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Brain Electrochemistry

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Abstract: Brain, as the source of neural activities such as perceptions and emotions, consists of the dynamic and complex networks of neurons that implement brain functions through electrical and chemical interactions. Therefore, analyzing and monitoring neurochemicals in living brain can greatly contribute to uncovering the molecular mechanism in both physiological and pathological processes, and to taking a further step in developing precise medical diagnosis and treatment against brain diseases. Through collaborations across disciplines, a handful of analytical tools have been proven to be befitting in neurochemical measurement, spanning the level of vesicles, cells, and living brains. Among these, electrochemical methods endowed with high sensitivity and spatiotemporal resolution provide a promising way to precisely describe the dynamics of target neurochemicals during various neural activities. In this review, we expand the discussion on strategies to address two key issues of *in vivo* electrochemical sensing, namely, selectivity and biocompatibility, taking our latest studies as typical examples. We systematically elaborate for the first time the rationale behind engineering electrode/brain interface, as well as the unique advantages of potentiometric sensing methods. In particular, we highlight our recent progress on employing the as-prepared *in vivo* electrochemical sensors to unravel the molecular mechanism of ascorbate in physiological and pathological processes, aiming to draw a blueprint for the future development of *in vivo* electrochemical sensing of brain neurochemicals.

Key words: *in vivo* electrochemical sensing; brain chemistry; selectivity; biocompatibility

1 Introduction

The human brain confers on us the capacities of thinking, feeling, learning and acting, which essentially emerges from the electrical and chemical interactions among tens of billions of neurons that compose the brain circuits. Through worldwide collaborations across disciplines, studies have been conducted to gain comprehensive understanding of the relationship between structural maps and functional maps, spanning the levels of vesicles, cells, circuits, and even higher levels[1-4]. Despite of these breakthroughs, it is still a long-term goal to obtain selective, quantitative, and real-time information on the kinetics of chemicals in living animal brain, providing promising ways to uncover molecular mechanism in both physiological and pathological processes. To date, a handful of analytical tools have been developed for measurement of targeted neurochemicals, which broadly fall into two categories, namely, noninvasive ones and invasive ones[5-7]. Noninvasive methods (e.g., functional magnetic resonance imaging and fluorescent assays) are capable of providing cross-scale pattern of neural activities while keeping the intact brain circuits injury-free[8-12]. On the other hand,

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invasive methods (e.g., *in vivo* electrochemistry) endowed with superior spatiotemporal resolution are capable of providing precise and dynamic pattern of specific targets during various neural activities [13-18]. This review aims to briefly outline our studies in tissue-implantable electrochemical sensing technologies in terms of molecular selectivity, biocompatibility, and potential applications in brain sciences. We will summarize for the first time the rationale behind engineering interfacial electron transfer and the development of potentiometric systems to control over the electrochemical selectivity and the biocompatibility of the *in vivo* sensors, with a specific highlight on employing these sensors for revealing molecular mechanism in physiological and pathological processes.

2 Developing Highly Selective *In Vivo* Sensors

Central nervous system (CNS) as sort of biological systems is highly dynamic and chemically complex, posing grand challenges for selective measurement of desired species. Ideally, analytes with electrochemical activity can be electrochemically transformed at electrode surface and generate a current or voltage change as readout for selective detection and quantification. However, the coexistence of structurally and electrochemically similar species such as their precursors and metabolites often present the big interference for highly selective measurement.

The emerging of fast scan cyclic voltammetry (FSCV) represents a great step forward to *in vivo* monitoring of the fast kinetics of neurochemicals sensitively, selectively and quantitively[19-21]. In specific, the electrochemical kinetics at carbon fiber electrode (CFE) among different neurochemicals becomes distinguishable at ultrahigh potential sweeping speed, resulting in distinct redox peak potentials for qualitative measurements. Over years of development and improvement, FSCV has greatly facilitated the understanding of rapid release of neurotransmitters in some pathological and physiological processes. Notably, these rapid release processes often bring with concurrent change of other neurochemicals (e.g., local pH). Therefore, with imperative need of eliminating mutual interferences and acquiring accurate measurements, strict requirements have been put forward on concurrent multicomponent analysis. Confronted with this challenge, the ongoing improvements in computational methods provide immense opportunities[22-25]. Recently, we proposed a deep learning-based voltammetric (DLV) platform for *in vivo* analysis of multiple neurochemicals by using vapor grown carbon fiber microelectrodes (VGCFEs). Dopamine (DA), ascorbate (AA) and ions were selected as a typical neurotransmitter, an important neuromodulator, and main component of extracellular fluid in CNS, respectively[26]. This method enables us to record the interplaying concentration changes in living rat brain during spreading depression (SD), which indicates a bright perspective not only in molecular mechanism discovery, but also in medical diagnosis and treatment.

In addition to voltammetry, potential-controlled amperometry as another important branch of *in vivo* electrochemical methods is well suited for continuous sensing of neurochemicals. Specifically, amperometry measures the concentration-dependent current transient over time at a constant potential, which quantitatively measures the rapid dynamics of target analytes. To achieve selective sensing, dedicated efforts have been devoted into constructing electrode/brain interfaces to exclusively permit specific electrochemical reactions[27, 28]. One common strategy is to immobilize recognition elements onto electrode surface, exemplified by biomacromolecules such as enzymes and aptamers. Besides, electrocatalysts modified onto the electrode could also achieve selective sensing by either accelerating the adsorption/desorption process or altering electron transfer kinetics and pathways of specific neurochemicals. More recently, ion transport-based sensors also provide another complementary strategy for selective sensing, greatly expanding the scope of detectable neurochemicals to encompass not only electroactive but also electroinactive ones. We herein highlight our latest progress on tuning electrochemical behavior of target-

ed neurochemicals, with a focus on unraveling the rationale behind engineering electrode/brain interface.

2.1 Biological Recognition Elements

Natural enzymes featured with high specificity and quick response are befitting recognition elements for *in vivo* biosensing[29]. Oxidases and dehydrogenases are the most widely utilized natural enzymes in design and construction of sensing interfaces[30-37]. After years of exploring and practicing, we gradually established the oxidase/dehydrogenase-based online electrochemical systems (OECSs) that realize *in vivo* biosensing of important neurochemicals (*e.g.*, glucose, lactate, hypoxanthine), which have been comprehensively reviewed previously[38-42]. However, interferences coming from oxygen or cofactors hinder these oxidase/dehydrogenase-based biosensors from *in vivo* neurochemical biosensing.

Confronted with these challenges, we believe that exploring new types of enzymes can push the boundaries of interference-free *in vivo* biosensing. Recently, we introduced ferredoxin-dependent glutamate synthase (Fd-GltS) as a new candidate to catalyze both glutamate synthesis and glutamate oxidation through different mediated electron transfer pathways (Figure 1(A))[43]. Specifically, Fd-GltS can catalyze bio-electrosynthesis of glutamate from glutamine and 2-oxoglutarate when using methyl viologen as the mediator. While using mediators with high redox potential, oxygen-independent bio-electrooxidation of glutamate was realized in the presence of Fd-GltS. With the redox center close to its surface, Fd-Glts holds great potential in the design of glutamate-based *in vivo* biosensor through direct electron transfer between the enzyme and electrode.

Besides both natural and artificial enzymes, aptamers also provide a powerful tool for developing high selective *in vivo* electrochemical biosensors[44]. Aptamers are short, synthetic single-stranded nucleic acids that possess recognition ability of specific target molecules with high affinity. Generally, aptamers undergo conformational changes upon binding with analyte molecules, resulting in an altered electron transfer pathway between electrodes and redox moieties modified on aptamers. Our early attempt of developing aptamer-based biosensor provides a dual recognition unit strategy for selective ATP sensing *in vivo*, by incorporating polyimidazolium-brush (PimB) and aptamers together to realize selective recognition of both triphosphate moieties and A nucleobase simultaneously[45]. Following that, we made more efforts on developing interfacial functionalization strategies and extending application of aptamer-based *in vivo* sensor. More recently, we demonstrated that the aptamer-modified CFE (aptCFE) shows a high selectivity for *in vivo* sensing of DA in living rat brain (Figure 1(B)). This study opens up a versatile strategy in the design of electrode/brain interface for exploring brain chemistry[46].

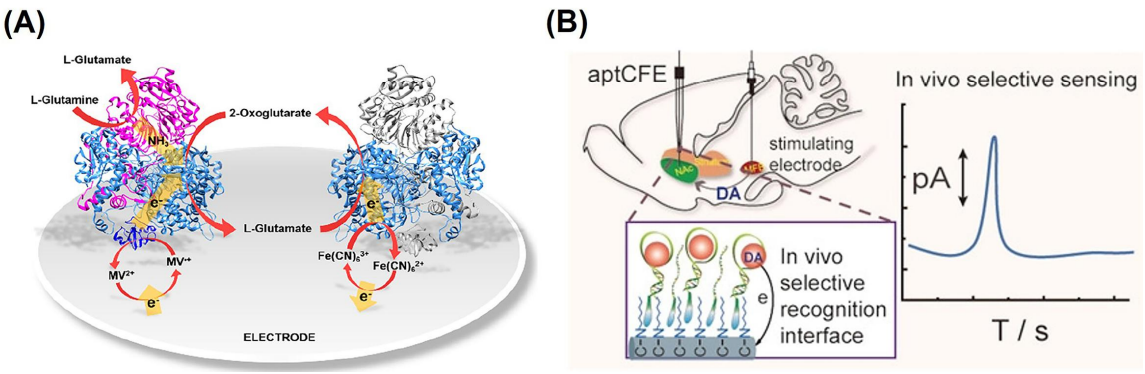

Figure 1 (A) Schematic illustration of mediated electron transfer pathways catalyzed by Fd-Glts[43]. Reproduced with permission of Ref. 43, copyright 2018 American Chemical Society. (B) Schematic illustration of aptCFE and typical current response of *in vivo* DA dynamics upon electrical stimulation[46]. Reproduced with permission of Ref. 46, copyright 2020 WILEY-VCH. (color on line)

2.2 Interface Design Guided by Formal Potential Sequence

In addition to biological entities that are endowed with high specificity, electrocatalysis with functional materials also provide promising opportunities for *in vivo* sensing[47-49]. Then, the question was asked: how could we identify the materials that can selectively recognize target analytes among numerous coexisting neurochemicals? In this case, we implemented the idea of guiding the interface design based on the formal potential ($E^{0'}$) of the neurochemicals. In general, for the molecules with low $E^{0'}$, such as ascorbic acid (AA), we demonstrate that by constructing the electrode surface with catalysts that can sufficiently accelerate the electrochemical processes, the "optimal selectivity" would be realized. AA, mostly known as the name of vitamin C, gains intense interests in brain research as it has been revealed recently to act more than simply a "micronutrient" in the CNS[50, 51]. Accumulating evidence suggests a close association between AA and neurochemical processes involved in both cognitively intact and impaired brain, which was almost unrecognized due to the lack of *in vivo* and qualitative assessment of this chemically unstable molecule in living systems[52, 53]. The availability of selective and reliable sensing approach essentially offers great opportunities for *in vivo* sensing of AA in both fundamental and clinical researches.

We found that the confinement of carbon nanotubes (CNTs) onto electrode surface remarkably accelerates the electrochemical oxidation of AA, enabling the oxidation to occur almost without an overpotential[54-57]. The excellent electrochemical activity of CNTs made it possible for us to selectively monitor AA without interference from other co-exiting species in biological systems. Further mechanistic study revealed that both chemical and electronic properties of CNTs are dominant factors for providing the sensing selectivity toward AA[56].

We further extended our studies on designing electrochemical sensors for molecules with high $E^{0'}$. We reasoned that electrocatalysts that are highly reactive for analytes, while show minimal reactivity to the interfering species, could fulfill the selectivity requirement. In this case, single-atom catalysts (SACs), featuring maximum atom utilization and chemically tunable coordination environment, are appealing candidates for rationally designing electrocatalyst with tailor-made selectivity and catalytic activity[58, 59]. Taken oxygen, a highly important biomolecule with high $E^{0'}$, as a typical target, we demonstrated that through tailoring catalytic metal atom-adsorbates (including both O_2 and H_2O_2) interactions, single-atom electrocatalysts can realize selective catalytic activity toward O_2 reduction without interference from H_2O_2. Specifically, we found that with designed Co-N_4/C catalyst, H_2O_2 shows very weak adsorption on Co centers, enabling the priority of direct four-electron reduction of O_2 on the electrode[60]. The excellent selectivity toward O_2 reduction endows the Co-N_4/C-based sensor with a high selectivity for O_2 sensing without interference from H_2O_2, offering a novel avenue to selective O_2 sensing in living animals (Figure 2(A)). Subsequent studies using different SACs further outline a more general role of tailoring catalytic metal atom-adsorbates interaction in achieving high selectivity, especially in (bio)sensing of physiological important chemicals with high $E^{0'}$ values, such as nitric oxide[61] (Figure 2(B)) and glucose[62] (Figure 2(C)).

In addition to the attempts to modulate interfacial electron transfer property of the neurochemicals with single atom catalysts, we recently tried to modulate the electron transfer pathway of different neurochemicals. Graphdiyne (GDY), featuring unique chemical and electronic properties, is an appealing target for this line of research[63]. For example, this two-dimensional carbon allotrope shows a low reduction potential and highly conjugated electronic structure. We reasoned that these would make GDY an efficient reducing agent and stabilizer for synthesizing nanoparticles on the surface, therefore, generating new type of catalyst with tunable catalytic activity[64]. Using Pd as an example, we demonstrated that GDY enables electroless deposition of ultrafine Pd clusters on its surface, yielding an excellent composite catalyst with superior selectivity and activity toward the reduction

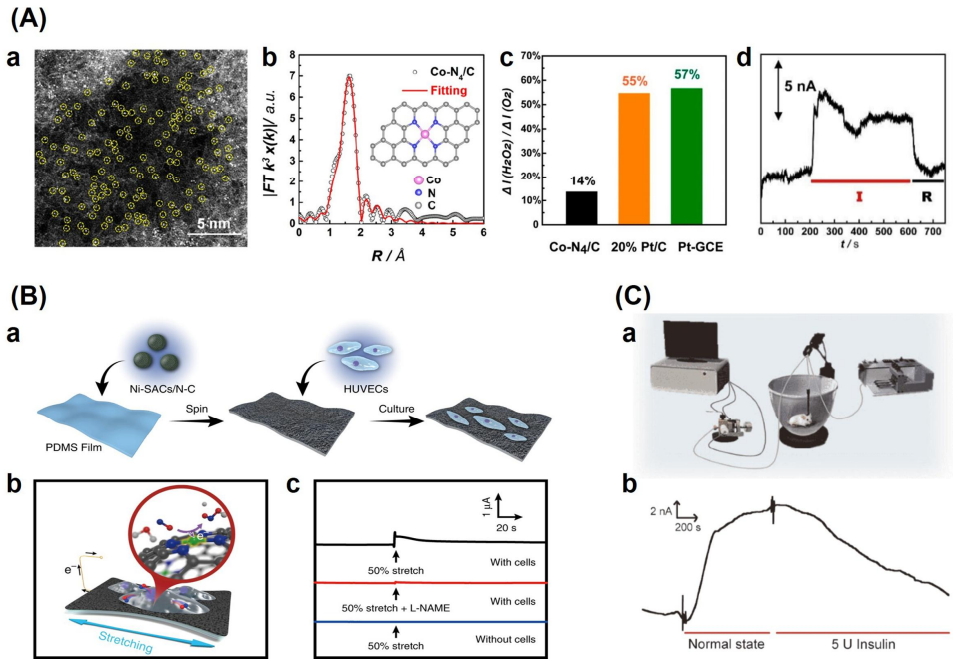

Figure 2 (A) (a-b) HAADF-STEM image (a) and EXAFS fitting curve (b) of Co-N_4/C. (c) Relative current ratio of H_2O_2 to O_2 recorded by three catalysts shown in the figure, and (d) *in vivo* O_2 fluctuation recorded by Co-N_4/C-based sensor[60]. Reproduced with permission of Ref. 60, copyright 2020 American Chemical Society. (B) (a-b) Schematic illustration of fabrication process (a) and stretching process (b) of Ni SAC/N-C-based sensor. (c) Real-time monitoring of NO release[61]. Reproduced with permission of Ref. 61, copyright 2020 Springer Nature. (C) (a) Schematic illustration of online electrochemical system (OECS) with a SAC-based electrochemical sensor for continuous glucose monitoring, and (b) typical amperometric response of OECS toward microdialysate *in vivo* sampled from rat brain[62]. Reproduced with permission of Ref. 62, copyright 2019 Springer. (color on line)

of 4-nitrophenol (Figure 3(A)). Giving a variety of metals are available, the electroless deposition approach provides a versatile platform for designing of electrocatalysts with tailorable selectivity, paving new avenues to modulating electron transfer for diverse selectivity needs in *in vivo* sensing applications. Furthermore, we have established a liquid phase exfoliation method with lithium hexafluorosilicate (Li_2SiF_6) for GDY, which is envisaged useful to understand the intrinsic property of GDY[65] (Figure 3(B)). The mechanism behind this efficient exfoliation probably attributed to the strong non-covalent inter actions between SiF_6^{2-} and GDY, and the easy diffusion of the cation (Li^+ and K^+) into the interlayers, thus synergistically weaken the attractive forces between GDY interlayers. With this method, damage-free single-, and few-layered GDY flakes can be obtained under ambient and mild conditions. Moreover, the exfoliated GDY flakes in the form of solvent suspension allow an immediate utilization for spin-coating or any other solution processing to address crucial prospects for sensor construction. Besides GDY, its oxidized form named graphdiyne oxide (GDYO) that possesses not only acetylenic bonds but also various forms of oxygen-containing functional groups on its surface also exhibits attractive properties for *in vivo* sensing. The exploration of the structure-activity relationship of GDYO greatly improves our understanding of the mechanism behind modulating the interfacial electron transfer, contributing to the development of a highly selective GDYO-based humidity sensor for potential human health and disease monitoring[66] (Figure 3(C)).

Based on these studies, we demonstrated that the electron transfer pathway can be further regulated by interfacing GDY and redox molecules[67] (e.g., methylene green, MG). While the semiconducting GDY films decelerate the oxidation of AA, the intercalated MG molecules that relay electrons by fast self-exchange

can accelerate the oxidation of dihydronicotinamide adenine dinucleotide (NADH), making it possible to construct a selective bioelectrocatalytic interface. As a typical example, NAD^+-dependent glucose dehydrogenase (GDH) is immobilized onto the MG-intercalated GDY nanosheets. The resulted GDH-MG/GDY-based biosensor shows great selectivity of glucose, free from the interference from coexisting neurochemicals including AA, DA and serotonin (5-HT) (Figure 3(D)). This study provides a universal strategy for tuning electrochemical properties of semiconducting or insulating materials, greatly broadening the studies on *in vivo* sensing sciences.

2.3 Ion Transport-Based *In Vivo* Sensor

In the complex environment of living brains, the electroactive neurochemicals are only the minority. For the other neurochemicals, their sluggish electrochemical reaction kinetics poses grand challenges on *in vivo* sensing by direct electrolysis. To circumvent this issue, ion transport-based sensing method opens a new paradigm in design of *in vivo* neurochemical sensors[68]. For example, ion current rectification (ICR), an ion transport behavior featured with unequal current intensities at the negative/positive potential with the same magnitude, reflects specific interaction between analytes and recognition elements modified on the inner-surface of nanopipettes. However, nanopipettes are inapplicable to be directly implanted into the living brain, mainly due to their fragileness and nanometer size. To address this issue, we prepared polyimidazolium-brush (PimB)-modified micropipettes and successfully extended ICR from nanoscale to microscale in symmetric electrolyte solution[69, 70]. For deep understanding of the experimental observations, we proposed a three-layer model consisting of a charged layer (CL), a double layer (DL) and a bulk layer (BL) (Figure 4(A)). Validated by both numeric simulation and experimental results, it is demonstrated

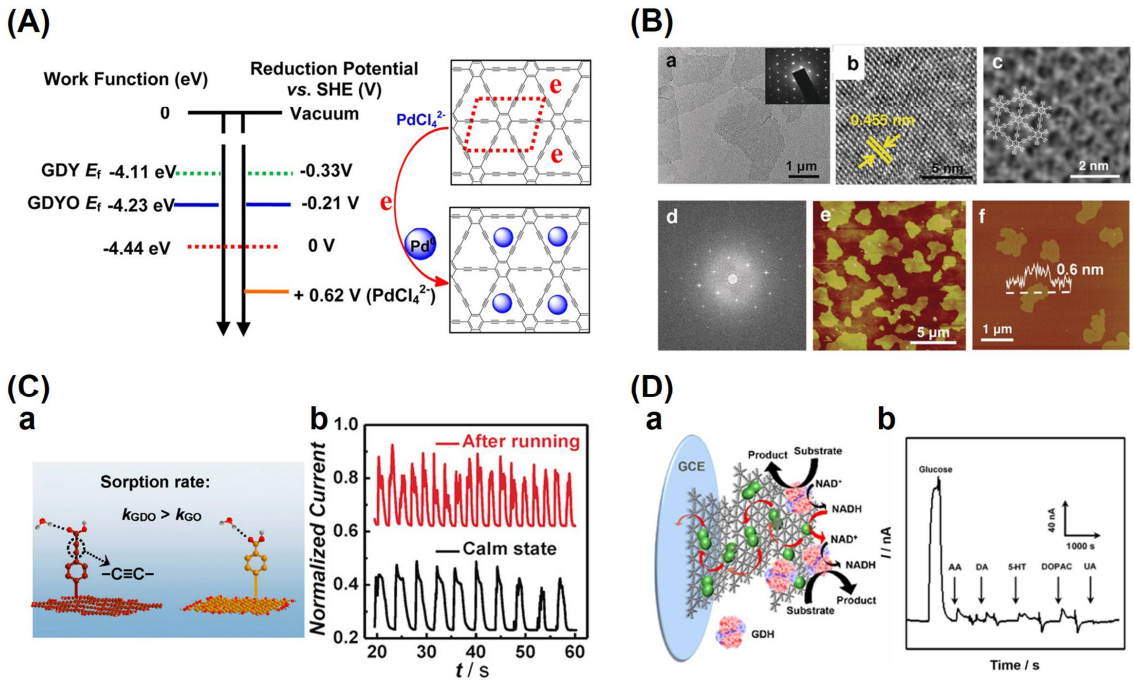

Figure 3 (A) Schematic illustration of Pd/GDY formation via electroless deposition[64]. Reproduced with permission of Ref. 64, copyright 2015 American Chemical Society. (B) TEM image (a), HRTEM image (b), STEM image (c), FFT pattern (d) and AFM images (e-f) of exfoliated GDY[65]. Reproduced with permission of Ref. 65, copyright 2019 WILEY-VCH. (C) (a) Schematic illustration of bonding model between water molecules and carbon nanomaterials (i.e., GO and GDYO). (b) Normalized current responses of GDYO-based sensor towards different human respiratory patterns[66]. Reproduced with permission of Ref. 66, copyright 2018 WILEY-VCH. (D) (a) Construction of the mediated biosensor interface and (b) typical amperometric responses to glucose and other neurochemicals[67]. Reproduced with permission of Ref. 67, copyright 2020 American Chemical Society. (color on line)

that influencing factors including the length of PimB, the concentration of electrolyte and the radius of micropipette collectively account for the observed microscale ion current rectification (MICR). With sufficient mechanical robustness and a designable inner surface, this MICR-based sensor unlocks the potential for ion transport-based *in vivo* neurochemical sensing. By coupling ATP aptamer and MICR-based sensors, we were able to determine the basal level of ATP in brain cortex[71].

We envisioned that MICR-based sensing methods may be capable of monitoring the dynamics of electrochemically inactive neurochemicals in the living brains. To improve the spatiotemporal resolution of MICR-based sensors, we developed the first transient ion transport-based microsensor by applying high-frequency square-wave pulse potential[72]. In general, the rapid association/dissociation of specific analytes on the modification alters surface charge density of the inner surface of the micropipette, resulting in variation of ion current output with temporal resolution at milliseconds level. Taking pH as a model target, we prepared poly(N-vinylimidazole)-brush (PvimB)-modified micropipette sensors, which exhibited high temporal resolution, sensitivity, selectivity, repeatability and stability (Figure 4(B)). The as-prepared microsensor was then implanted into the living rat brain, successfully monitoring pH variation during acid-base disturbance upon CO_2 inhalation. We proposed that this study established a versatile sensing platform based on transient ion transport behavior, opening up a new avenue for *in vivo* monitoring of transient process of neurochemical dynamics through rationale interface design.

3 Developing Highly Biocompatible *In Vivo* Sensors

The voltammetric and amperometric sensors enable selective and sensitive analyses of biomolecules

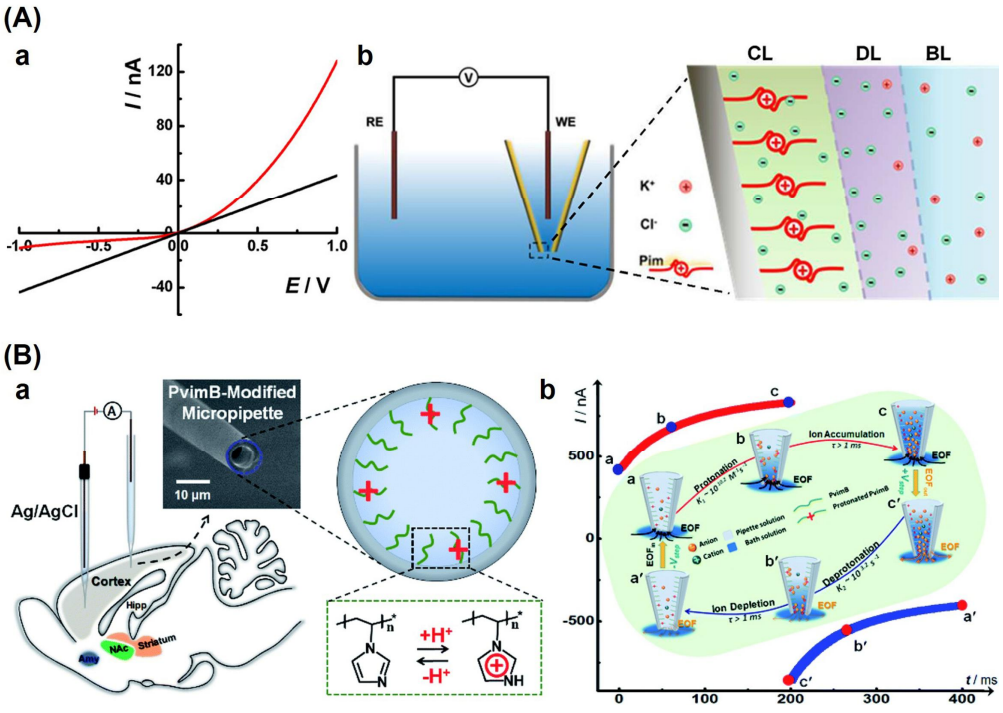

Figure 4 (A) (a) Typical current-voltage responses acquired by using bare micropipette (black curve) and PimB-modified micropipette (red curve). (b) Schematic illustration of proposed three-layer model[69]. Reproduced with permission of Ref. 69, copyright 2017 American Chemical Society. (B) Schematic illustrations of (a) experimental setup of *in vivo* pH sensing by using PvimB-modified micropipette and (b) transient ion transport behaviors and generated ion currents under high-frequency pulse potential[72]. Reproduced with permission of Ref. 72, copyright 2021 Royal Society of Chemistry. (color on line)

in living systems, showing great potential in unraveling the physio-pathological role of neurochemicals in brain. However, as these sensors are based on electrolytic-cell mechanism, an external voltage is always required to drive the oxidation/reduction of the analyte. This inevitably generates a current flow in the electrolytic cell, and potentially changes the activity of cells that is sensitive to electrical stimulus, such as neurons in the CNS. For example, it was observed that low-voltage-activated currents decrease the firing frequency of neurons[73].

To tackle the biocompatibility issue, potentiometric methods offer a possible solution that determines analyte concentrate by potential difference according to the Nernst equation. Under equilibrium conditions, the preferred transport of target analytes across the selective membrane introduces a mensurable electromotive force (EMF) between an indicating electrode and a reference electrode, as the readout of relative concentration of analytes. Based on this principle, potentiometric sensors with different ion selective membranes, termed as ion-selective electrode (ISE), make a powerful tool for ion analysis[74]. To explore the application of ISEs in *in vivo* ion sensing, we coupled H^+-selective membrane with CFEs (CF-H+ISEs)[75]. This as-prepared potentiometric sensor showed not only high selectivity and reversibility but also strong antifouling property against proteins, therefore allowing us to investigate pH changes in living rat brains during quick acid-base disturbances. We thereafter utilized carbon materials as transducing layers to establish a more stable sensing interface, realizing extended applications in monitoring the dynamics of extracellular K^+[76] and Ca^{2+}[77] *in vivo*.

However, there are only limited choices of permselective membranes, posing a significant challenge to potentiometric sensing methods to be well-suited for sensing the majority of neurochemicals. More recently, we proposed a galvanic cell-based sensing mechanism that realizes the sensing of neurochemicals under nearly zero current flow. Generally, a GRP-based sensing system consists of two separated compartments (Figure 5(A)). In different compartments, reductants and oxidants undergo electro-redox rection at an anode (i.e., the indicating electrode) and a cathode (i.e., the reference electrode), respectively. For spontaneous redox reactions, the established potential difference measured by a voltmeter with high internal resistance could provide information of target redox pairs. In practice, the potential readout is determined by the concentration and electrode kinetics of analytes, presence of coexisting redox species, and other factors. Therefore, the designs of cathodes and anodes are essential for making a self-driven galvanic cell[78]. We first demonstrated that laccase can undergo an efficient direct electron transfer (DET) and show efficient electrochemical catalysis on carbon nanotube (CNT)-modified electrodes. This initiates the employment of laccase based direct electron transfer systems at the cathode of biofuel cells. Moreover, with controlling the orientation of the laccase at CNT electrodes using ethanol-assisted immobilization, a dramatic enhancement of the catalytic activity was observed as revealed by 600% increase of oxygen reduction current (Figure 5(B))[79]. As oxygen can be reduced at the highest potential where the thermodynamics allowed, laccase electrode provides the foundation for the establishment of ideal galvanic redox potentiometric (GRP) cathode. Based on these results, we developed the first GRP sensor with laccase/CNT and CNT electrode as a cathode and an anode, respectively. The as-designed two-electrode GRP sensor shows high sensitivity and selectivity to AA, enabling the reliable measurement of the basal level of cortical AA in living rat brain with excellent biocompatibility (Figure 5(C))[80].

Encouraged by these studies, we took a further step to develop a single-carbon-fiber-powered GRP microsensor for neurochemical monitoring[81]. Specifically, we combined the bipolar electrochemistry and GRP to fabricate a single-electrode powered GRP sensor. We selected AA as the model neurochemical and modified the anodic pole with multi-walled carbon nanotubes (MWNTs), on which the onset potential of AA oxidation is -0.10 V *vs*. Ag/AgCl. The micropipette was backfilled with 3 mol·L^{-1} KCl solution

containing $K_3Fe(CN)_6/K_4Fe(CN)_6$ with higher reduction potential (i.e., +0.30 V) to accomplish the spontaneous reaction. The as-designed bipolar GRP sensor exhibits high selectivity and sensitivity to AA, and most importantly, shows undetectable effects on transient or lasting, excitatory or inhibitory activity of neurons as closely investigated with electrophysiological recording (Figure 5(D)). The excellent neuronal compatibility of the GRP sensor diminishes the potential crosstalk between multimodal recording systems, paving an effective way to study the correlation between chemical and electrical signaling of neurons in brain research.

4 *In Vivo* Neurochemical Sensing: from Basic Research to Application

Our bodies and the systems that comprise them are very complex, rendering results obtained from *in vitro* studies must be considered carefully. It is also clear

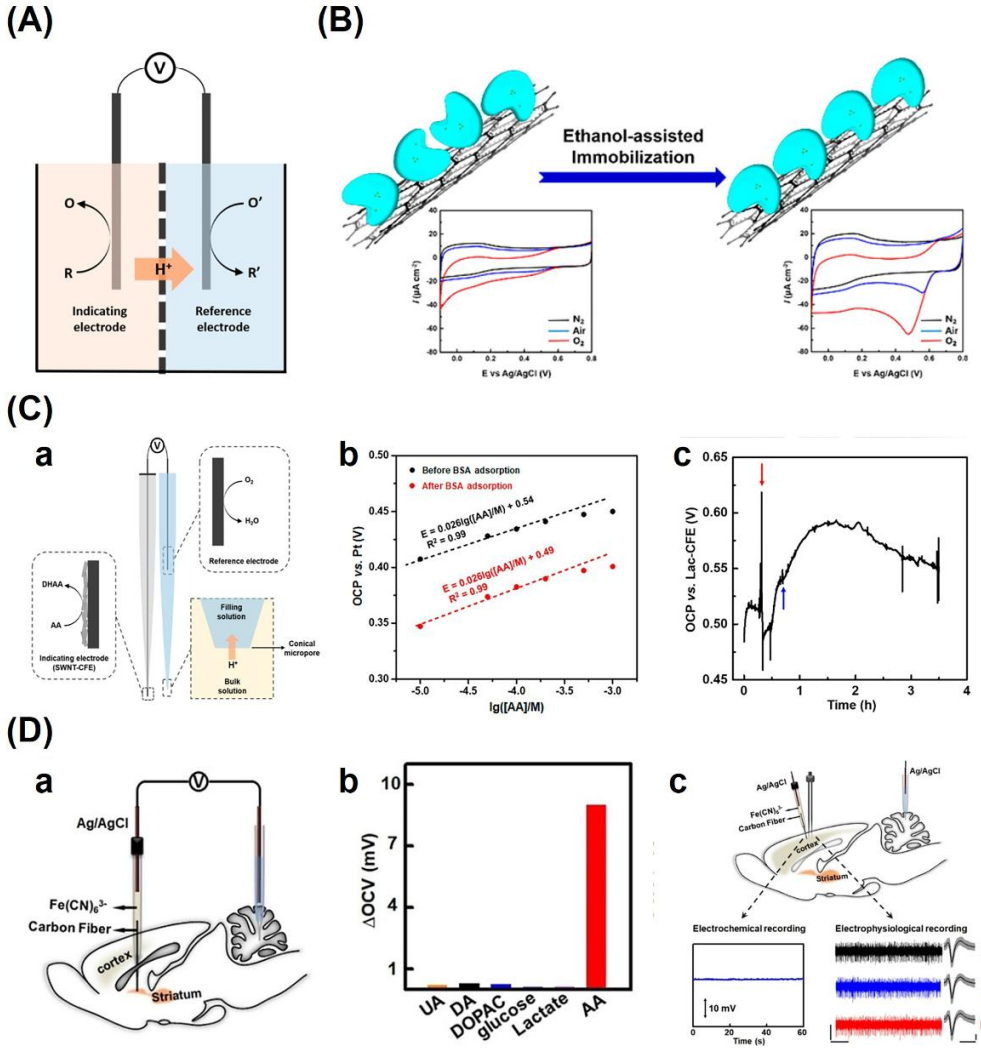

Figure 5 (A) Schematic illustration of GRP sensor[80]. (B) Schematic illustration of orientation and cyclic voltammograms of untreated and ethanol-treated laccase on SWCNT/GCE[79]. Reproduced with permission of Ref. 79, copyright 2017 American Chemical Society. (C) (a) Schematic illustration of GRP sensor for *in vivo* sensing of AA. (b) Calibration curves of the GRP sensor before and after BSA absorption. (c) Real-time recording of cortical AA level during global cerebral ischemia (red arrow) and reperfusion (blue arrow)[80]. Reproduced with permission of Ref. 80, copyright 2018 American Chemical Society. (D) (a) Schematic illustration of the single-carbon-fiber-powered microsensor and *in vivo* sensing of AA with the microsensor. (b) Change in open circuit voltage (OCV) after adding AA and interferents. (c) *In vivo* synchronous OCV measurement with the microsensor and electrophysiological recording by the MEA[81]. Reproduced with permission of Ref. 81, copyright 2020 WILEY-VCH. (color on line)

that neurochemicals in physiological and pathological processes are largely different in terms of their quantitative, spatial and temporal information. Therefore, *in vivo* analysis can provide critical information for understanding the intrinsic molecular mechanism of brain function, as well as the cause of neurodegenerative disorders.

We have demonstrated above that our micro-sized electrochemical sensors, featured with implantable to specific region, high selectivity of target neurochemicals and high biocompatibility of nervous system, hold great promise for *in vivo* sensing of neurochemicals in living animals. Indeed, with these sensors, we have successfully monitored the dynamic changes of a great variety of neurochemicals including AA[55, 56], dopamine[46], oxygen[60], ATP[44, 71], glucose[30, 31, 33], lactic acid[36], catecholamine[82], K^+[76], Ca^{2+}[77] and Mg^{2+}[83] in living brain of animals with high temporal and spatial resolution.

Taken *in vivo* sensing of AA as an example here to demonstrate how we started from molecule detection to revealing molecular mechanism. With the brain-implantable ascorbate sensor (CFEAA1.0 and CFEAA2.0), we reported the first observation on the release of ascorbate in response to spreading depression[84] (Figure 6(A)) and cytotoxic edema[85] (Figure 6(B)), both frequently happened during brain injury. Further mechanistic studies with inhibitors revealed that the AA release may undergo through volume sensitive organic anion channels. Interestingly, by combining electroanalytical chemistry with single-cell amperometry, we also found that AA may also be released by vesicular-mediated exocytosis[86].

More excitingly, with the controlled fabrication, our *in vivo* sensors show great potential for commercialization, and many of our *in vivo* sensing system have been widely adopted by doctors in dozens of hospitals, and validated their effectiveness with clinically relevant animal models. We together observed the dynamic changes of AA[87-91], DA[92], norepinephrine[93], glutamic acid[94-96], Mg^{2+}[97] and other biomolecules in tinnitus, vertigo, olfactory dysfunction, spinal cord injury, and many other disease related animal models, providing rich information to reveal the molecular mechanism of related diseases. Taken together, our *in vivo* sensors can reliably monitor the neurochemicals in the complicated central nervous system, providing essential means for understanding brain function and disease.

5 Summary and Outlooks

To gain insight into essential features of the brain, it is of great importance to provide series of sensing science and technology that fill a vital need for understanding the chemistry nature behind physiological and pathological processes. We have been working in this field for about 20 years, devoting to constructing

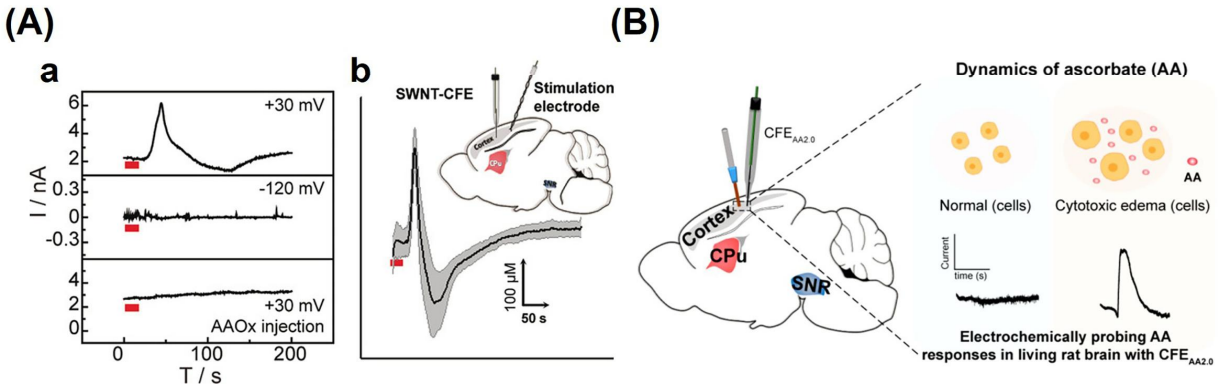

Figure 6 (A) (a) Current signals recorded with CFEAA1.0 under different bias voltages and (b) *in vivo* sensing of AA in the rat cortex during SD process[84]. Reproduced with permission of Ref. 84, copyright 2019 WILEY-VCH. (B) Schematic illustration and typical current responses of *in vivo* AA release in rat brain by using CFEAA2.0[85]. Reproduced with permission of Ref. 85, copyright 2020 American Chemical Society. (color on line)

new principles of electrochemistry in designing platforms for dynamic analysis and sensing *in vivo* with high selectivity and biocompatibility. To fulfill the selectivity requirement, we construct the sensing interface by modulating electrochemical reaction kinetics, for example, via tailoring catalytic active site-analyte interactions and via building an electron transfer way specifically for the target analytes. To tackle the biocompatibility issue of electrolytic cell-based methods, we develop the first galvanic redox potentiometric sensor that provides not only excellent sensing performance but also superior neuronal compatibility. Along with these progresses, we have realized the spatiotemporal resolution analysis and sensing of neurochemicals with high selectivity, which greatly promotes the understanding of brain chemistry.

Nevertheless, we must note that these *in vivo* sensors still face many challenges. One urgent need is to minimize the sensors design and improve its biocompatibility to overcome the body's natural rejection response[98]. Another important aspect is to realize high-throughput and multimodal analysis, where artificial intelligence, physiological techniques, and wireless technology can be combined to resolving multiple biological parameters, and allowing remote monitoring and assessments in real-time. In addition, electrochemical systems can be further improved to be multifunctional, incorporating sensors and then applying a drug or intervention based on the sensor data obtained for precise medicine. Taken together, with the development of chemistry, materials science, micro-processing technology, information engineering and other related technologies, the establishment of highly selective, highly sensitive, high spatial and temporal resolution sensors for *in vivo* analysis of multi-species will become the focus of research in this field. The *in vivo* sensing system will greatly contribute to the deep understanding of brain function, and promote the development of accurate diagnosis and assist in treatment decision.

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脑神经电化学研究

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摘要: 大脑是认知、情感等神经活动的物质基础。脑内神经元通过化学信号及电信号相互连接,共同构成动态而复杂的神经信号网络,实现各项神经活动。因此,对于脑神经化学分子的分析与检测有助于揭示神经生理、病理过程中的分子机制,进而发展神经系统疾病的精准诊断及治疗手段。随着各学科的融合与发展,已有多种分析技术在不同层次实现神经分子的检测。其中,电化学分析方法具有高灵敏、高时空分辨等优势,有望在活体层次上精准描述特定神经分子在神经生理或病理过程中的动态变化。本文围绕选择性以及生理兼容性两大关键问题展开,以本课题组最新研究进展为例,系统阐述了电极界面的构筑原则以及电位型检测方法的独特优势,着重介绍了抗坏血酸在神经生理和病理过程中的动态变化规律,并对脑神经电化学分析领域的发展前景进行了展望。

关键词: 活体电化学传感;脑神经化学;选择性;生理兼容性