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Ratiometric Electrochemical Sensor for Effective and Reliable Detection of Ascorbic Acid in Living Brains

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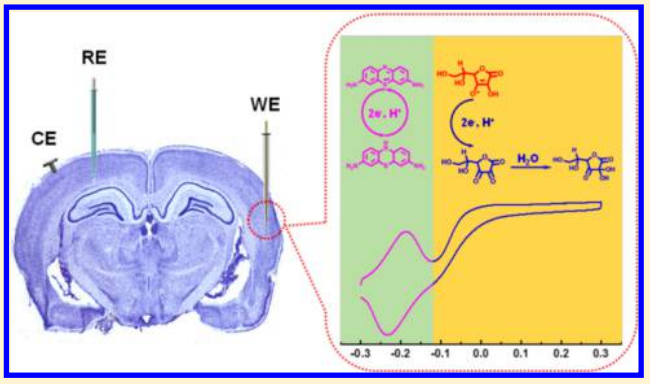
Supporting Information

ABSTRACT: The *in vivo* detection of ascorbic acid (AA), one of the physiologically important cerebral neurochemicals, is critical to probe and understand brain functions. Electrochemical sensors are convenient for AA detection. However, conventional electrochemical sensors usually suffer from several challenges, such as sluggish electron transfer kinetics for AA oxidation and poor reproducibility. To address these challenges, here we report ratiometric electrochemical sensors for effective and reliable detection of AA in living brains. The sensors were constructed by immobilizing preassembled thionine/Ketjen black (KB) nanocomposites onto glassy carbon (GC) electrodes or carbon fiber microelectrodes (CFMEs). The KB in the rationally functionalized nanocomposites efficiently facilitated AA oxidation at a relatively negative potential (∼−0.14 V) without particular physical or chemical pretreatment, forming the basis of selective measurement of AA. With a well-defined and reversible pair of redox wave at −0.22 V, the assembled thionine acted as an internal reference to substantially alleviate the lab-to-lab, person-to-person, and electrode-to-electrode variations. The *in vitro* experiments demonstrated that the sensors exhibited extremely high reproducibility and stability toward selective measurement of AA. More, with operational simplicity and robustness in analytical performance, the designed sensors were successfully applied to *in vivo* effectively, selectively, and reliably monitor the dynamic change of cerebral AA associated with pathological processes (i.e., salicylate-induced tinnitus as the model) in living rats' brains. This study not only offers a new strategy for construction of ratiometric electrochemical sensors but also opens a new way for selective and reliable detection of neurochemicals for probing brain functions.



As one of the most important biomolecules, ascorbic acid (AA, vitamin C) has been demonstrated to play critical roles in various physiological and pathological processes, from antiscurvy and neuroprotection in brain injuries/dysfunctions to modulating neurological functions and even promoting stem cell differentiation.[1−8] It is therefore of paramount importance to developing facile analytical methods for highly selective and reliable measurement of AA. Among the methods so far developed, the electrochemical approaches are convenient and suitable for AA detection because of their unique features, such as high sensitivity, low cost, and ease of miniaturization. Unfortunately, though AA is electrochemically active, its sluggish electron-transfer kinetics and severe electrode fouling by its oxidized product present great barriers for selectively electrochemical measurement of AA.[9,10] It is even more challenging to perform the measurements in complicated biological systems like the central nervous systems (CNS).[11]

Early attempts used ascorbic acid oxidase (AAOx) to selectively differentiate the net electrochemical response for AA from the total electrochemical signals, which were time-consuming and complicated.[12−15] Recently, Mao et al. demonstrated that carbon nanotubes (CNTs), one of the promising carbon nanomaterials, efficiently facilitated the oxidation of AA, establishing novel platforms for selective AA detection.[16,17] The CNTs-modified electrodes were easily prepared. More, these improved platforms successfully minimized previous inevitable variations. To further improve the sensing performance, the carbon fibers with *in situ* grown carbon nanomaterials as pristine microelectrodes have been developed for selective AA monitoring.[18] Such an approach successfully avoided the manual electrode modification and thus the potential person-to-person and electrode-to-electrode deviations. Even so, the ever-increasing interest in brain function probing still presents a pressing need to establish more effective methods for selective and reliable AA detection in living brains.

Recently, great effort has been made onto the development of ratiometric electrochemical biosensors.[19−23] The introduction of an independent redox probe provides a built-in correction toward the signal transduction during the bio-recognition process.[24] The obtained ratiometric response

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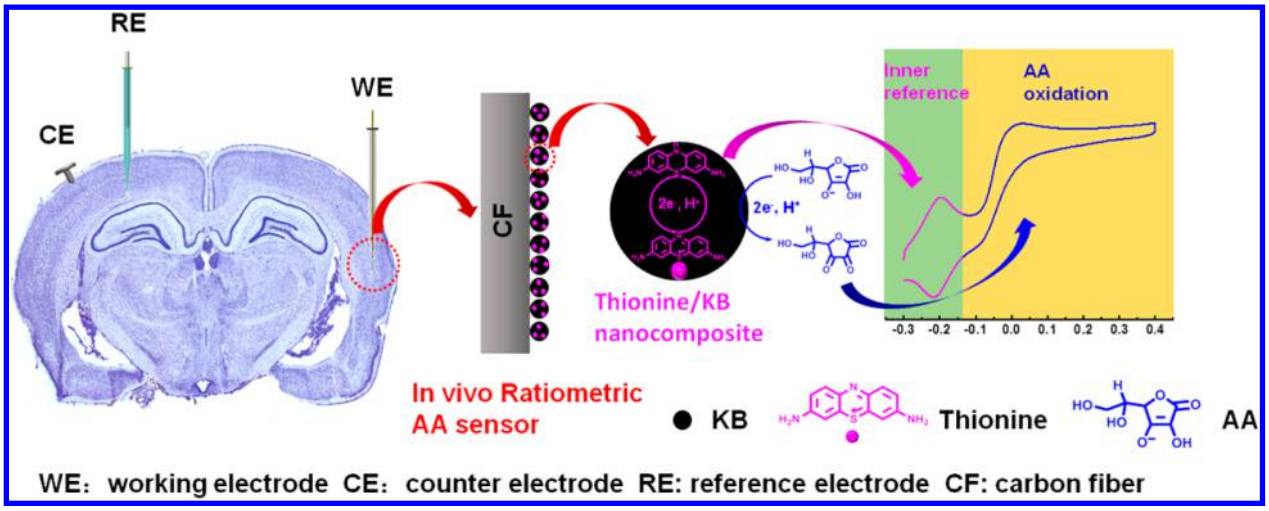
Scheme 1. Schematic Illustration of Rationally Designed Ratiometric Electrochemical Sensor for Selective and Reliable Measurement of Cerebral AA in Living Brains



between the internal reference and the analyte can substantially overcome intrinsic systematic errors of electrochemical sensors derived from environmental and personal factors and thus lead to remarkably enhanced reproducibility and robustness. For instance, ferrocene (Fc) and methylene blue (MB) were adopted as internal references for ratiometric and selective detection of metal ions, DNA, and others with much improved robustness and reproducibility.[19,20,22]

While the intrinsic properties of ratiometric electrochemical sensors make them promising for *in vivo* analysis, there are still several key challenges to be tackled to rationally design such sensors for selective and reliable measurement of cerebral AA in living brains. First, the electrode materials used should have good electrochemical catalytic activity toward AA oxidation, which can well distinguish the oxidation signals of AA from other electroactive neurochemicals. Second, the selected internal reference redox probe should exhibit reversible electrochemical activities. Third, the selected internal reference must possess high physical and chemical stability. Since ratiometric electrochemical sensors use the current ratio between the target and the internal reference instead of the absolute current value of the target for indicating target recognition, the potential dissociation or leakage of internal reference would lead to inevitable variations. Fourth, the internal reference should possess an appropriate redox potential, which does not disturb AA oxidation (Figure S1).

To address these challenges, in this study, we demonstrate a rationally designed ratiometric electrochemical sensor for *in vivo* selective and reliable detection of cerebral AA in the brain of living rats (Scheme 1). Carbon black (Ketjen black, KB), one of the promising carbon nanomaterials, was chosen as the electrode substrate due to its good conductivity, high surface-to-area ratio, and low cost.[25−27] Interestingly, we found that KB was able to readily facilitate the oxidation of AA at a relatively negative potential. To introduce an internal reference for ratiometric AA detection, KB was further functionalized by assembling with thionine, an azine dye that has also been widely used as a redox mediator in enzyme-based electrochemical biosensors.[28−31] It was shown that thionine was tightly immobilized onto KB through hydrophobic and $\pi-\pi$ stacking interactions. The assembled thionine/KB nanocomposites were then used to fabricate the ratiometric electrochemical sensors for AA detection by coating them on a glassy carbon (GC) electrode surface (or carbon fiber microelectrodes, CFMEs). Since thionine has a reversible redox potential at −0.22 V, it can act as a proper internal reference for AA detection (Scheme 1 and Figure S1). Our results indeed showed that the as-designed ratiometric electrochemical sensors exhibited excellent selectivity toward AA detection with high robustness, reproducibility, and stability. The promising application was successfully validated for *in vivo* selective measurement of cerebral AA in living rat brains under both health and pathological conditions. With the simplicity in design and the robustness in analytical performance, the demonstrated design strategy here is expected to offer a facile and general method to construct ratiometric electrochemical sensors for a wide range of other physiologically important chemicals in living systems.

EXPERIMENTAL SECTION

Chemicals and Materials. Ketjen black (KB) was purchased from Shanghai Cuike Chemical Co. Ltd. (Shanghai, China) and used without further purification. Ascorbic acid oxidase (AAOx) (Sigma-Aldrich), thionine (Alfa-Aesar), ascorbic acid (AA) (J&K Scientific), uric acid (UA) (J&K Scientific), dopamine (DA) (Adamas), 3,4-dihydroxyphenylacetic acid (DOPAC) (Accela), and 5-hydroxytryptamine (5-HT) (Alfa-Aesar) were used as received. Artificial cerebrospinal fluid (aCSF) was prepared by mixing NaCl (126 mM), KCl (2.4 mM), KH_2PO_4 (0.5 mM), $MgCl_2$ (0.85 mM), $NaHCO_3$ (27.5 mM), Na_2SO_4 (0.5 mM), and $CaCl_2$ (1.1 mM) into Milli-Q water, and the solution pH was adjusted to 7.4. All aqueous solutions were prepared with Milli-Q water (18.2 MΩ cm, Millipore).

Preparation of Thionine/KB Nanocomposites. The thionine/KB nanocomposites were prepared as follows. Typically, 1 mg of KB and 2 mg of thionine were dispersed into 1 mL of Milli-Q water. The thionine/KB nanocomposites were formed by at least a 5 h sonication at room temperature. The formed nanocomposites were then purified by centrifugation and sequential water washing for 3 times. The obtained thionine/KB nanocomposites were redispersed in 1 mL of Milli-Q water for further experiments.

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Fabrication of Thionine/KB-Modified Glassy Carbon (GC) Electrodes. Before assembling the thionine/KB nanocomposites, GC electrodes (3 mm in diameter, Bioanalytical Systems, Inc.) were first polished with aqueous slurries of fine alumina powders (0.3 and 0.05 μm) on a polishing cloth and then sonication-cleaned in water and alcohol, respectively (each for 5 min). To assemble the thionine/KB nanocomposites onto the above-treated GC electrode, 4 μL of thionine/KB nanocomposites aqueous suspension was drop-coated onto the GC electrode and then air-dried.

Fabrication of Thionine/KB-Modified Carbon Fiber Microelectrodes (CFMEs). The modified CFMEs were fabricated as follows. Briefly, a glass capillary (i.d. 1.5 mm, length 100 mm) was pulled on a microelectrode puller into two conic capillaries with sharp tips (10−30 μm in diameter). A single carbon fiber (9 μm in diameter) was attached to a copper wire with silver conducting paste and dried. Then, the carbon fiber was inserted into a conic capillary with the fiber exposed to the sharp open end of the capillary and the Cu wire exposed to the other open end of the capillary. Both the open ends of the capillary were sealed with 1:1 (v:v) of epoxy resin and ethylenediamine. The epoxy on the exposed fiber was removed with acetone. After a treatment at 100 °C for 1 h, the exposed carbon fiber of the CFMEs was cut to a length of 500−1000 μm.

Prior to the modification with thionine/KB nanocomposites, the above-obtained CFMEs were first cleaned by sequential sonication in acetone, 3.0 M HNO_3, 1.0 M KOH, and Milli-Q water, each for 10 s. The cleaned CFMEs were then electrochemically activated by sequentially subjecting to potential-controlled amperometry (+2.0 V for 30 s and −1.0 V for 10 s) and cyclic voltammetry (scanning from 0 to 1.0 V at a scan rate of 0.1 V s^{-1} until a stable voltammogram was obtained) in 0.5 M H_2SO_4.

To assemble thionine/KB nanocomposites onto the as-prepared CFMEs, one drop of thionine/KB dispersion was applied onto a clean glassy plate, and the CFMEs were carefully immersed into the droplet and rolling for 1 min. *Caution:* the carbon fibers could be easily broken in this step. The thionine/KB-modified CFMEs were then air-dried and rinsed with Milli-Q water for further use.

Electrochemical Measurements. Electrochemical experiments were performed on a computer-controlled electrochemical analyzer (CHI 660E, CHI Instrument). For *in vitro* measurements, the thionine/KB-modified GC electrodes or thionine/KB-modified CFMEs were used as the working electrodes with an Ag/AgCl (KCl-saturated) as the reference electrode and a platinum wire as the counter electrode. For *in vivo* measurements, the thionine/KB-modified CFMEs, a homemade, implantable miniaturized Ag/AgCl electrode, and an embedded stainless steel wire were used as the working, reference, and counter electrodes, respectively (Scheme 1).

Instrumentation. Scanning electron microscopy (SEM) was performed on a S-4800 instrument (Hitachi, Tokyo, Japan). X-ray photoelectron spectroscopy (XPS) was conducted with a PHI-5000 VersaProbe system (Ulvac-Phi, Japan) using Al Kα as the X-ray source. UV−visible spectra were recorded at room temperature on a TU-1900 spectrophotometer (Beijing Purkinje General Instrument Co. Ltd., China).

***In Vivo* Monitoring of AA in Living Brains.** Adult male Sprague−Dawley rats (250−300 g) were purchased from Jiesijie Laboratory Animal Co. (Shanghai, China). Rats' surgeries were performed as follows. Briefly, the animals were anaesthetized with chloral hydrate (345 mg/kg, i.p.) and positioned onto a stereotaxic frame. A thionine/KB-modified CFME was carefully implanted into the auditory cortex of the rat (AP = 3.84 mm, L = 6.50 mm from bregma, V = 3.50 mm from dura) using a standard stereotaxic. The miniaturized Ag/AgCl reference electrode was positioned into the dura of the brain and secured with tooth cement (Scheme 1). Cyclic voltammetry (CV) was employed for *in vivo* measurement of AA in rat brains.

To examine the selectivity of the thionine/KB-modified CFME for *in vivo* voltammetric measurement of AA, the responses of exogenously infused aCSF, AA, or AA followed by AAOx were recorded. These exogenously infused solutions were pumped into the cortex via a fine silicon capillary (i.d. 75 μm), which was in parallel combined with the thionine/KB-modified CFME and coimplanted into the brain. The outlet of the capillary was ∼0.5 mm higher than the tip of the microelectrode, and they were spaced by ∼0.5−1 mm. The pumping was performed by a microinjection pump (CMA 402, CMA Microdialysis AB, Stockholm, Sweden) with gas-impermeable syringes and tetrafluoroethylene hexafluoropropene (FEP) tubing (Scheme S1).

RESULTS AND DISCUSSION

Characterization of Thionine/KB Nanocomposites and Thionine/KB-Modified CFMEs. To function as an internal reference, the selected thionine should be efficiently assembled onto KB as the nanocomposites. Thionine was assembled onto KB via hydrophobic and $\pi-\pi$ stacking interactions. The formation of the thionine/KB nanocomposites was first confirmed by UV−visible spectroscopy. As shown in Figure 1A, the nanocomposites exhibited two peaks at

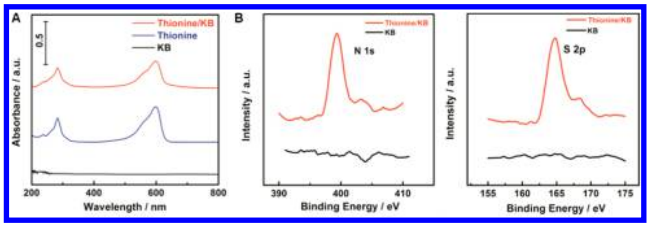

Figure 1. (A) UV−visible absorption spectra of KB, thionine, and thionine/KB nanocomposites. (B) XPS spectra of KB and thionine/KB nanocomposites.

around 283 and 600 nm, which matched well with the characteristic peaks of monomeric thionine. The absorption band at 283 nm was attributed to the $\pi-\pi^*$ transition of the phenothiazine ring while the 600 nm peak was assigned to the $n-\pi^*$ transitions of the C=N bond.[32] As a comparison, plain KB alone only exhibited featureless absorption.

The assembly of thionine onto KB was further demonstrated by XPS analysis. As shown in Figure 1B, N 1s and S 2p peaks located at 399 and 165 eV were clearly observed from thionine/KB nanocomposites, while no peaks were obtained from plain KB. These results substantially demonstrated the successful assembly of thionine onto the KB surface to form the thionine/KB nanocomposites.

Figure 2 shows typical SEM images of plain CFMEs and thionine/KB-modified CFMEs. The plain CFMEs possessed a relatively smooth surface with a diameter of about 9 μm. After the functionalization, the resultant electrode surface was well covered with the thionine/KB nanocomposites (Figure 2B). As

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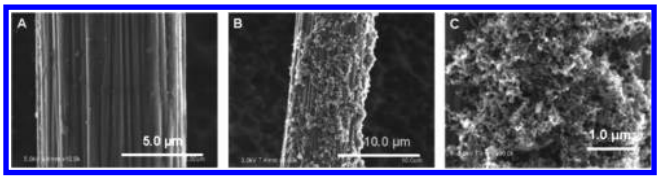

Figure 2. SEM images of plain CFME (A) and thionine/KB-modified CFME (B). (C) Higher magnification image of thionine/KB-modified CFME.

displayed in Figure 2C, the typical size of the assembled thionine/KB nanocomposites was around 50 nm, which would not only significantly improve the electrode surface but also efficiently facilitate the fast electron-transfer kinetics for AA oxidation (*vida infra*).

Evaluation of Thionine/KB-Modified GC Electrodes. As mentioned above, at least four criteria should be met to prepare a robust ratiometric electrochemical sensor for AA detection. Each of them was evaluated using thionine/KB-modified GC electrodes (or KB-modified GC electrodes) as follows.

First, the KB-facilitated oxidation of AA was studied at a KB-modified GC electrode in aCSF. The CVs in Figure 3A shows

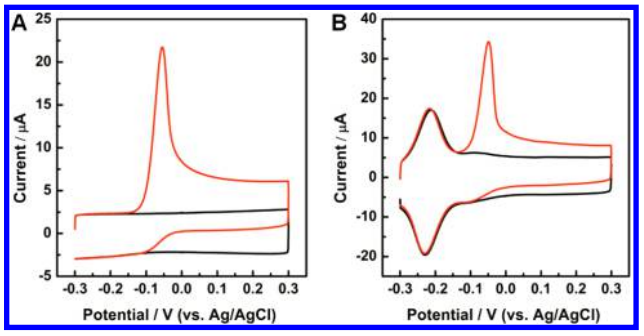

Figure 3. Typical CVs recorded at KB- (A) and thionine/KB-modified (B) GC electrodes in aCSF in the absence (black curves) or presence (red curves) of 1 mM AA. Scan rate: 10 mV s^{-1}.

that the oxidation of AA occurred at ∼−0.14 V and reached its maximum at −0.05 V. Such a negative onset potential demonstrated that KB indeed enabled the fast electron-transfer kinetics for AA oxidation.[16,17] More, when the AA oxidation was performed on a thionine/KB-modified GC electrode, the onset potential of AA oxidation was almost the same as that on a KB-modified GC electrode (Figure 3B). This suggests that the functionalization of KB with thionine has negligible influence on its capability in accelerating electron-transfer of AA oxidation. The results in Figure 3 clearly showed that KB was an ideal electrode substrate for effective AA measurements.

Then the electrochemical behaviors of thionine as the built-in internal reference on thionine/KB-modified GC electrodes were assessed. As shown in Figure 3B (black curve), the thionine/KB-modified GC electrode exhibited a pair of well-defined redox waves at a formal potential of −0.22 V, which was ascribed to the reversible redox processes of thionine assembled onto the KB surface (Scheme 1). When the thionine/KB-modified GC electrode was scanned in the presence of AA, the redox waves of thionine remained nearly the same (Figure 3B, red curve), indicating that the assembled thionine did not take part in and thus did not disturb the KB-facilitated oxidation of AA. More importantly, the oxidation peaks of thionine and AA were well separated by ∼165 mV and no overlap between them was observed.

The stability of the thionine/KB nanocomposites on GC electrodes was also studied. As shown in Figures S2 and S3, the thionine/KB-modified GC electrode exhibited excellent stability under either continuous CV scanning for at least 50 cycles or continuously dipping the electrode in aCSF for at least 4 h. These results strongly suggested that the thionine/KB nanocomposites can be employed to develop ratiometric electrochemical sensors for selective and reliable AA detection by using the redox peaks of thionine as the internal reference.

Ratiometric Electrochemical Detection of AA with Thionine/KB-Modified GC Electrodes. The performance of the as-designed thionine/KB-modified GC electrodes toward ratiometric detection of AA was studied. Figure 4 depicts

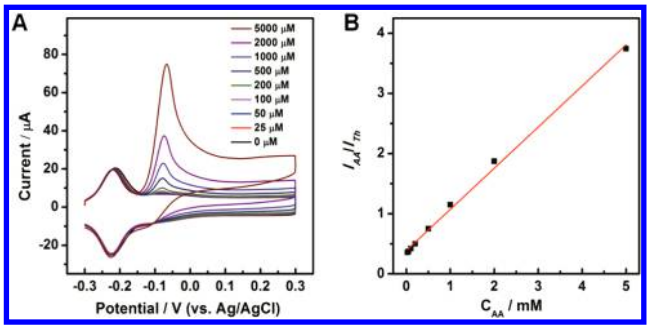

Figure 4. Ratiometric electrochemical detection of AA with a thionine/KB-modified GC electrode: (A) typical CVs of a thionine/KB-modified GC electrode in aCSF in the presence of different concentrations of AA. Scan rate was 10 mV s^{-1}. (B) I_{AA}/I_{Th} as a function of AA concentration.

typical CVs of a thionine/KB-modified GC electrode in aCSF containing different concentrations of AA. Interestingly, the oxidation peak of AA at ∼−0.05 V (denoted as I_{AA}) increased with the increasing concentration of AA while the oxidation peak of thionine −0.21 V (denoted as I_{Th}) remained constant, resulting in a ratiometric approach to AA detection. The ratio between the two peak currents (i.e., I_{AA}/I_{Th}) showed a good dynamic range for the concentration of AA from 0.025 to 5 mM. The detection limit for AA was as low as 25 μM. Such a dynamic range toward AA detection well covers the physiological concentrations of AA in the CNS (∼200−400 μM),[1,17,18] substantially validating its application for *in vivo* detection of cerebral AA in living brains.

To demonstrate the unique advantages of the as-developed ratiometric electrochemical sensor over conventional electrochemical sensors in AA detection, we intentionally varied the amounts of thionine/KB nanocomposites (or KB alone as controls) confined onto the GC electrode surface. Then the performance of both types of electrochemical sensors toward AA detection was interrogated. As shown in Figure 5A, though various amounts of thionine/KB nanocomposites were applied, the obtained response curves for the three sensors were almost the same. In contrast, when various amounts of KB were applied, totally different response curves were obtained with the three KB-modified GC electrodes (Figure 5B), suggesting that the amount of KB confined onto the electrode surface had a significant influence on the AA detection performance. Such variations are hard-to-avoid for conventional electrochemical sensors because both environmental and personal factors are readily involved in fabricating the sensors. The introduction of an internal reference (thionine in this case), however, efficiently reduces the environmental and personal disturbance and thus

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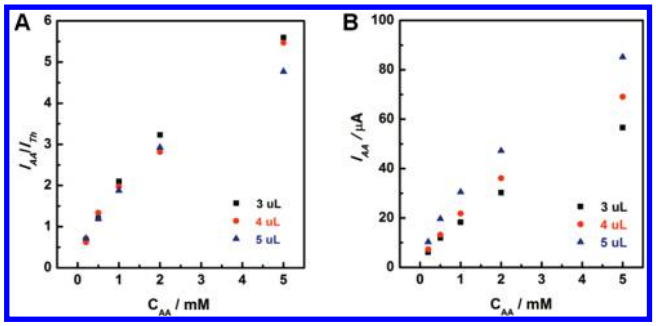

Figure 5. Plots of electrochemical response versus AA concentrations with different amounts of thionine/KB nanocomposites modified GC electrodes (A) and different amounts of KB modified GC electrodes (B).

successfully enhances the robustness of electrochemical sensors toward target detection.

For conventional electrochemical sensors, the electrode-to-electrode differences also introduce the variations. However, such variations can be eliminated using the developed ratiometric electrochemical sensors. As shown in Figure S8, three independent thionine/KB-modified GC electrodes exhibited almost identical responses toward AA detection. The robustness of the ratiometric sensor against "harsh" treatments was also tested. The results in Figure S9 demonstrated that the ratiometric sensors had excellent robustness and reliability compared to the conventional ones.

To use our ratiometric electrochemical sensor for measurement of cerebral AA in living brains, it is of critical importance to evaluate its selectivity because of the chemical and physiological complexity in CNS. Here the selectivity against several commonly coexisting electroactive species in the cerebral systems was investigated. As displayed in Figure S10, the oxidation of these electroactive neurochemicals like DOPAC, DA, UA, and 5-HT occurred at 0.10, 0.12, 0.24, and 0.27 V, respectively. These potentials were more positive than and well separated from that for AA oxidation, forming the basis of *in vivo* selective measurement of AA in living brains with the developed ratiometric electrochemical sensor.

***In Vivo* Measurement of Cerebral AA with Thionine/KB-Modified CFMEs.** After establishing that thionine/KB-modified GC electrodes can be used for ratiometric electrochemical detection of AA with excellent robustness and selectivity, the use of the thionine/KB nanocomposites for *in vivo* measurement of AA in living brains was then studied. For this purpose, the modified CFMEs instead of modified GC electrodes were used due to their much smaller size that would induce minimal tissue damage as well as faster electron-transfer kinetics and better sensitivity (see Figure S11). As shown in Figure S12, the oxidation of AA at thionine/KB-modified CFMEs occurred at ~−0.10 V due to fast electron-transfer kinetics, which was similar to the thionine/KB-modified GC electrode. As expected, the assembled thionine exhibited a stable well-defined redox wave at −0.21 V, which was well separated from the AA oxidation peak and thus could be employed as the internal reference. When tested in aCSF containing different concentrations of AA, the obtained calibration curves for different thionine/KB-modified CFMEs were also almost the same, further confirming the good reproducibility of the developed ratiometric sensors.

To demonstrate *in vivo* performance of the developed ratiometric electrochemical sensors, a thionine/KB-modified CFME was carefully implanted into the cortex of a rat brain. Figure 6 displays typical CVs recorded every 5 min with the

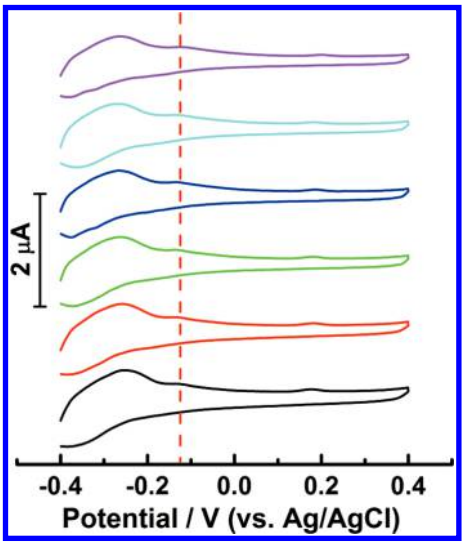

Figure 6. Consecutive CV curves *in vivo* recorded every 5 min at a thionine/KB-modified CFME implanted in the cortex of a rat brain (from bottom to top). Scan rate: 10 mV s^{-1}.

implanted CFME. Compared with CVs of *in vitro* experiments, the recorded redox waves of thionine broadened, which might be ascribed to severe electrode fouling as well as remarkably enhanced resistance in cerebral systems. The consecutive CVs remained unchanged with a stable I_{AA}/I_{Th} ratio during a long-run monitoring, revealing the good stability and reproducibility of the ratiometric sensor for endogenous AA measurements. According to the postcalibration, the basal level of cortical AA under normal conditions was estimated to be 0.28 ± 0.07 mM (n = 3). To further confirm the recorded oxidation current at ~−0.12 V resulted from endogenous AA oxidation, 100 mM AA was exogenously microinjected into the vicinity of the implanted electrode through a capillary at a flow rate of 1 μL min^{-1}. As shown in Figure S13B, the oxidation current at −0.10 V gradually increased with a continuous AA infusion. As a control, the infusion of aCSF did not produce any obvious changes (Figure S13A). When the microinjected AA was replaced with AAOx, the oxidation current at −0.10 V gradually decreased due to the AAOx catalyzed oxidation of AA (Figure S13C). These *in vivo* results substantially demonstrated that the developed ratiometric electrochemical sensor was able to *in vivo*, selectively and reliably measure the extracellular AA in living brains.

The validity of this ratiometric electrochemical sensor in probing brain functions was illustrated by continuously monitoring the dynamic changes of AA in auditory cortex during salicylate-induced tinnitus. As displayed in Figure 7 and Figure S14, the AA concentration in auditory cortex remained constant when the rats were under normal conditions. After the rats were administrated with salicylate-induced tinnitus, the AA concentration sharply increased to ~150% of its basal levels after 4 h. Although the exact neurochemical mechanisms for salicylate-induced tinnitus are still unclear, the instant increment of AA may be mediated by the excitotoxicity of *N*-methyl-D-aspartic acid (NMDA) receptor which is activated by the accumulation of arachidonic acid, efflux of calcium ion, production of reactive oxygen species (ROS), etc.[33−37]

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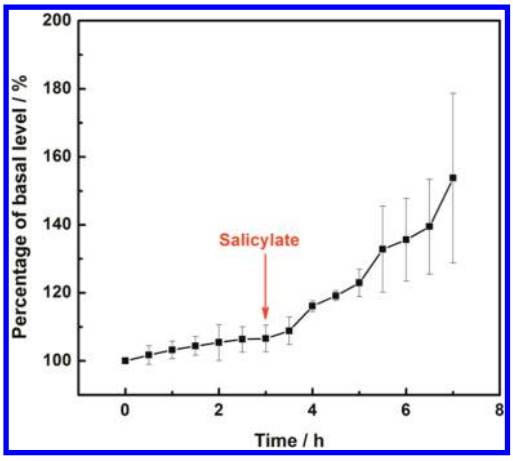

Figure 7. Dynamic change of AA responses consecutively recorded with a thionin/KB-modified CFME implanted in the auditory cortex of a rat brain following salicylate-induced tinnitus.

CONCLUSIONS

In this study, we have rationally designed a ratiometric electrochemical sensor for *in vivo* effective, selective, and reliable detection of cerebral AA in living brains. To construct the sensor, the redox probe thionine was first assembled onto KB to form the thionine/KB nanocomposites. The resultant nanocomposites not only efficiently facilitated AA oxidation but also provided a built-in internal reference for ratiometric sensing. After immobilizing the nanocomposites onto GC electrodes (or CFMEs), the obtained ratiometric electrochemical sensors exhibited good selectivity, excellent stability, and high reproducibility toward AA detection. The developed ratiometric electrochemical sensors successfully minimized lab-to-lab, person-to-person, and electrode-to-electrode deviations. More, with operational simplicity and robustness in analytical performance, the designed sensors were successfully applied to *in vivo* effectively and reliably monitor the dynamic change of AA associated with pathological processes. This study not only offers a new strategy for construction of ratiometric electrochemical sensors but also establishes a novel platform for neurochemical detection to probe brain functions with good stability and reliability.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b02014.

Additional figures and the associated discussion (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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