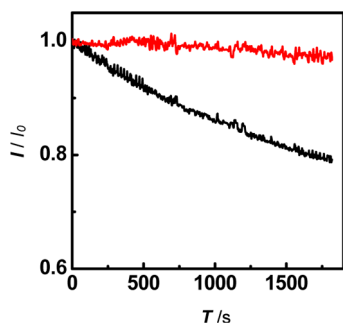
that, similar to the bare CF microelectrode, the VACNT-CF microelectrode also showed an ill-defined voltammetric response toward ascorbate (Figure 3B), though a reasonably good response to the  $K_3Fe(CN)_6$  redox probe was observed (Figure 3B, black line). The observed difference in electron transfer for ascorbate and  $K_3Fe(CN)_6$  was attributable to their different electrochemical behaviors. Indeed, an early study has demonstrated that  $K_3Fe(CN)_6$  is relatively insensitive at micro-sized fiber electrodes,<sup>6b</sup> while ascorbate is an inner-sphere reaction species with electron-transfer kinetics highly sensitive to the microstructure, cleanliness, and chemistry of the electrode surface.<sup>4</sup>

To improve electron transfer with ascorbate, we pretreated VACNT-CF microelectrodes through electrochemical oxidation to produce surface oxides, remove impurities, and activate the electrodes, as is the case with conventional carbon-based electrodes (e.g., GC, highly oriented pyrolytic graphite, carbon fiber).<sup>4d,5,12</sup> As a first try, we electrochemically pretreated VACNT-CF microelectrodes in 0.5 M  $H_2SO_4$  as we did for CF microelectrodes. Unfortunately, such pretreatment did not effectively improve electron transfer of ascorbate at VACNT-CF microelectrodes but increased the background current (13.7 nA, Figure 3C) resulting from formation of a transparent layer of oxidized carbon by electrochemical activation of the electrode in acidic solution.<sup>13</sup> We then electrochemically pretreated VACNT-CF microelectrodes in alkaline solution (i.e., 1.0 M NaOH) at +1.5 V for 80 s. To our surprise, we found that oxidation of ascorbate was largely improved; a well-defined steady-state current response was obtained at 0.03 V with a half-peak potential of ca.  $-0.11\text{ V}$  versus Ag/AgCl (aCSF) and the background current was even lower than that of the VACNT-CF microelectrodes (i.e., 1.98 nA) (Figure 3D). These results clearly revealed that electrochemical pretreatment in 1.0 M NaOH remarkably increased the electron-transfer kinetics for ascorbate oxidation at VACNT-CF microelectrodes.

To understand the mechanism underlying the improved electron transfer of ascorbate at aCNT-CF microelectrodes, we performed SEM studies on the VACNT-CFs before and after electrochemical pretreatment in 1.0 M NaOH, and we found that the nanotube tips of the treated VACNT-CFs were

opened, as shown in Figure 1D, which was not observed at untreated aCNT-CFs (Figure 1C). Thus, the pretreatment-induced nanotube tip opening of the VACNT-CFs could significantly increase the electron transfer of ascorbate, consistent with an early study in which we found that the electron transfer of ascorbate was much more favorable at the oxidized tip of CNTs with respect to the CNT sidewall.<sup>14</sup>

In addition, improved electron transfer of ascorbate and reduced background current were also considered to result from the removal of graphite oxides and surface redox couple at the VACNT-CF surface and minimal adsorption of the chemical species onto electrode surface.<sup>15</sup> As reported previously, adsorption of the ascorbate oxidation product onto the electrode surface often leads to electrode fouling, and hence a gradual decrease in the sensitivity.<sup>16</sup> Fortunately, the VACNT-CF microelectrode used in this study exhibited good resistance against electrode fouling with only 2.0% decrease in the current after continuous measurement of 0.5 mM ascorbate in aCSF at +0.05 V for 30 min (red curve in Figure 4). This



**Figure 4.** Amperometric current response for 0.5 mM ascorbate recorded with VACNT-CF (red curve) at +0.05 V and CF (black curve) microelectrodes at +0.2 V.  $I_0$  and  $I$  were current values at starting time and given time, respectively.

value compares very favorably with that of CF microelectrode (i.e., 21%), providing not only strong support for the aforementioned mechanism but also validation for the use of VACNT-CFs as pristine microelectrodes for in vivo electrochemical monitoring of ascorbate to be described below. Although use of CFs as pristine microelectrodes could also facilitate the oxidation of ascorbate after electrochemical pretreatment in 1.0 M NaOH, CFs cannot be used to constitute an analytical protocol for in vivo monitoring of

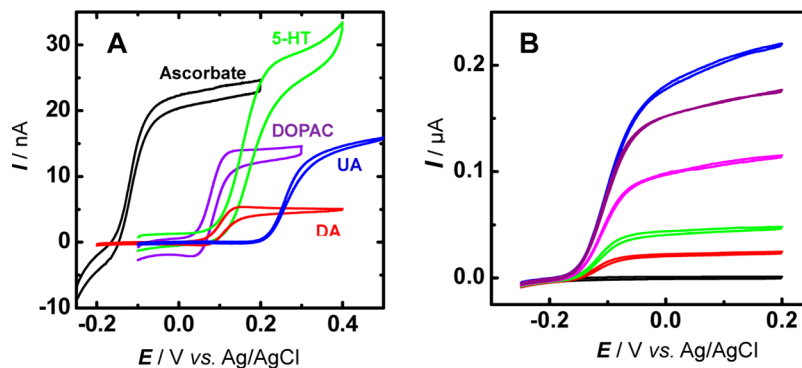
ascorbate due to their poor stability, as shown in Figure 4 (black curve).

#### Selectivity, Linearity, Stability, and Reproducibility.

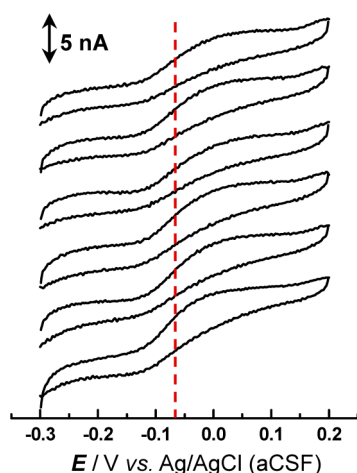
The excellent electrochemical properties of VACNT-CF microelectrode make possible the selective detection of ascorbate from its electrochemical oxidation even with other electroactive species coexisting in cerebral systems. As shown in Figure 5A, other electrochemically active species in the central nervous system including 5-HT, UA, DA, and DOPAC also produce well-defined quasi-steady-state current responses at the VACNT-CF microelectrode with  $E_{1/2}$  values of 0.17, 0.27, 0.10, and 0.08 V, respectively. These potentials are more positive than that for ascorbate oxidation at the same electrode. The well-separated ascorbate oxidation potential from those of other coexisting electrochemically active species seen in Figure 5A provides good validation for the in vivo selective measurements of ascorbate in rat brain by the newly developed VACNT-CF microelectrodes.

In addition to high selectivity toward ascorbate, the VACNT-CF microelectrode also showed good linearity for measurement of ascorbate. As can be seen in Figure 5B, the steady-state currents increased proportionally with increasing ascorbate concentration from 0.10 to 1.00 mM [ $I$  (nanoamperes) =  $177.50C_{\text{ascorbate}}$  (millimolar) + 6.44,  $\gamma = 0.9991$ ]. We have also investigated the reproducibility of VACNT-CF microelectrodes by comparing the current responses to ascorbate for electrodes prepared by different people from different batches. We found that, for all VACNT-CF microelectrodes, a well-defined sigmoid-shaped voltammogram was obtained for ascorbate oxidation with almost the same current response (data not shown), suggesting that stable microelectrodes could be easily and reproducibly fabricated from VACNT-CF starting materials. The unique electrochemical properties of VACNT-CF microelectrodes, together with their demonstrated selectivity, stability, and linearity, makes CF-supported VACNTs particularly attractive for in vivo monitoring of ascorbate in, for example, rat brain.

To demonstrate in vivo performance of VACNT-CF microelectrodes in rat brain, we studied in vivo sensitivity and stability of the microelectrodes. Figure 6 shows typical cyclic voltammograms for ascorbate oxidation at the VACNT-CF microelectrode in the striatum of an anesthetized rat brain measured successively every 5 min immediately after electrode implantation. As can be seen, all voltammograms recorded in vivo exhibited a well-defined sigmoid shape, which was quite



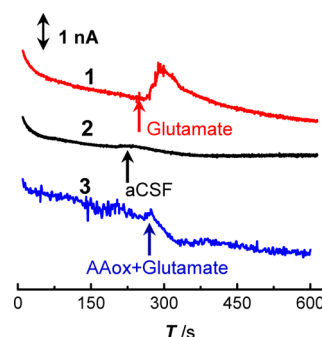
**Figure 5.** (A) CVs at VACNT-CF microelectrode in aCSF containing ascorbate, 5-HT, UA, DA, and DOPAC. Concentration for each species was 0.10 mM. Scan rate was  $10 \text{ mV}\cdot\text{s}^{-1}$ . (B) Typical CVs obtained at VACNT-CF microelectrode in aCSF (pH 7.4) containing ascorbate with different concentrations of 0.0, 0.1, 0.2, 0.5, 0.8, and 1.0 mM (from bottom to top).



**Figure 6.** CV responses consecutively recorded in vivo every 5 min at VACNT-CF microelectrode implanted in the striatum of anesthetized rats (from bottom to top). Scan rate was  $10 \text{ mV}\cdot\text{s}^{-1}$ .

similar to that recorded in vitro (Figure 3D, red line). Moreover, we found that the implanted VACNT-CF microelectrode has a capability of maintaining steady-state behavior and the same half-peak oxidation potential during successive in vivo measurements; after consecutively running the measurements six times with a time interval of 5 min, the current response decreased by ca. 20%, a value that is smaller than those reported previously for other electrodes.<sup>8a</sup> In addition, VACNT-CF microelectrode showed good reproducibility for in vivo measurements of ascorbate in striatum with a relative standard deviation calculated to be 6.4% ( $n = 8$ ). The excellent reproducibility and durability, along with the well-maintained sigmoid shape of the voltammograms recorded in vivo, revealed once again that VACNT-CF microelectrodes showed a high capability against electrode fouling even in rat brain. The basal level of striatum ascorbate for VACNT-CF electrodes in anesthetized rats was estimated to be  $0.25 \pm 0.06 \text{ mM}$  ( $n = 3$ ), which agreed well with reported values.<sup>3e,f,5e,8a</sup>

**In Vivo Observation of Striatum Ascorbate Release Evoked by Glutamate Infusion.** To further demonstrate in vivo application of the VACNT-CF microelectrodes developed in this study, one capillary (i.d.  $50 \mu\text{m}$ ) was implanted into the striatum together with the VACNT-CF microelectrode for exogenously infusing glutamate or aCSF into the brain. These solutions were delivered from syringes and pumped through FEP tubing by a microinjection pump. Figure 7 displays typical dynamic current responses recorded with the VACNT-CF microelectrode at  $+50 \text{ mV}$  in the rat striatum when  $2.0 \mu\text{L}$  of  $100 \mu\text{M}$  glutamate was exogenously infused into the vicinity of the implanted electrode at a flow rate of  $1 \mu\text{L}\cdot\text{min}^{-1}$ . A sharp yet transient increase in the current response was recorded (curve 1, Figure 7), suggesting an increase in the extracellular level of ascorbate after glutamate was consecutively infused into the rat brain, possibly through an ascorbate/glutamate heteroexchange mechanism at the glutamate uptake site.<sup>17</sup> As a control, we have also infused aCSF into the brain and did not see any obvious change in the current response (curve 2, Figure 7). To further verify that the increased response after glutamate infusion is entirely due to extracellular ascorbate change, we conducted an identical experiment in which excess ascorbate oxidase (AAox) was coinfused with glutamate. As shown in Figure 7 (curve 3), the infusion of a mixture of glutamate and



**Figure 7.** Amperometric responses for ascorbate recorded in vivo with VACNT-CF microelectrode during local microinfusion of  $2.0 \mu\text{L}$  of  $100 \mu\text{M}$  glutamate (curve 1),  $2.0 \mu\text{L}$  of aCSF (curve 2), and  $2.0 \mu\text{L}$  of  $100 \mu\text{M}$  glutamate containing AAox ( $40 \text{ units}\cdot\text{mL}^{-1}$ ) (curve 3), with  $1.0 \mu\text{L}\cdot\text{min}^{-1}$  into rat striatum. The electrode was polarized at  $+0.05 \text{ V}$ . Arrows represent the beginning of the microinfusion.

AAox did not lead to an increase in extracellular ascorbate level as observed with the VACNT-CF microelectrode; only an expected decline in the baseline level of ascorbate was recorded due to the enzymatic oxidation. These results were consistent with a previous report<sup>5b</sup> and demonstrate that the newly developed electrochemical method with VACNT-CFs as pristine microelectrodes could be used for reliable in vivo measurements of ascorbate in rat brain, in particular, and should be useful for physiological and pathological investigations in general.

## CONCLUSIONS

In summary, we have demonstrated a new strategy using VACNT-CFs as pristine microelectrodes for real-time, in vivo monitoring of ascorbate in rat brain. This strategy essentially alleviates manual surface modification and thus largely minimizes person-to-person and electrode-to-electrode deviations for fabrication of in vivo microelectrodes. Both in vitro and in vivo experiments demonstrated that VACNT-CF microelectrodes possess high selectivity, good reproducibility, and stability useful for selective and reliable measurements of ascorbate in rat brain. Furthermore, the methodology demonstrated in this study should be applicable to general in vivo measurements, since VACNT-CFs could be further rationally modified to have various functionalities in a controlled manner. Thus, this study offers a new analytical platform for in vivo measurements that is of great importance in understanding of chemical events involved in important physiological and pathological processes.

## AUTHOR INFORMATION

### Corresponding Authors

\*E-mail mnzhang@ruc.edu.cn.

\*E-mail lxd115@case.edu.

\*Fax +86-10-6255-9373; e-mail lqmao@iccas.ac.cn.

### Notes

The authors declare no competing financial interest.

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