ACS SENSORS

Review

Cite This: ACS Sens. 2019, 4, 3102−3118

pubs.acs.org/acssensors

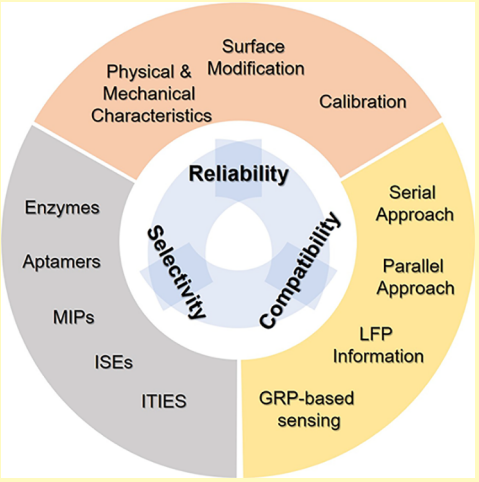
In Vivo Electrochemical Sensors for Neurochemicals: Recent Update

Cong Xu,[†,‡] Fei Wu,[†,‡] Ping Yu,[†,‡] and Lanqun Mao*[,†,‡]

[†]Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, The Chinese Academy of Sciences, Beijing 100190, China

[‡]University of Chinese Academy of Sciences, Beijing 100049, China

ABSTRACT: *In vivo* electrochemical sensing based on implantable microelectrodes is a strong driving force of analytical neurochemistry in brain. The complex and dynamic neurochemical network sets stringent standards of *in vivo* electrochemical sensors including high spatiotemporal resolution, selectivity, sensitivity, and minimized disturbance on brain function. Although advanced materials and novel technologies have promoted the development of *in vivo* electrochemical sensors drastically, gaps with the goals still exist. This Review mainly focuses on recent attempts on the key issues of *in vivo* electrochemical sensors including selectivity, tissue response and sensing reliability, and compatibility with electrophysiological techniques. *In vivo* electrochemical methods with bare carbon fiber electrodes, of which the selectivity is achieved either with electrochemical techniques such as fast-scan cyclic voltammetry and differential pulse voltammetry or based on the physiological nature will not be reviewed. Following the elaboration of each issue involved in *in vivo* electrochemical sensors, possible solutions supported by the latest methodological progress will be discussed, aiming to provide inspiring and practical instructions for future research.



KEYWORDS: *In vivo electrochemical sensors, analytical neurochemistry, enzyme-based biosensor, aptamer-based biosensor, ion current rectification, galvanic redox potentiometry, solid-state ion-selective electrodes, molecularly imprinted polymers, tissue response, biofouling, electrophysiological techniques*

In vivo sensors directly established on systems maintaining biological activity offer promise in both fundamental research and practical applications.[1−5] Through continuous monitoring of certain analytes or biological processes, *in vivo* sensors not only enable researchers to understand biological structure and functions close to the physiological condition, but also enable users to set up personal databases for customized health management.

Brain endows people with the abilities of cognition, perception, and action through a complicated and dynamic network, which essentially subjects them to transmission processes of neurochemicals. To take an in-depth look at the brain, groundbreaking research has been conducted at different scales: quantitative measurement of neurotransmitters at the molecular scale,[6−9] dynamic monitoring of nervous processing at the cellular scale,[10−13] investigation of normal and abnormal states of metabolism and behavior at higher scales, and so forth.[14−16] In past decades, research that focus on neurochemistry have received increasing attention and sustained development. For example, quantitative correlation between neurochemicals and neural activity in both physiological and pathological processes that has been a longstanding goal is gradually established.[17−19] This correlation, in turn, provides guidance in molecular regulation of neural networks.[20] However, along with the better knowledge of brain structure and function, increased understanding of insufficiency and technical limitations is also acquired, especially when facing neurogenic and psychiatric disorders.

Neurons and neuroglia cells collectively constitute the dynamic network, in which various chemicals interact continuously. Neurotransmitters and neuromodulators are two major categories of neurochemicals. Typically, neurotransmitters serve as messengers across the synapse, establishing direct communication between neighboring neurons. Neuromodulators often participate in modulation of neurotransmission. Besides, neurochemicals also include small ions, energy suppliers, reactive oxygen species (ROS), gases, peptides, proteins, and nucleic acids, which corporately regulate activity and dynamic structure of vesicles, neurons, and circuits. This complex and dynamic environment provides opportunities for *in vivo* neurochemical analysis, owing to its improved reliability and time resolution compared with conventional *in vitro* analysis. However, the complexity of the brain network also proposes high demands about *in vivo* analysis generally in two aspects: (a) sufficient spatiotemporal resolution, sensitivity, reliability, and selectivity are necessary to monitor specific neurochemicals; and (b) disturbance of the brain is required to be minimized.

Received: September 3, 2019
Accepted: November 13, 2019
Published: November 13, 2019

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

Imaging and electrochemical sensing are two typical *in vivo* analytical tools at the forefront. *In vivo* imaging is a strong driving force behind neurochemical research because this noninvasive method enables a three-dimensional (3D) visualization of neural activity across broad spatial scales, from the molecular level to intact brain. Common imaging techniques include functional magnetic resonance imaging (fMRI), positron emission tomography (PET), mass spectrometry (MS), and fluorescence.[21] More recently, these methods are reaching greater sensitivity (micromolar range) and larger imaging depth (millimeters level), with the development of multiphoton microscopy, novel fluorescent and luminescent probes, and other technologies.[22] Aside from imaging, *in vivo* electrochemical sensing also contributes to fundamental insights in analytical neurochemistry, owing to its excellent spatial and temporal resolution.[23,24] Typically, electroactive neurotransmitters undergo redox reactions at the surface of implanted microelectrodes, generating current or potential signals that reflect characteristics of neurotransmitters qualitatively and/or quantitatively. Fast-scan cyclic voltammetry (FSCV), amperometry, and galvanic redox potentiometry are representative examples.[3,24−30]

Herein, we will restrict the scope of this Review to implantable *in vivo* electrochemical sensors. Embarking from the actual experiments, we summarize three key issues that have decisive significance in sensor design: (a) selectivity; (b) tissue response and sensing reliability; (c) compatibility with electrophysiological techniques. In each section, we will elaborate on the issues and their causes. We will also provide possible solutions to these issues, supported by the latest progress. Furthermore, we will present a brief outlook on *in vivo* electrochemical sensing in the last section.

SELECTIVITY

Selectivity is the determining factor of sensor performance. Different from the situation in solution, identifying analyte selectively in the brain is commonly confronted with considerable challenges owing to the myriad of coexisting chemicals in the complex and ever-changing microenvironment. Interference from electroactive species raises a critical requirement on selectivity, as they often have similar chemical structures and electrochemical properties. A typical example could be given with catecholamines and their metabolites, which undergo similar 2-electron oxidation reactions that occur at almost the same potential in physiological solutions.[24] Beside this, other coexisting reductants at relatively high concentrations, such as ascorbic acid (AA),[31] are also inevitable hurdles on selective sensing. Moreover, some of these species fluctuate accompanying physiological and pathological processes, putting forward additional challenges for selective sensing. Typical examples include glucose[32] and lactate[33] dynamics with increased metabolic demand under normal conditions, and ascorbate[19] and oxygen[18] fluctuation during spreading depolarization (SD).

To achieve selective sensing for electroactive neurochemicals, one essential thought is to regulate thermodynamics and kinetics of particular electrochemical processes, leading to rational design of the electrode/brain interface.[34] A good example is to use carbon nanotubes (CNTs) to enhance electron-transfer kinetics of AA oxidation and thus reduce its activation overpotential to avoid undesired oxidative interference, establishing a selective platform for *in vivo* sensing of AA.[35−38] While modulating interfacial electron transfer kinetics is an attractive strategy to tune sensing selectivity, utilizing recognition elements is one of the most effective way for obtaining selectivity for *in vivo* sensing.[39] Thereby, we will highlight the latest progress on the electrode/brain interface design with recognition elements and an emerging technique in this section, aiming to provide some useful inspiration.

Recognition Elements. *Enzymes.* High selectivity of enzymes endows them with distinctive advantages in the detection in a complex environment. The successful immobilization of glucose oxidase on electrodes heralded the explosive growth of an enzyme-based electrochemical biosensor.[40] Typically, an enzyme can selectively catalyze a substrate and then transfer electrons to an electrode directly (direct electron transfer, DET) or through mediators (mediated electron transfer, MET).

Theoretically, DET-based biosensors (or third-generation electrodes) can fit in well with the complex *in vivo* environment, without introducing additional mediators or being interfered with by oxygen concentration. However, stringent requirements on enzyme immobilization with proper orientation, low current response, and enzyme activity problems hinder its application in biosensing.[41] To solve these problems, increasing efforts have been made in developing enzyme immobilization methods,[42,43] and fabricating electrode materials with higher conductivity and better biocompatibility.[44−46] Immobilization methods generally include adsorption, covalent binding, cross-linking, entrapment, and affinitive binding.[47] In order to improve electron transfer efficiency, tuning the surface-immobilized enzyme orientation is one of the great concerns in the biosensor research community. Inspiring strategies to solve this problem include the use of nanoscale materials,[47,48] introduction of site-specific binding peptides,[49] and protein engineering.[48,50] Interestingly, organic solvents are also found to play important roles in regulating enzyme orientation by balancing surface wetting as well as protein denaturing.[51] Beside enzyme immobilization, recent years have witnessed emerging development of biocompatible and conductive electrode materials, such as biomimetic membranes,[52] nanomaterials,[53,54] conducting polymers,[55] and their hybrid composites.[56,57] However, *in vivo* sensing of neurochemicals by DET-based biosensor is still in its infancy. One encouraging example is the Cytochrome C (Cyt c)-based *in vivo* sensor, which has been applied to measurement of extracellular superoxide anion (O_2^-) in the rat brain.[58] In order to improve selectivity, Tian et al. investigated the properties of copper−zinc superoxide dismutase (Cu, Zn-SOD) for selective detection of O_2^-.[59−61] To do this, nanomaterials (i.e., differently shaped gold nanostructures,[62] TiO_2 microneedles,[63] and ZnO nanodisks[64]) were utilized to immobilize SOD onto the electrode surface for accelerating DET. Further improvements on electrode miniaturization and SOD stabilization call for *in vivo* O_2^- monitoring in rat brain.[65]

For MET-based biosensors including both the first- and second-generation biosensors, oxidases have been utilized in monitoring neurochemicals in the central nervous system (CNS). The first-generation biosensors detect the consumed O_2 or produced hydrogen peroxide (H_2O_2), thus determining the analytes that are catalyzed by specific oxidase. An excellent example is the adenosine microbiosensor reported by Dale et al.,[66] who combined adenosine metabolizing enzymes (i.e., xanthine oxidase, purine nucleoside phosphorylase, and adenosine deaminase) within a composite matrix on a platinum (Pt) microelectrode. The resulting biosensor

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

possessed characteristics of small size (25−100 μm diameter), quick response (~2 s), high sensitivity (100−222 mA M^{-1} cm^{-2}), and stability (>5 days), showing good performance to monitor the release of adenosine *in vivo*. Taking a further step, they developed an adenosine triphosphate (ATP) biosensor based on an enzyme cascade of glycerol kinase and glycerol-3-phosphate oxidase that converted ATP to glycerone phosphate and detectable H_2O_2.[67] Exhibiting a quick response (<10 s), fast temporal resolution (in millisecond scale), and wide linear response range (20 nM − 50 μM), this biosensor was utilized to detect ATP release in the nervous system, unraveling the participation of ATP in mediating chemosensory transduction in CNS.[68]

Poor selectivity is one of the most serious problems for the first-generation biosensors mainly because of the high potential employed for the oxidation of H_2O_2, at which other neurochemicals (e.g., AA) can be oxidized as well. Improvement has been achieved by introducing the permselective membrane, reducing the oxidation potential of H_2O_2, and employing other techniques.[69−82] For instance, Gerhardt et al. developed the self-referencing microelectrode arrays (MEAs).[83] On the active sites, the oxidase can selectively respond to targeted analytes, whereas on the reference sites, the chemically inactive proteins bovine serum albumin (BSA) serve as the reporter of background signals and interfering noises. By offline subtraction, self-referencing MEAs make it possible to real-time monitor neurochemicals, such as L-glutamate (L-Glu), glucose, lactate, choline, and acetylcholine (ACh), in anesthetized, awake, or freely moving animals (rats and monkeys).[84−95] Different electrode materials were utilized to fulfill various needs in monitoring diverse chemical events *in vivo*. For example, the response time requirement for studying neuro-metabolism is usually not as strict as that for monitoring neurotransmission. Thus, polymers like the polyurethane membrane were used to expand the linear detection range at the cost of response speed.[85,95] Although second-by-second sensing can accomplish *in vivo* measurements in many cases, higher temporal resolution is still a critical goal for acquiring more detailed neurochemical information.

The second-generation biosensors use artificial mediators to replace O_2 for shuttling the electron transfer of enzymes, including organic (e.g., quinone compounds), inorganic (e.g., ferricyanide), and metal−organic (e.g., ferrocene derivatives) structures. By this means, selectivity and sensitivity are greatly improved due to low polarization potential, O_2-insensitivity, and high enzyme utilization. Being one critical factor, immobilization of mediators and enzymes onto the electrode surface impacts redox potential of mediators, enzyme activity, as well as electron transfer between mediators and enzymes, therefore dramatically influencing the sensor performance. Attempts have been made on both immobilization matrixes and processes.[96−102] As an example to highlight novel immobilization matrixes, Ma et al. first applied the metal−organic framework to coimmobilize mediator and enzyme on the electrode surface to prepare *in vivo* biosensors.[103]

Despite the above-mentioned breakthroughs, challenges still exist. For instance, oxidase-based electrochemical biosensors can hardly exclude interference from O_2 completely because of the fast chemical reaction between oxidase and O_2. While dehydrogenases are generally independent of O_2, it is difficult to integrate both cofactors NAD^+ and electrocatalyst for the oxidation of β-nicotinamide adenine dinucleotide (NADH) on the surface during the fabrication of dehydrogenase-based biosensors.

To confront these challenges, an enlightening idea that exploring new enzymes has attracted increasing attention. Recently, Wu et al. introduced ferredoxin-dependent glutamate synthase (Fd-GltS).[104] Fd-GltS can specifically catalyze the reversible transformation between 2-oxoglutarate and L-Glu, being free from problems of O_2 dependency and H_2O_2 interference (Figure 1). Furthermore, the flavin mononucleotide (FMN) which accepts electrons during L-Glu oxidation is embedded in Fd-Glts, indicating that there is little need to introduce extra cofactors like other dehydrogenases do. Instead of searching for natural alternatives, synthetic nanomaterials mimicking enzymatic catalysts, termed nanozymes, have also attracted increasing attention.[105] As for more details and remarkable applications of nanozymes in the field of biosensing, please refer to the review published recently.[106]

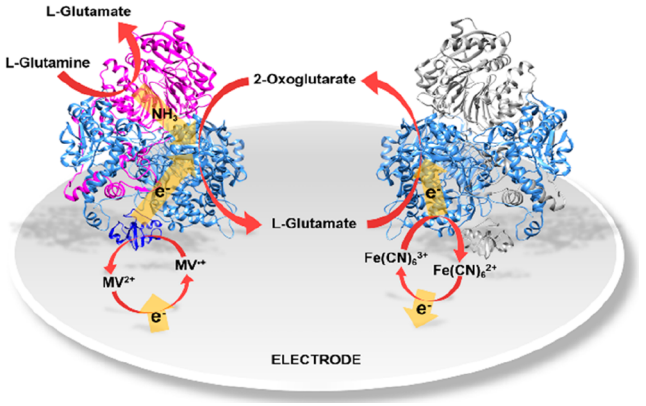


Figure 1. Schematic illustration of mediated bioelectocatalysis by Fd-Glts.

Aptamers. As an alternative of enzymes, aptamers are synthetic nucleic acids that possess high affinity with specific ligands including small molecules, proteins, and even cells.[107−109] The development of SELEX (systematic evolution of ligands by exponential enrichment) and PCR (polymerase chain reaction) techniques enables aptamers to be simple, flexible, and low-cost recognition elements. Inspired by electrochemical DNA (E-DNA) sensors,[110] Xiao et al. first adopted aptamer in electrochemical sensing,[111] arousing great interest in the field of electrochemical aptamer-based (E-AB) sensors. Typically, aptamers modified on the surface of electrodes undergo conformational reorganization upon aptamer-ligand binding, thus changing electron transfer process between electrodes and redox moiety modified on aptamers.[112] Besides its successful applications in whole blood[113,114] and blood serum,[115] research on selective analysis of dopamine (DA),[116] serotonin,[117] and other neurochemicals[118] have also been gaining prominence because of the fast response (<1 s, for DA aptasensor) and excellent sensitivity (62 nA μM^{-1} cm^{-2}, for DA aptasensor). Moreover, aptamer superstructures are proven to further improve detection selectivity and sensitivity by amplifying signals.[119]

Field-effect transistor (FET) can also serve as a highly sensitive platform for sensing of small molecules. In the latest breakthrough, Nakatsuka et al. combined FETs with aptameric stem-loop receptors that enable specific detection of dopamine, serotonin, glucose, and sphingosine-1-phosphate

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

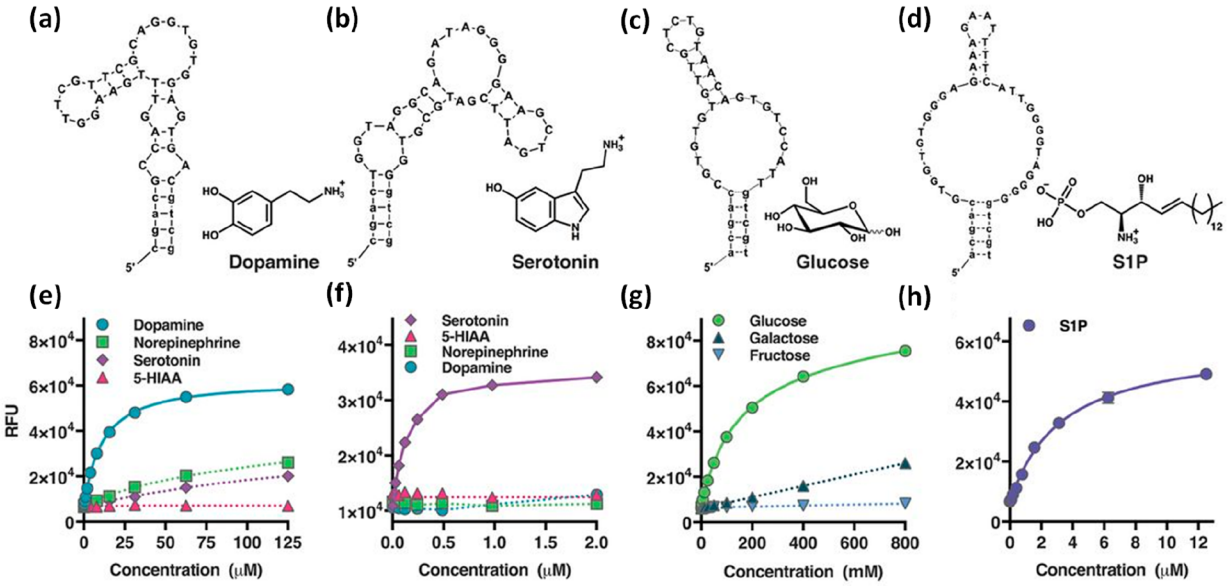


Figure 2. (a–d) Aptamers for dopamine, serotonin, glucose, and S1P. (e–h) Fluorescence responses indicate selectivity of aptamers.[120] (Reprinted (adapted) with permission from *Science*. Copyright 2018, American Association for the Advancement of Science).

(S1P) in the presence of various interfering species (Figure 2). More importantly, these aptamer-FETs not only presented quick responses (in the order of seconds) in wide ranges of analyte concentrations (10^{-14} to 10^{-9} M), but also maintained stability when continuously exposed to brain tissue for 4 h.[120]

It is a difficult task to directly obtain exclusive aptamers for targeted small molecules. With ATP as a typical example, although some aptamer-based biosensors can selectively detect ATP by recognizing A nucleobase, these aptamers show similar affinity to sugar and triphosphate moieties, adding difficulties to further distinguish ATP from adenosine diphosphate (ADP) and adenosine monophosphate (AMP). In this case, Yu et al. developed a dual recognition unit strategy (DRUS) by incorporating polyimidazolium-brush (PimB) and aptamers together to realize selective recognition of both A nucleobase and triphosphate moieties simultaneously.[121] On the basis of this strategy, they constructed a highly sensitive and selective ATP-biosensor and utilized this sensor to detect cerebral ATP coupling with *in vivo* microdialysis. Shortly afterward, He et al. found an ion current rectification (ICR) phenomenon in the PimB-modified micropipette, termed micrometer-scale ion current rectification (MICR).[122] Inspired by MICR and DRUS, Zhang et al. constructed a selective online ATP sensor that can deliver rectification ratio signals in a linear relationship with *in vivo* cerebral ATP concentration (5 nM to 100 nM).[123] Compared with that of nanopipettes, tips at the micrometer scale possess higher mechanical strength, which provides the micropipette-based sensors great potential in further applications on *in vivo* sensing as implantable devices.

Another critical need is to reach sufficient temporal resolution, which is mainly limited by the property of aptamers as well as adopted electrochemical methods. To enhance temporal resolution, Idili et al. prepared an E-AB sensor by re-engineering the aptamers and introducing another aptamer for larger signal gain.[124] This E-AB sensor achieved a 20 s resolved measurement of irinotecan *in vivo*, making possible a high-precision view of pharmacokinetics. In addition to aptamers, changing electrochemical methods also helps improve temporal resolution. A 300 ms resolved measurement of small molecules was achieved by employing chronoamperometry, an order of magnitude faster than previous E-AB sensors.[125] Though performed in blood, their pioneering work is of certain significance for *in vivo* monitoring neurochemical dynamics in high resolution.

Molecularly Imprinted Polymers (MIPs). MIP-based sensors have also been developed because of their inherent advantages in biocompatibility, high selectivity and sensitivity, chemical/mechanical stability, reusability, and facile and inexpensive preparation.[126,127] Typically, MIPs are polymerized together with target molecules (as the templates). These target templates are extracted after polymerization, leaving recording sites on MIPs which are complementary to the templates. In the past decade, various MIP-based sensors were constructed for measuring neurochemicals[127−129] including DA,[130,131] AA,[132] uric acid (UA),[133] tryptamine,[134,135] and noradrenaline (NE).[136,137] For example, Li et al. successfully detected DA at trace levels in serum and brain samples by using an MIP-based sesnsor.[138] To be specific, MIPs was electro-polymerized on the nanoporous Au−Ag alloy microrods (NPAMR), which were obtained via dealloying Au−Ag alloy microrods (AMR). This MIP/NPAMR system showed outstanding sensing performance such as wide linear detection range (2×10^{-13} to 2×10^{-8} M) and low detection limit (7.63×10^{-14} M, S/N = 3). While most research focused on analyzing biosamples *in vitro*, Tsai et al. first proposed a MIP-based microsensor for *in vivo* sensing.[139] Taking the DA-imprinted overoxidized polypyrrole (OPPy) as MIPs, the microsensor achieved a low detection limit (4.5 nM), good reproducibility and excellent temporal resolution at the subsecond scale, and was applied for DA detection in rat striatum under administration of 3,4-dihydroxy-L-phenylalanine (L-DOPA).

Recognition Elements for Ions. Ions are an indispensable part in the brain for cell functioning. For example, local pH of the brain microenvironment is closely correlated with many physiological and pathological processes such as ion transport, cell growth and apoptosis, and even neurodegenerative disorders. Ca^{2+}, acting as one of the second messengers,

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

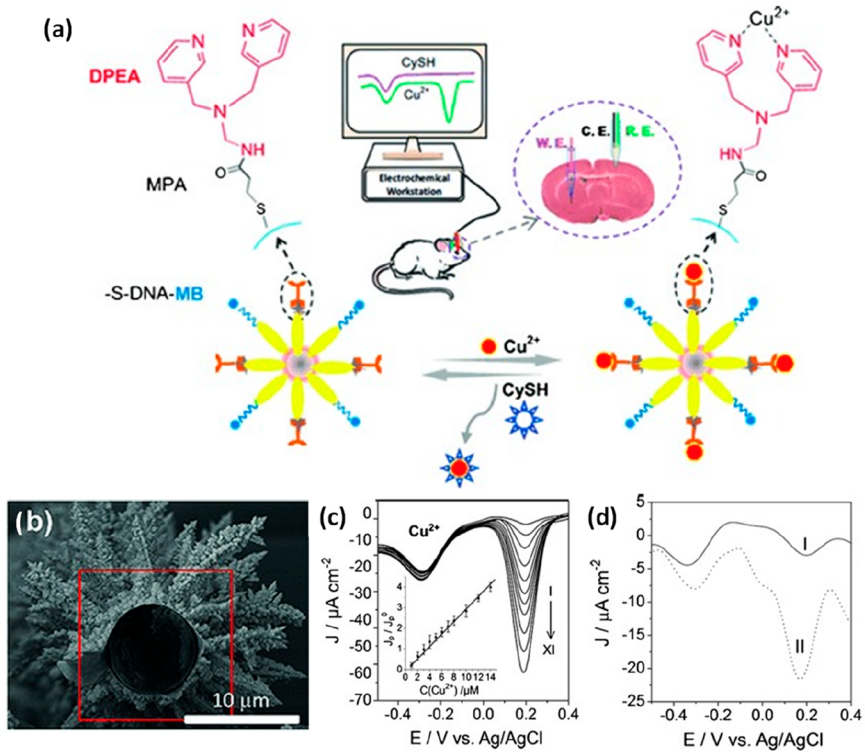


Figure 3. (a) Schematic illustration of dual-channel ratiometric electrochemical sensor for monitoring Cu^{2+} and L-cysteine (CySH) in live rat brains. (b) SEM image of the cross section of the electrochemical sensor. (c) Differential pulse voltammetry (DPV) responses obtained in artificial cerebrospinal fluid (aCSF) with different concentration of Cu^{2+}. (d) DPV responses obtained in (I) normal rat brain and (II) rat brain with Alzheimer's Disease (AD).[143] (Reprinted (adapted) with permission from *Angew. Chem., Int. Ed.* Copyright 2015, WILEY-VCH Verlag GmbH & Co. KGaA.)

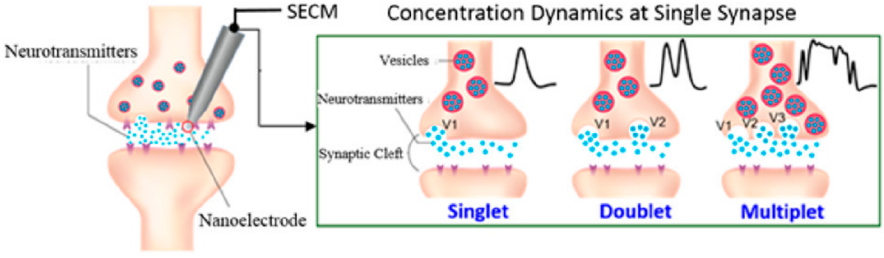


Figure 4. Schematic illustration of a nanoITIES pipet electrode positioned around the synaptic cleft by SECM to monitor concentration dynamics at single synapse.[153] (Reprinted (adapted) with permission from *J. Am. Chem. Soc.* Copyright 2018, American Chemical Society).

participates in regulating neurotransmitter release and synapse formation. In addition, Mg^{2+} plays a significant role in learning and memory. Surface/interface design is keeping pace with method innovation and paves an effective way to realize ion detection with high selectivity and stability. Over the past 10 years, an increasing amount of research has endeavored to accomplish *in vivo* electrochemical sensing directly on implantable sensors. Two enlightening strategies respectively based on dual-channel ratiometric electrochemical biosensors and solid-state ion-selective electrodes (ISEs) are emerging. The dual-channel ratiometric electrochemical biosensor initially adopts two independent working electrodes, which are modified with specific recognition elements, respectively.[140,141] To reduce brain injury during operation and inaccuracy between two electrodes, recognition and reference molecules are integrated onto a single electrode, realizing *in vivo* qualitative measurement of ions such as H^+[142] and Cu^{2+}[143] (Figure 3). Different from the ratiometric strategy that offers insights into design principles, ISE as one of the most well-developed potentiometric sensors is experiencing its rebirth in the field of *in vivo* electrochemical sensing.[144] Compared with the great amount of applications on wearable devices, applications on *in vivo* neurochemical sensors are just emerging in these years. For example, Hao et al. demonstrated a H^+-ISE for monitoring *in vivo* pH change during acid−base disturbances.[145] With the aim to meet the requirements of miniaturization as well as mechanical robustness, they replaced the conventional glassy electrode with a carbon fiber microelectrode (CFE) to be the conducting substrate coated by an antifouling H^+ ion-selective membrane. The proposed H^+-ISE showed reversible, sensitive (58.4 mV/pH) and fast (<1 s) response toward pH. This example together with other successful applications on *in vivo* sensing of K^+[146,147] and Ca^{2+}[148] proved the wide applicability of ISEs in the future.

Emerging Technique. *Liquid−Liquid Interface-Based Sensing.* Ion transfer behavior across the interface between

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

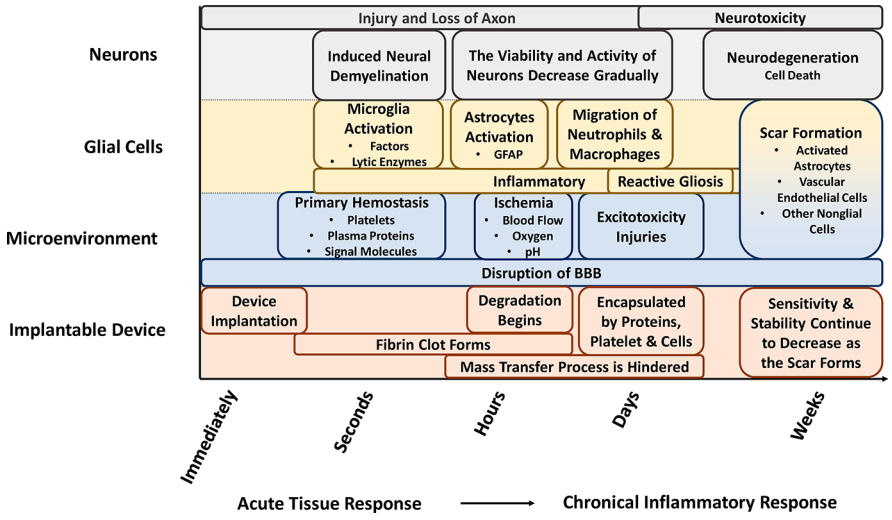


Figure 5. Simplified scheme of brain tissue responses after implantation of microelectrodes.

two immiscible electrolyte solutions (ITIES) reveals a difference between similar molecules, endowing ITIES-based sensing methods with unexpected selectivity. Typically, the liquid−liquid interface is formed between organic solution filling in the pipet and the testing biological fluid. Under appropriate polarization conditions, charged analytes transfer from the aqueous phase to the organic phase, and can be determined by cyclic voltammetric mode (CV) or amperometric mode qualitatively and quantitively. In this decade, ITIES based on nanopipette (nanoITIES) has increased the applicability in neurochemical analysis because of the enhanced spatial resolution.[149] Based on nanoITIES, Shen et al. successfully identified and quantified ACh, tryptamine (T), and serotonin (5-HT),[150] and subsequent γ-aminobutyric acid (GABA)[151] and DA.[152] Improved selectivity was realized by modulating solvent and pH of the filling solution, and introducing different types and concentration of ionophores as well. Taking a further step, they employed the scanning electrochemical microscopy (SECM) to position the nanoITIES pipet electrode, and observed diverse patterns of cholinergic concentration dynamics at single synapse (Figure 4).[153] These works marked that nanoITIES-based sensing is consolidating its status during *in vivo* neurochemical analysis.

TISSUE RESPONSE AND SENSING RELIABILITY

In the history of *in vivo* electrochemical sensing, tissue response to implants has been thought of as a major constraint for high reliability. This decade has witnessed a rapid growth of literature on the brain tissue response to implanted sensors; books and reviews elaborated the whole reaction process, biochemical pathway, biological reaction of different cell types, and improvement of existing approaches as well.[154−161] In this section, we will tease out the brain tissue response after sensor implantation and highlight inspiring strategies and innovative research that achieved a higher degree of sensing reliability.

Tissue Response. Tissue response can be generally divided into two overlapping stages: acute tissue response and chronical inflammatory response (Figure 5). The implantation of a sensor into brain is inevitably accompanied by disruption of blood-brain barrier (BBB) and activation of nearby glia cells, triggering an acute tissue response.[159] Primary hemostasis occurs within seconds, as collagen fibers on damaged endothelial cells expose and adhere to platelets, induced by a coagulation cascade.[157] Upon platelets attaching to the injury site, they rapidly aggregate, activate, and degranulate, aimed to generate a fibrin clot. Furthermore, a wealth of released plasma proteins and signal molecules can nonspecifically adsorb on the sensing surface during clot formation, leading to the initial electrode biofouling and passivation.[154] These adsorbed proteins and molecules also trigger an accelerated cascade of inflammatory and immune response. For example, neutrophils and macrophages successively migrate to the injury site within 24 h and clear foreign substance for days by phagocytosis or by releasing chemicals (e.g., superoxide and chemotactic cytokine).[159]

Besides, device insertion and released plasma proteins (e.g., albumin and fibrinogen) also activate glial cells through various pathways.[155] Microglia primarily play the roles of killing pathological cells or degrading cellular debris. Once activated, they begin to proliferate, and stretch out the lamellipodia toward the device within 30 min. They can rapidly hinder mass transfer processes on the sensing surface, leading to decreased sensitivity. Moreover, these cells can persistently secrete factors and lytic enzymes that initiate and facilitate foreign body degradation, resulting in structural integrity failure of the electrode; these factors and enzymes also induce neural demyelination, apoptotic cell death, and electrode-modified biomaterials destruction.[159] In addition to microglia, astrocytes are another important glial cell type, which mainly participate in maintaining normal neurochemical environment and buffering signal molecules during synaptic transmission.[157] In response to injury, the activated astrocytes upregulate glial fibrillary acid protein (GFAP), and swell osmotically *in situ*.[158] Within several days after insertion, the implanted sensor is gradually encapsulated by a mixture of proteins, platelet plug, and cells. Moreover, the viability and activity of the surrounding neurons is also a challenge as the extracellular environment and supporting glial cells gradually deviate from the normal physiological state.

The persistent presence of implanted sensors is considered as the main reason for the chronical inflammatory response, which includes both repairing and remodeling processes.[157] Chronical response in CNS can be basically characterized by generation of cytokines and chemokines, eruption of reactive gliosis, and formation of capillary. Sustained expression of cytokines can not only impede BBB healing, but also lead to

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

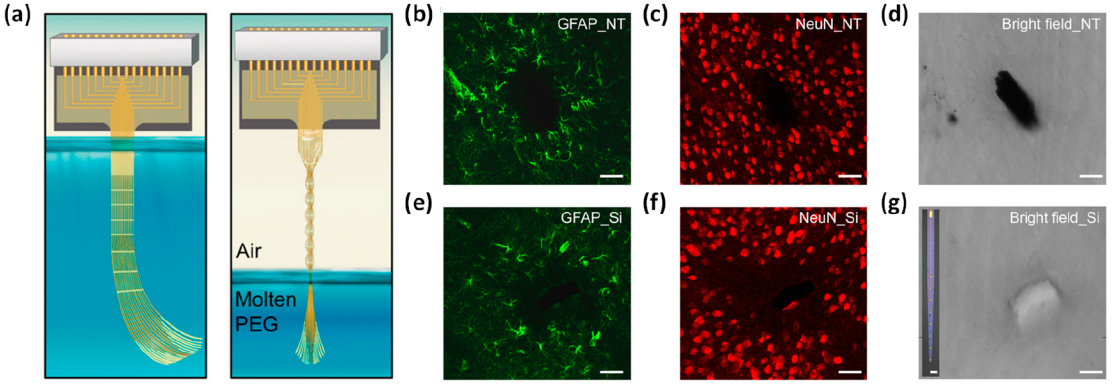


Figure 6. (a) Schematics of Neurotassel; (b–g) Immunohistochemical staining and bright-field images of a brain slice after 5-week implantation of a Neurotassel (upper row) and a silicon probe (lower row), respectively.[175] (Reprinted (adapted) with permission from *Sci. Adv.* Copyright 2019, American Association for the Advancement of Science).

neurotoxicity and neurodegeneration, especially when combined with ischemia and excitotoxicity.[159] Reactive gliosis refers to an ongoing process in which glial cells become hypertrophic, and also actively proliferate and migrate. Typically, the activated astrocytes intertwine tightly and encircle around the lesion cores compactly by 2–4 weeks after insertion and remain intact after 6 weeks, forming a structural and functional barrier between the lesion core and viable neuronal tissue. While this astrocyte scar prevents further neuronal damage and inappropriate connections, it dramatically attenuates sensing sensitivity and stability as well. Much of the sensing signals are lost during fibrous encapsulation. Besides, vascular endothelial cells and other nonglial cells are also involved in scar formation, resulting in accelerated angiogenesis and inhibition of injured axon regrowth.[157]

Improvement of Sensing Reliability. Although brain inflammatory response can have an impact on *in vivo* electrochemical sensing, strategies have subsequently emerged along with better understanding of this issue. Aimed at minimizing tissue damage, alleviating inflammatory response, or reducing its impact on sensing, improved aspects including the physical and mechanical characteristics (e.g., size, shape, flexibility) of sensors, surface modification (e.g., hydrophilic polymers, superwettability-based polymers), and calibration methods will be discussed below.

Physical and Mechanical Characteristics. It is generally accepted that minimizing tissue damage during implantation can essentially alleviate both short-term and long-term inflammatory response. To attenuate acute response events and neuronal damage, physical and mechanical characteristics of the implanted sensor are primary concerns. Flexibility (or compliance), as a key parameter correlated with mechanical strain, leads the sensor design toward smaller size[162] and with softer materials.[163] For instance, chronic inflammatory response brings considerable difficulties to long-term neurochemical monitoring. Recently, chronic *in vivo* dopamine (DA) recording in rodents[164] and primates[165] was achieved by using a 7-μm-diameter CFE, reinforcing previous study demonstrating that using microelectrodes with less than 12 μm in diameter can dramatically reduce tissue encapsulation. Aside from CFEs, carbon nanopipette electrodes (CNPEs) with a submicrometer diameter tip can be utilized to realize DA detection in *Drosophila* brain[166] and rat brain slices.[167]

Additionally, researchers also observed decreased inflammatory response when establishing detection on implantable sensors made from polymers with low elastic modulus.[168] However, these flexible sensors pose new challenges in batch fabrication and accurate insertion. To enhance the reproducibility, batch fabrication strategies such as photolithography and 3D-printing received increasing attention. Yang et al. lately fabricated 3D-printed carbon microelectrodes for the first time and successfully applied these microelectrodes to DA detection in brain slice and *in vivo*.[169] In addition, flexible sensors are likely to buckle or deflect during implantation, resulting in the wrong inserted location and additional tissue damage. Attempts have been made to solve this problem with the assistance of dissolvable or removable stiff shuttle during insertion. The development of polymers provides new possibilities in this area. One typical example is poly(ethylene glycol) (PEG), a biocompatible polymer with high solubility in biofluid. Another impressive example is the mechanically adaptive polymer, of which elastic modulus can be controlled by temperature or degree of water-saturation.[170−174] Functionalizing these polymer materials with microelectrodes makes it possible to accurately positioning and stable chronic neural recording. Lately, Guan et al. developed a Neurotassel that consists of an array of flexible microelectrode filaments, each with cross section at neurite scale (Figure 6a). The stiff PEG adhered on the Neurotassel surface will dissolve after implantation, exposing the Neurotassel probes which have comparable elastic modulus with the brain tissue (Figure 6b–g). Experiments on mice shown that Neurotassel was able to not only record multiple neurons stably up to 6 weeks, but also be assembled with optical fibers, achieving optogenetic stimulation and neuronal activity recording simultaneously.[175]

Surface Modification. Owing to the important role of the interface where electrochemical reactions commonly take place, surface modification plays a weighty role in electrochemical sensing.[176−180] To improve the biocompatibility of surface modification, it is of necessity to qualitatively describe the nonspecific protein adsorption process and quantitatively determine possible parameters. Water-mediated hydrophobic and hydration forces were the main parameters leading to "Berg's limit", a concept that ascribed the surface biocompatibility to its hydrophobic or hydrophilic properties. Thereafter, various polar (e.g., hydrogen bonds) and nonpolar (e.g., van der Waals) factors were taken into accounts. Whitesides et al. proposed empirical rules to describe properties of protein-

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

resistant monolayers: the presence of polar functional groups and hydrogen bond acceptor groups; the absence of hydrogen bond donor groups and net charge.[181] Although exceptional examples cannot be ignored, these rules effectively established a theoretical foundation for surface modification, spurring the growth of biocompatible surface materials including carbon-based materials,[182−184] polymeric and nonpolymeric films,[106,185−188] protein films,[189] metallic nanoparticles,[190,191] hydrogels,[192] peptides,[193−195] cell membrane,[196] and thiolated self-assembled monolayers (SAMs).[197,198] One representative example is CNT, which shows great compatibility with mammalian cells and neurons.[199] It has also been proven that some of these surface modification materials can drastically improve the stability of *in vivo* electrochemical sensing. For example, Liu et al. reported a cell-membrane-mimic film by electropolymerizing ethylenedioxythiophene tailored with phosphorylcholine (PEDOT−PC) (Figure 7a,b).[200] Impressively, this ultrathin film showed excellent

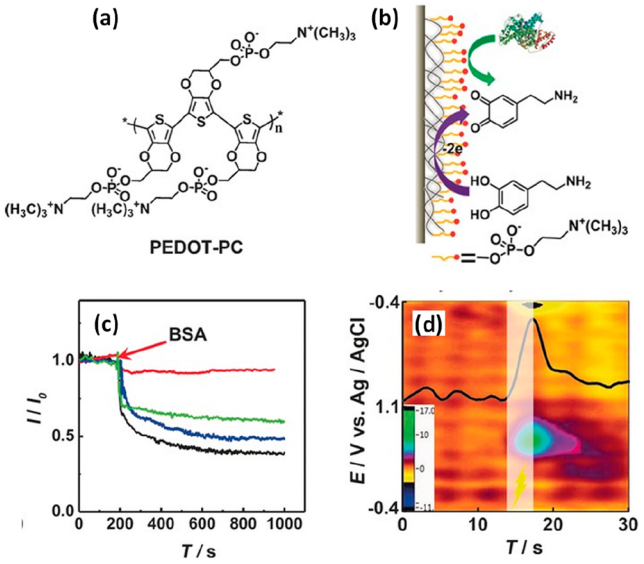


Figure 7. (a) Structure of PEDOT−PC. (b) Schematic illustration of microelectrode interface functionalized with PEDOT−PC. (c) Amperometric current responses toward 20 μM DA by CFE (black), PEDOT/CFE (blue), PEDOT−OH/CFE (green), and PEDOT−PC/CFE (red) at +0.20 V upon indicated addition of 10 mg/mL BSA. (d) *In vivo* FSCV response obtained with PEDOT−PC/CFE.[200] (Reprinted (adapted) with permission from *Angew. Chem., Int. Ed.* Copyright 2017, WILEY-VCH Verlag GmbH & Co. KGaA.)

capability to resist protein adsorption with sustained sensitivity and time response for *in vivo* detecting of DA (Figure 7c,d). More recently, Su et al. proposed a kind of silica nanoporous membrane (SNM) that was modified on CFEs. The uniform nanochannels effectively prevent direct contact between the electrode surface and proteins through steric effect, and thus enable continuous monitoring of cerebral oxygen under *in vivo* condition with enhanced stability.[201] Interestingly, in addition to the above-mentioned modified materials, E-AB sensors also exhibit attractive antifouling properties because they are insensitive to nonspecific protein adsorption to a great extent, supporting a stable measurement lasting for hours.[124]

Besides, it is demonstrated that the surface roughness and topography can affect the interaction between brain tissue and electrode surface,[202−205] and even regulate differentiation of neural stem cells.[206] Thus, microstructure of surface modification has gained considerable attention in these years. One typical example is a superwettability-based antifouling surface. Nanostructure on these bioinspired surfaces can greatly resist adhesion of proteins and cells by trapping air cushion or liquid layers.[207,208] Overall, understanding and strategies unceasingly shed new light on surface modification of implantable sensor, allowing investigators to address the challenge of inflammatory responses with broader perspectives.

Calibration. Aiming at accurate quantification, calibration is an essential step of *in vivo* electrochemical sensing because of the variability of each microelectrode surface, which mainly comes from handmade process, as well as complex tissue responses. Typically, the microelectrode is calibrated in the homogeneous electrolyte solution right after being removed from brain tissue. However, postcalibration is based on the assumptions of unaltered sensitivity, surface properties, and electrochemical processes during and after implantation. These assumptions cause increasing concern that the postcalibration cannot truly reflect the experimental process in the immensely complex *in vivo* environment. In these years, literature has emerged that provides alternative methods of calibration, including *in situ* calibration and precalibration for single CFE.

In situ calibration takes nonfaradic background charging current as a reference of electrode sensitivity to specific analytes.[209] To extract chemical information out of the background waveform, mathematical tools such as multiple linear regression,[210] and subsequently principle component regression,[211] were reasonably adopted. Recently, Meunier et al. employed a model circuit which mimicked surface fouling by additional impedance, and validated this impedance model by oxidation potential shifts.[212] On the other hand, precalibration was designed to improve calibration accuracy by minimizing the impact of biofouling. The term precalibration was first proposed to describe the process that CFEs were pretreated with BSA before implantation.[189] Although BSA adsorption affects mass transport and electron transfer, BSA-pretreated CFEs bear good reliability in maintaining sensitivity during *in vivo* electrochemical sensing of DA. From this perspective, this simple strategy possesses the capability to realize basal level detection of neurochemicals such as ascorbic acid (AA) and O_2.

COMPATIBILITY WITH ELECTROPHYSIOLOGICAL TECHNIQUES

Integrating electrochemical and simultaneous electrophysiological signals together can enrich the knowledge of the relationship between neuromodulator systems and neural interactions under physiological and pathological conditions,[213−215] thus advancing the development of clinical monitoring and treatment.[216] However, combining these two powerful tools together is not easy because of compatibility issues. One compatibility issue stems from the interference on spontaneous neuronal activity during *in vivo* electrochemical sensing. Hefti et al. performed an early experiment that combined electrophysiological recording with *in vivo* chronoamperometric (CA) measurements based on a carbon-paste electrode simultaneously in same brain region.[217] Their results suggested that CA recordings at potential below +0.55 V did not influence firing rates of adjacent neurons. For potential above +0.55 V, the initially high current interrupted spontaneous activity of neurons. Lowering the operation

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

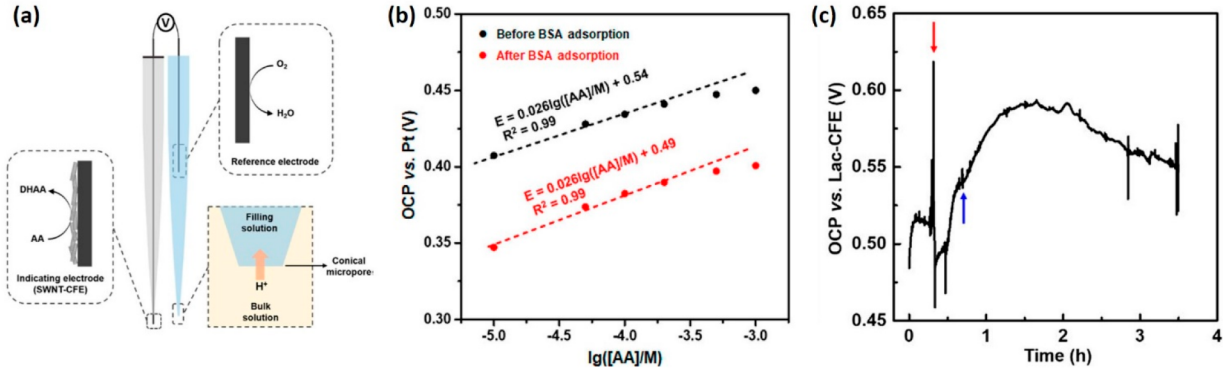


Figure 8. (a) Schematic illustration of GRP sensor design for *in vivo* AA measurement. (b) Calibration linear curves before and after BSA adsorption. (c) *In vivo* monitoring of cortical AA concentration by GRP sensor during global cerebral ischemia (red arrow) and reperfusion (blue arrow).[230] (Reprinted (adapted) with permission from *Anal. Chem.* Copyright 2018, American Chemical Society).

potential, using small-sized working electrode and adopting voltammetric methods are effective strategies to avoid large circuit current. Indeed, following research based on CFEs and MEAs indicated that no significant influence on neuronal firing rates was observed during DPV, CV, CA, and constant-potential amperometry at even higher applied potential.[218−220] However, unusual inhibition or activation of some neurons can still happen when direct interference of applied potential on the recording electrode is excluded.[217] Owing to the unclear effects, it is still an indispensable part of *in vivo* electrochemical sensing to examine the influence on neuronal activity, especially when coupled with electrophysiological measurements.

The other compatibility issue is the signal interference between the two methods. In the past decades, two typical approaches, namely, serial approach and parallel approach, have been commonly employed to record neurotransmitter dynamics and single-unit spike neuronal activity.[221,222] For the serial approach, two types of recording are realized alternately at the same microelectrode.[213,223] Although problems such as undersampling and electrical artifacts are inevitable, the serial approach can extract both types of signal from the same location. For the parallel approach, two separated microelectrodes are employed to record electrochemical and electrophysiological signals continuously.[17,224] While careful insertion is required to make sure that two recording sites are precisely located at the same neural circuit, parallel approach enables complete sampling information and more reliable data interpretation. Along with the development of micromachining and locating technology, the parallel approach has been applied to plenty of research mainly focused on DA,[213] Glu,[92] and AA.[17]

More recently, local field potential (LFP) information with lower frequency has gained increasing attention in simultaneous electrochemical and electrophysiological measurements. Zhang et al. proposed a simple method to extract LFP information from amperometric recording signals.[225] They found that high frequency components of amperometric signals only reflected LFP information in several aspects, suggesting that both electrochemical and LFP information can be acquired from one single electrochemical sensor simultaneously. Based on the finding, they successfully coupled the phasic ACh release and theta oscillation *in vivo* for the first time.[226] Soon after, Viggiano et al. presented another feasible method to reconstruct LFPs by using Fast Fourier Transform (FFT) and inverse FFT under a resistor−capacitor circuit-based model.[227] Lately, LFP has also fulfilled its potential on epilepsy research.[228,229] By decomposing the amperometric signals with high sampling rates, each individual recording sites on the MEA could simultaneously report changes of local pO_2 and LFP-related currents. This work extended the horizon of MEAs as *in vivo* sensors that record electrochemical and LFT information simultaneously at multiple sites.

However, uncertain influence on neuronal activity is still an unavoidable issue for both amperometry and voltammetry that cause concentration gradient and electric field polarization around the electrodes. Confronted with this issue, galvanic redox potentiometry (GRP), measuring interfacial chemical concentration under thermodynamic equilibrium circumstances, can serve as a new breakthrough.[230] Typically, GRP is established on a galvanic cell consisting of an indicating electrode (E_I) as the anode, a reference electrode (E_R) as the cathode, and a voltammeter with high impedance. Based on the Nernst equation, this self-driven sensor can obtain concentration-dependent information as reactants are able to establish equilibrium potential spontaneously under infinitesimal current when the potential difference ($E = E_R - E_I$) is positive.

High selectivity and stability remain the biggest challenges in GRP that restrict its application for *in vivo* electrochemical sensing. One effective solution is the interfacial modification on electrodes. Lately, Wu et al. pioneered a GRP-based sensor that achieves *in vivo* AA sensing selectively. To be specific, the indicating CFE was modified with a single-walled carbon nanotube (SWCNT) to selectively oxidize AA, and the reference CFE was modified with laccase to stably reduce O_2 (Figure 8). Their inspiring results showed high selective measurement of AA in complex environment with electroactive neurochemicals coexisting, suggesting that the GRP-based sensor endows the potential to be a universal strategy for *in vivo* neurochemical detections.[230]

CONCLUSION AND OUTLOOKS

In vivo electrochemical analysis has raised close attention of various fields including electrochemistry, physiology, materials science, and micromachining. In this Review, we discussed three key issues that lie at the heart of *in vivo* electrochemical sensors. The issue of selectivity promotes the development of electrochemical methods and electrode interfacial design. Recognition elements such as enzymes, aptamers, MIPs, and

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

ion-selective membranes possess specific selectivity to neurochemicals of interest. Response time and temporal resolution of these recognition elements varies from subseconds to minutes, commensurate with the requirements of different physiological and pathological processes monitoring. To gain more precise neurochemical information, considerable interest has been paid to developing novel recognition elements and electrochemical methods. Moreover, ion transfer at ITIES, as an emerging technique, sheds new light on *in vivo* electrochemical sensing. Besides, ion transport-based sensing which takes ionic currents rather than electron currents as output signals has also expanded the concept of electrochemical sensing. Although there are still few *in vivo* experiments conducted by the ion transport-based sensors, their application on the level of single cells afford researchers a bright future in this field.[231] Besides selectivity, brain tissue response remains a challenging issue, because it inevitably makes a continuous impact on both neuronal activity and implanted sensors. Flexible materials, advanced surface modification, and innovated calibration are all effective solutions to minimize tissue damage, alleviate inflammatory response, and reduce impact of tissue response on sensing. Furthermore, a compatibility issue as the premise of sensing attracts grave concern. Followed by rapid development of the serial approach and the parallel approach, acquiring LFP information from amperometric signals extended the horizon of electrochemical/electrophysiological compatibility. However, both amperometry and voltammetry pose uncertain problems on their compatibility with neuronal activity. From this perspective, GRP-based sensors afford researchers a new thinking direction.

Although herein we tried to discuss these issues separately, *in vivo* electrochemical sensing can only be realized when taking the whole spectrum of aspects into consideration. For example, the outstanding performance of selective modification is usually established on its antifouling characteristics, and advanced micromachining technology is one main driving force behind development of novel microelectrodes. As a kind of emerging technology, *in vivo* electrochemical sensing is rapidly growing to be a promising approach for neurochemical detection with high selectivity, spatiotemporal resolution, sensitivity, and reliability.

AUTHOR INFORMATION

Corresponding Author

*E-mail: lqmao@iccas.ac.cn.

ORCID

Lanqun Mao: 0000-0001-8286-9321

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge financial support from the National Natural Science Foundation of China (Grant No. 21790390, 21790391, 21621062, 21435007 for L. Mao; 21775151 for P. Yu; 21874139 for F. Wu), and National Key Research and Development Project of China (Grant No. 2018YFE0200800), and the Chinese Academy of Sciences (QYZDJ-SSW-SLH030).

ABBREVIATIONS

ROS, reactive oxygen species; 3D, three-dimensional; fMRI, functional magnetic resonance imaging; PET, positron emission tomography; MS, mass spectrometry; FSCV, fast-scan cyclic voltammetry; AA, ascorbic acid; O_2, oxygen; SD, spreading depolarization; DET, direct electron transfer; MET, mediated electron transfer; Cyt c, Cytochrome C; O_2^-, superoxide anion; Cu, Zn-SOD, copper−zinc superoxide dismutase; CNS, central nervous system; H_2O_2, hydrogen peroxide; Pt, platinum; ATP, adenosine triphosphate; MEAs, microelectrode arrays; BSA, bovine serum albumin; L-Glu, L-glutamate; ACh, acetylcholine; NADH, β-nicotinamide adenine dinucleotide (reduced form); Fd-GltS, ferredoxin-dependent glutamate synthase; FMN, flavin mononucleotide; SELEX, systematic evolution of ligands by exponential enrichment; PCR, Polymerase Chain Reaction; E-DNA, electrochemical DNA; E-AB, electrochemical aptamer-based; DA, dopamine; FET, field-effect transistor; S1P, sphingosine-1-phosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; DRUS, dual recognition unit strategy; PimB, polyimidazolium-brush; ICR, ion current rectification; MICR, micrometer-scale ion current rectification; MIP, molecularly imprinted polymer; UA, uric acid; NE, noradrenaline; NPAMR, nanoporous alloy microrods; AMR, alloy microrods; OPPy, overoxidized polypyrrole; L-DOPA, 3,4-dihydroxy-L-phenylalanine; ISEs, solid-state ion-selective electrodes; CFE, carbon fiber microelectrode; CySH, L-cysteine; DPV, differential pulse voltammetry; aCSF, artificial cerebrospinal fluid; AD, Alzheimer's Disease; ITIES, interface between two immiscible electrolyte solution; CV, cyclic voltammetry; nanoITIES, ITIES based on nanopipette; T, tryptamine; 5-HT, serotonin; GABA, gamma-aminobutyric acid; SECM, scanning electrochemical microscopy; BBB, blood-brain barrier; GFAP, glial fibrillary acid protein; CNPEs, carbon nanopipette electrodes; PEG, polyethylene glycol; SAMs, self-assembled monolayers; PEDOT, poly(3,4-ethylenedioxythiophene); PC, phosphorylcholine; SNM, silica nanoporous membrane; CA, chronoamperometry; LFP, local field potential; FFT, Fast Fourier Transform; GRP, galvanic redox potentiometry; SWCNT, single-walled carbon nanotube

VOCABULARY

Field-effect transistor, a semiconductor device whose output current can be controlled by an electric field; Ion current rectification, a nonlinear current−voltage response that the ion current in one direction is higher than that in the opposite direction; Molecularly imprinted polymers, synthetic polymers with cavities which can specifically response to their "template" molecules; Ion-selective electrodes, electrodes that determine the concentration or activity of a specific ion by measuring the electric junction potential; Galvanic redox potentiometry, an analytical method measuring the concentration-dependent electrochemical potential at near-zero current

REFERENCES

(1) Rong, G.; Corrie, S. R.; Clark, H. A. In Vivo Biosensing: Progress and Perspectives. *ACS Sens.* **2017**, *2*, 327−338.

(2) Soto, R. J.; Hall, J. R.; Brown, M. D.; Taylor, J. B.; Schoenfisch, M. H. In Vivo Chemical Sensors: Role of Biocompatibility on Performance and Utility. *Anal. Chem.* **2017**, *89*, 276−299.

(3) Xiao, T.; Wu, F.; Hao, J.; Zhang, M.; Yu, P.; Mao, L. In Vivo Analysis with Electrochemical Sensors and Biosensors. *Anal. Chem.* **2017**, *89*, 300−313.

(4) Wu, F.; Yu, P.; Mao, L. Self-Powered Electrochemical Systems as Neurochemical Sensors: Toward Self-Triggered In Vivo Analysis of Brain Chemistry. *Chem. Soc. Rev.* **2017**, *46*, 2692−2704.

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

(5) Laing, S.; Jamieson, L. E.; Faulds, K.; Graham, D. Surface-Enhanced Raman Spectroscopy for In Vivo Biosensing. *Nat. Rev. Chem.* **2017**, *1*, 0060.
(6) Ewing, A. G.; Bigelow, J. C.; Wightman, R. M. Direct In Vivo Monitoring of Dopamine Released from 2 Striatal Compartments in the Rat. *Science* **1983**, *221*, 169−171.
(7) Cahill, P. S.; Walker, Q. D.; Finnegan, J. M.; Mickelson, G. E.; Travis, E. R.; Wightman, R. M. Microelectrodes for the Measurement of Catecholamines in Biological Systems. *Anal. Chem.* **1996**, *68*, 3180−3186.
(8) Pihel, K.; Walker, Q. D.; Wightman, R. M. Overoxidized Polypyrrole-Coated Carbon Fiber Microelectrodes for Dopamine Measurements with Fast-Scan Cyclic Voltammetry. *Anal. Chem.* **1996**, *68*, 2084−2089.
(9) Zhang, Q.; Liu, B.; Wu, Q.; Liu, B.; Li, Y.; Sun, S.; Wang, Y.; Wu, X.; Chai, Z.; Jiang, X.; Liu, X.; Hu, M.; Wang, Y.; Yang, Y.; Wang, L.; Kang, X.; Xiong, Y.; Zhou, Y.; Chen, X.; Zheng, L.; Zhang, B.; Wang, C.; Zhu, F.; Zhou, Z. Differential Co-Release of Two Neurotransmitters from a Vesicle Fusion Pore in Mammalian Adrenal Chromaffin Cells. *Neuron* **2019**, *102*, 173.
(10) Cartmell, J.; Schoepp, D. D. Regulation of Neurotransmitter Release by Metabotropic Glutamate Receptors. *J. Neurochem.* **2000**, *75*, 889−907.
(11) Phillips, P. E. M.; Stuber, G. D.; Heien, M.; Wightman, R. M.; Carelli, R. M. Subsecond Dopamine Release Promotes Cocaine Seeking. *Nature* **2003**, *422*, 614−618.
(12) Navarro, X.; Vivo, M.; Valero-Cabre, A. Neural Plasticity after Peripheral Nerve Injury and Regeneration. *Prog. Neurobiol.* **2007**, *82*, 163−201.
(13) Patriarchi, T.; Cho, J. R.; Merten, K.; Howe, M. W.; Marley, A.; Xiong, W.; Folk, R. W.; Broussard, G. J.; Liang, R.; Jang, M. J.; Zhong, H.; Dombeck, D.; von Zastrow, M.; Nimmerjahn, A.; Gradinaru, V.; Williams, J. T.; Tian, L. Ultrafast Neuronal Imaging of Dopamine Dynamics with Designed Genetically Encoded Sensors. *Science* **2018**, *360*, No. eaat4422.
(14) Schultz, W. Multiple Dopamine Functions at Different Time Courses. *Annu. Rev. Neurosci.* **2007**, *30*, 259−288.
(15) Yang, G.; Wu, L.; Jiang, B.; Yang, W.; Qi, J.; Cao, K.; Meng, Q.; Mustafa, A. K.; Mu, W.; Zhang, S.; Snyder, S. H.; Wang, R. H_2S as a Physiologic Vasorelaxant: Hypertension in Mice with Deletion of Cystathionine Gamma-lyase. *Science* **2008**, *322*, 587−590.
(16) Bartz, J. A.; Zaki, J.; Bolger, N.; Ochsner, K. N. Social Effects of Oxytocin in Humans: Context and Person Matter. *Trends Cognit. Sci.* **2011**, *15*, 301−309.
(17) Cheng, H.; Xiao, T.; Wang, D.; Hao, J.; Yu, P.; Mao, L. Simultaneous In Vivo Ascorbate and Electrophysiological Recordings in Rat Brain following Ischemia/Reperfusion. *J. Electroanal. Chem.* **2016**, *781*, 90−96.
(18) Xiao, T.; Li, X.; Wei, H.; Ji, W.; Yue, Q.; Yu, P.; Mao, L. In Vivo Monitoring of Oxygen Fluctuation Simultaneously at Multiple Sites of Rat Cortex during Spreading Depression. *Anal. Chem.* **2018**, *90*, 13783−13789.
(19) Xiao, T.; Wang, Y.; Wei, H.; Yu, P.; Jiang, Y.; Mao, L. Electrochemical Monitoring of Propagative Fluctuation of Ascorbate in the Live Rat Brain during Spreading Depolarization. *Angew. Chem., Int. Ed.* **2019**, *58*, 6616−6619.
(20) McIntyre, C. C.; Anderson, R. W. Deep Brain Stimulation Mechanisms: the Control of Network Activity via Neurochemistry Modulation. *J. Neurochem.* **2016**, *139*, 338−345.
(21) Phan, N. T. N.; Li, X.; Ewing, A. G. Measuring Synaptic Vesicles using Cellular Electrochemistry and Nanoscale Molecular Imaging. *Nat. Rev. Chem.* **2017**, *1*, 0048.
(22) Wu, F.; Yu, P.; Mao, L. Analytical and Quantitative In Vivo Monitoring of Brain Neurochemistry by Electrochemical and Imaging Approaches. *ACS Omega* **2018**, *3*, 13267−13274.
(23) Wilson, G. S.; Johnson, M. A. In-Vivo Electrochemistry: What Can We Learn about Living Systems? *Chem. Rev.* **2008**, *108*, 2462−2481.
(24) Robinson, D. L.; Hermans, A.; Seipel, A. T.; Wightman, R. M. Monitoring Rapid Chemical Communication in the Brain. *Chem. Rev.* **2008**, *108*, 2554−2584.
(25) Bucher, E. S.; Wightman, R. M. Electrochemical Analysis of Neurotransmitters. *Annu. Rev. Anal. Chem.* **2015**, *8*, 239−261.
(26) Yu, P.; He, X.; Mao, L. Tuning Interionic Interaction for Highly Selective In Vivo Analysis. *Chem. Soc. Rev.* **2015**, *44*, 5959−5968.
(27) Wang, Y.; Mao, L. Recent Advances in Analytical Methodology for In Vivo Electrochemistry in Mammals. *Electroanalysis* **2016**, *28*, 265−276.
(28) Ganesana, M.; Lee, S. T.; Wang, Y.; Venton, B. J. Analytical Techniques in Neuroscience: Recent Advances in Imaging, Separation, and Electrochemical Methods. *Anal. Chem.* **2017**, *89*, 314−341.
(29) Roberts, J. G.; Sombers, L. A. Fast-Scan Cyclic Voltammetry: Chemical Sensing in the Brain and Beyond. *Anal. Chem.* **2018**, *90*, 490−504.
(30) Xue, Y.; Xiao, T.; Jiang, Y.; Wu, F.; Yu, P.; Mao, L. Progress of In Vivo Electrochemical Analysis of Brain Neurochemistry. *Chin. J. Anal. Chem.* **2019**, *47*, 1443−1454.
(31) Harrison, F. E.; May, J. M. Vitamin C Function in the Brain: Vital Role of the Ascorbate Transporter SVCT2. *Free Radical Biol. Med.* **2009**, *46*, 719−730.
(32) Smith, S. K.; Lee, C. A.; Dausch, M. E.; Horman, B. M.; Patisaul, H. B.; McCarty, G. S.; Sombers, L. A. Simultaneous Voltammetric Measurements of Glucose and Dopamine Demonstrate the Coupling of Glucose Availability with Increased Metabolic Demand in the Rat Striatum. *ACS Chem. Neurosci.* **2017**, *8*, 272−280.
(33) Smith, S. K.; Gosrani, S. P.; Lee, C. A.; McCarty, G. S.; Sombers, L. A. Carbon-Fiber Microbiosensor for Monitoring Rapid Lactate Fluctuations in Brain Tissue Using Fast-Scan Cyclic Voltammetry. *Anal. Chem.* **2018**, *90*, 12994−12999.
(34) Wei, H.; Wu, F.; Yu, P.; Mao, L. Advances in Electrochemical Biosensors for In Vivo Analysis. *Chin. J. Anal. Chem.* **2019**, *47*, 1466−1479.
(35) Zhang, M.; Liu, K.; Xiang, L.; Lin, Y.; Su, L.; Mao, L. Carbon Nanotube-Modified Carbon Fiber Microelectrodes for In Vivo Voltammetric Measurement of Ascorbic Acid in Rat Brain. *Anal. Chem.* **2007**, *79*, 6559−6565.
(36) Xiang, L.; Yu, P.; Hao, J.; Zhang, M.; Zhu, L.; Dai, L.; Mao, L. Vertically Aligned Carbon Nanotube-Sheathed Carbon Fibers as Pristine Microelectrodes for Selective Monitoring of Ascorbate In Vivo. *Anal. Chem.* **2014**, *86*, 3909−3914.
(37) Xiao, T.; Jiang, Y.; Ji, W.; Mao, L. Controllable and Reproducible Sheath of Carbon Fibers with Single-Walled Carbon Nanotubes through Electrophoretic Deposition for In Vivo Electrochemical Measurements. *Anal. Chem.* **2018**, *90*, 4840−4846.
(38) Ji, W.; Zhang, M.; Mao, L. Recent Advances on In Vivo Electrochemical Analysis of Vitamin C in Rat Brain. *Chin. J. Anal. Chem.* **2019**, *47*, 1559−1571.
(39) Ding, S.; Cao, S.; Liu, Y.; Lian, Y.; Zhu, A.; Shi, G. Rational Design of a Stimuli -Responsive Polymer Electrode Interface Coupled with In Vivo Microdialysis for Measurement of Sialic Acid in Live Mouse Brain in Alzheimer's Disease. *ACS Sens.* **2017**, *2*, 394−400.
(40) Updike, S. J.; Hicks, G. P. Enzyme Electrode. *Nature* **1967**, *214*, 986−988.
(41) Liang, B.; Guo, X.; Fang, L.; Hu, Y.; Yang, G.; Zhu, Q.; Wei, J.; Ye, X. Study of Direct Electron Transfer and Enzyme Activity of Glucose Oxidase on Graphene Surface. *Electrochem. Commun.* **2015**, *50*, 1−5.
(42) de Poulpiquet, A.; Ciaccafava, A.; Lojou, E. New Trends in Enzyme Immobilization at Nanostructured Interfaces for Efficient Electrocatalysis in Biofuel Cells. *Electrochim. Acta* **2014**, *126*, 104−114.
(43) Rasmussen, M.; Abdellaoui, S.; Minteer, S. D. Enzymatic Biofuel Cells: 30 Years of Critical Advancements. *Biosens. Bioelectron.* **2016**, *76*, 91−102.
(44) Zhang, L.; Wang, J.; Tian, Y. Electrochemical In-Vivo Sensors Using Nanomaterials Made From Carbon Species, Noble Metals, or Semiconductors. *Microchim. Acta* **2014**, *181*, 1471−1484.

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

(45) Das, P.; Das, M.; Chinnadayyala, S. R.; Singha, I. M.; Goswami, P. Recent Advances on Developing 3rd Generation Enzyme Electrode for Biosensor Applications. *Biosens. Bioelectron.* **2016**, *79*, 386−397.
(46) Moon, J.-M.; Thapliyal, N.; Hussain, K. K.; Goyal, R. N.; Shim, Y.-B. Conducting Polymer-Based Electrochemical Biosensors for Neurotransmitters: A Review. *Biosens. Bioelectron.* **2018**, *102*, 540−552.
(47) Pinyou, P.; Blay, V.; Muresan, L. M.; Noguer, T. Enzyme-Modified Electrodes for Biosensors and Biofuel Cells. *Mater. Horiz.* **2019**, *6*, 1336−1358.
(48) Xiao, Y.; Patolsky, F.; Katz, E.; Hainfeld, J. F.; Willner, I. Plugging into Enzymes": Nanowiring of Redox Enzymes by a Gold Nanoparticle. *Science* **2003**, *299*, 1877−1881.
(49) Lee, Y. S.; Baek, S.; Lee, H.; Reginald, S. S.; Kim, Y.; Kang, H.; Choi, I.-G.; Chang, I. S. Construction of Uniform Monolayer- and Orientation-Tunable Enzyme Electrode by a Synthetic Glucose Dehydrogenase without Electron-Transfer Subunit via Optimized Site-Specific Gold-Binding Peptide Capable of Direct Electron Transfer. *ACS Appl. Mater. Interfaces* **2018**, *10*, 28615−28626.
(50) Holland, J. T.; Lau, C.; Brozik, S.; Atanassov, P.; Banta, S. Engineering of Glucose Oxidase for Direct Electron Transfer via Site-Specific Gold Nanoparticle Conjugation. *J. Am. Chem. Soc.* **2011**, *133*, 19262−19265.
(51) Wu, F.; Su, L.; Yu, P.; Mao, L. Role of Organic Solvents in Immobilizing Fungus Laccase on Single Walled Carbon Nanotubes for Improved Current Response in Direct Bioelectrocatalysis. *J. Am. Chem. Soc.* **2017**, *139*, 1565−1574.
(52) Xu, Q.; Shen, Y.; Tang, J.; Xue, M.; Jiang, L.; Hu, X. Electrochemical Method Assisted Immobilization and Orientation of Myoglobin into Biomimetic Brij 56 Film and Its Direct Electrochemistry Study. *ACS Appl. Mater. Interfaces* **2015**, *7*, 11286−11293.
(53) Zhao, M.; Gao, Y.; Sun, J.; Gao, F. Mediatorless Glucose Biosensor and Direct Electron Transfer Type Glucose/Air Biofuel Cell Enabled with Carbon Nanodots. *Anal. Chem.* **2015**, *87*, 2615−2622.
(54) Roychoudhury, A.; Basu, S.; Jha, S. K. Dopamine Biosensor Based on Surface Functionalized Nanostructured Nickel Oxide Platform. *Biosens. Bioelectron.* **2016**, *84*, 72−81.
(55) Sun, C.; Wang, D.; Zhang, M.; Ni, Y.; Shen, X.; Song, Y.; Geng, Z.; Xu, W.; Liu, F.; Mao, C. Novel L-Lactic Acid Biosensors Based on Conducting Polypyrrole-Block Copolymer Nanoparticles. *Analyst* **2015**, *140*, 797−802.
(56) Luo, Z.; Yang, D.; Qi, G.; Yuwen, L.; Zhang, Y.; Weng, L.; Wang, L.; Huang, W. Preparation of Highly Dispersed Reduced Graphene Oxide Decorated with Chitosan Oligosaccharide as Electrode Material for Enhancing the Direct Electron Transfer of Escherichia coli. *ACS Appl. Mater. Interfaces* **2015**, *7*, 8539−8544.
(57) Qiao, Y.; Tahara, K.; Zhang, Q.; Song, X.; Kikuchi, J.-i. Cerasomes: Soft Interface for Redox Enzyme Electrochemical Signal Transmission. *Chem. - Eur. J.* **2016**, *22*, 1340−1348.
(58) Rahman, M. A.; Kothalam, A.; Choe, E. S.; Won, M.-S.; Shim, Y.-B. Stability and Sensitivity Enhanced Electrochemical In Vivo Superoxide Microbiosensor Based on Covalently Co-immobilized Lipid and Cytochrome c. *Anal. Chem.* **2012**, *84*, 6654−6660.
(59) Tian, Y.; Mao, L.; Okajima, T.; Ohsaka, T. Superoxide Dismutase-based Third-Generation Biosensor for Superoxide Anion. *Anal. Chem.* **2002**, *74*, 2428−2434.
(60) Tian, Y.; Mao, L. Q.; Okajima, T.; Ohsaka, T. Electrochemistry and Electrocatalytic Activities of Superoxide Dismutases at Gold Electrodes Modified with a Self-Assembled Monolayer. *Anal. Chem.* **2004**, *76*, 4162−4168.
(61) Luo, Y.; Tian, Y.; Zhu, A.; Rui, Q.; Liu, H. Direct Electron Transfer of Superoxide Dismutase Promoted by High Conductive TiO_2 Nanoneedles. *Electrochem. Commun.* **2009**, *11*, 174−176.
(62) Liu, H.; Tian, Y. Analytical Application of Pyramidal, Rodlike, and Spherical Gold Nanostructures: Simultaneous Detection of Ascorbic Acid and Uric Acid. *Electroanalysis* **2008**, *20*, 1227−1233.
(63) Luo, Y.; Tian, Y.; Zhu, A.; Rui, Q.; Liu, H. Direct Electron Transfer of Superoxide Dismutase Promoted by High Conductive TiO_2 Nanoneedles. *Electrochem. Commun.* **2009**, *11*, 174−176.
(64) Deng, Z.; Rui, Q.; Yin, X.; Liu, H.; Tian, Y. In Vivo Detection of Superoxide Anion in Bean Sprout Based on ZnO Nanodisks with Facilitated Activity for Direct Electron Transfer of Superoxide Dismutase. *Anal. Chem.* **2008**, *80*, 5839−5846.
(65) Zhang, L.; Tian, Y. Designing Recognition Molecules and Tailoring Functional Surfaces for In Vivo Monitoring of Small Molecules in the Brain. *Acc. Chem. Res.* **2018**, *51*, 688−696.
(66) Llaudet, E.; Botting, N. P.; Crayston, J. A.; Dale, N. A Three-Enzyme Microelectrode Sensor for Detecting Purine Release from Central Nervous System. *Biosens. Bioelectron.* **2003**, *18*, 43−52.
(67) Llaudet, E.; Hatz, S.; Droniou, M.; Dale, N. Microelectrode Biosensor for Real-Time Measurement of ATP in Biological Tissue. *Anal. Chem.* **2005**, *77*, 3267−3273.
(68) Gourine, A. V.; Llaudet, E.; Dale, N.; Spyer, K. M. ATP is a Mediator of Chemosensory Transduction in the Central Nervous System. *Nature* **2005**, *436*, 108−111.
(69) Baker, K. L.; Bolger, F. B.; Lowry, J. P. A Microelectrochemical Biosensor for Real-Time In Vivo Monitoring of Brain Extracellular Choline. *Analyst* **2015**, *140*, 3738−3745.
(70) Sardesai, N. P.; Ganesana, M.; Karimi, A.; Leiter, J. C.; Andreescu, S. Platinum-Doped Ceria Based Biosensor for in Vitro and In Vivo Monitoring of Lactate during Hypoxia. *Anal. Chem.* **2015**, *87*, 2996−3003.
(71) Conway, G. E.; Lambertson, R. H.; Schwarzmann, M. A.; Pannell, M. J.; Kerins, H. W.; Rubenstein, K. J.; Dattelbaum, J. D.; Leopold, M. C. Layer-by-Layer Design and Optimization of Xerogel-Based Amperometric First Generation Biosensors for Uric Acid. *J. Electroanal. Chem.* **2016**, *775*, 135−145.
(72) Baker, K. L.; Bolger, F. B.; Lowry, J. P. Development of a Microelectrochemical Biosensor for the Real-time Detection of Choline. *Sens. Actuators, B* **2017**, *243*, 412−420.
(73) Song, Y.; Su, D.; Shen, Y.; Liu, H.; Wang, L. Design and Preparation of Open Circuit Potential Biosensor for In Vitro and In Vivo Glucose Monitoring. *Anal. Bioanal. Chem.* **2017**, *409*, 161−168.
(74) Cordeiro, C. A.; Sias, A.; Koster, T.; Westerink, B. H. C.; Cremers, T. In Vivo "Real-Time" Monitoring of Glucose in the Brain with an Amperometric Enzyme-Based Biosensor Based on Gold Coated Tungsten (W-Au) Microelectrodes. *Sens. Actuators, B* **2018**, *263*, 605−613.
(75) Clay, M.; Monbouquette, H. G. A Detailed Model of Electroenzymatic Glutamate Blosensors to Aid in Sensor Optimization and in Applications In Vivo. *ACS Chem. Neurosci.* **2018**, *9*, 241−251.
(76) Hossain, I.; Tan, C.; Doughty, P. T.; Dutta, G.; Murray, T. A.; Siddiqui, S.; Iasemidis, L.; Arumugamr, P. U. A Novel Microbiosensor Microarray for Continuous Ex Vivo Monitoring o Gamma-Amino-butyric Acid in Real-Time. *Front. Neurosci.* **2018**, *12*, 500.
(77) Wilson, L. R.; Panda, S.; Schmidt, A. C.; Sombers, L. A. Selective and Mechanically Robust Sensors for Electrochemical Measurements of Real-Time Hydrogen Peroxide Dynamics In Vivo. *Anal. Chem.* **2018**, *90*, 888−895.
(78) Ganesana, M.; Trikantzopoulos, E.; Maniar, Y.; Lee, S. T.; Venton, B. J. Development of a Novel Microbiosensor for In Vivo Monitoring of Glutamate Release in the Brain. *Biosens. Bioelectron.* **2019**, *130*, 103−109.
(79) Zhou, J.; Ma, Z.; Hong, X.; Wu, H. M.; Ma, S. Y.; Li, Y.; Chen, D. J.; Yu, H. Y.; Huang, X. J. Top-Down Strategy of Implantable Biosensor Using Adaptable, Porous Hollow Fibrous Membrane. *ACS Sens.* **2019**, *4*, 931−937.
(80) Tang, H.; Cai, D.; Ren, T.; Xiong, P.; Liu, Y.; Gu, H.; Shi, G. Fabrication of a Low Background Signal Glucose Biosensor with 3D Network Materials as the Electrocatalyst. *Anal. Biochem.* **2019**, *567*, 63−71.
(81) Hou, H.; Mao, J.; Han, Y.; Wu, F.; Zhang, M.; Wang, D.; Mao, L.; Li, Y. Single-Atom Electrocatalysis: a New Approach to In Vivo

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

Electrochemical Biosensing. *Sci. China: Chem.* **2019**, 1 DOI: 10.1007/s11426-019-9605-0.
(82) Baker, K. L.; Bolger, F. B.; Doran, M. M.; Lowry, J. P. Characterisation of a Platinum-based Electrochemical Biosensor for Real-time Neurochemical Analysis of Choline. *Electroanalysis* **2019**, *31*, 129−136.
(83) Burmeister, J. J.; Gerhardt, G. A. Self Referencing Ceramic Based Multisite Microelectrodes for the Detection and Elimination of Interferences from the Measurement of L-glutamate and other Analytes. *Anal. Chem.* **2001**, *73*, 1037−1042.
(84) Burmeister, J. J.; Pomerleau, F.; Palmer, M.; Day, B. K.; Huettl, P.; Gerhardt, G. A. Improved Ceramic-Based Multisite Microelectrode for Rapid Measurements of L-glutamate in the CNS. *J. Neurosci. Methods* **2002**, *119*, 163−171.
(85) Burmeister, J. J.; Palmer, M.; Gerhardt, G. A. L-lactate Measures in Brain Tissue with Ceramic-Based Multisite Microelectrodes. *Biosens. Bioelectron.* **2005**, *20*, 1772−1779.
(86) Day, B. K.; Pomerleau, F.; Burmeister, J. J.; Huettl, P.; Gerhardt, G. A. Microelectrode Array Studies of Basal and Potassium-Evoked Release of L-glutamate in the Anesthetized Rat Brain. *J. Neurochem.* **2006**, *96*, 1626−1635.
(87) Rutherford, E. C.; Pomerleau, F.; Huettl, P.; Stromberg, I.; Gerhardt, G. A. Chronic Second-by-Second Measures of L-glutamate in the Central Nervous System of Freely Moving Rats. *J. Neurochem.* **2007**, *102*, 712−722.
(88) Hascup, K. N.; Hascup, E. R.; Pomerleau, F.; Huettl, P.; Gerhardt, G. A. Second-by-Second Measures of L-glutamate in the Prefrontal Cortex and Striatum of Freely Moving Mice. *J. Pharmacol. Exp. Ther.* **2008**, *324*, 725−731.
(89) Burmeister, J. J.; Pomerleau, F.; Huettl, P.; Gash, C. R.; Wemer, C. E.; Bruno, J. P.; Gerhardt, G. A. Ceramic-Based Multisite Microelectrode Arrays for Simultaneous Measures of Choline and Acetylcholine in CNS. *Biosens. Bioelectron.* **2008**, *23*, 1382−1389.
(90) Konradsson-Geuken, A.; Gash, C. R.; Alexander, K.; Pomerleau, F.; Huettl, P.; Gerhardt, G. A.; Bruno, J. P. Second-by-Second Analysis of Alpha 7 Nicotine Receptor Regulation of Glutamate Release in the Prefrontal Cortex of Awake Rats. *Synapse* **2009**, *63*, 1069−1082.
(91) McLamore, E. S.; Mohanty, S.; Shi, J.; Claussen, J.; Jedlicka, S. S.; Rickus, J. L.; Porterfield, D. M. A Self-Referencing Glutamate Biosensor for Measuring Real Time Neuronal Glutamate Flux. *J. Neurosci. Methods* **2010**, *189*, 14−22.
(92) Stephens, M. L.; Pomerleau, F.; Huettl, P.; Gerhardt, G. A.; Zhang, Z. Real-Time Glutamate Measurements in the Putamen of Awake Rhesus Monkeys using an Enzyme-Based Human Microelectrode Array Prototype. *J. Neurosci. Methods* **2010**, *185*, 264−272.
(93) Cao, H.; Li, A.; Nguyen, C. M.; Peng, Y.; Chiao, J. An Integrated Flexible Implantable Micro-Probe for Sensing Neurotransmitters. *IEEE Sens. J.* **2012**, *12*, 1618−1624.
(94) Burmeister, J. J.; Davis, V. A.; Quintero, J. E.; Pomerleau, F.; Huettl, P.; Gerhardt, G. A. Glutaraldehyde Cross-Linked Glutamate Oxidase Coated Microelectrode Arrays: Selectivity and Resting Levels of Glutamate in the CNS. *ACS Chem. Neurosci.* **2013**, *4*, 721−728.
(95) Lourenco, C. F.; Ledo, A.; Gerhardt, G. A.; Laranjinha, J.; Barbosa, R. M. Neurometabolic and Electrophysiological Changes During Cortical Spreading Depolarization: Multimodal Approach Based on a Lactate-Glucose Dual Microbiosensor Arrays. *Sci. Rep.* **2017**, *7*, 6764.
(96) Vasylieva, N.; Barnych, B.; Meillerd, A.; Maucler, C.; Pollegioni, L.; Lin, J.-S.; Barbier, D.; Marinesco, S. Covalent Enzyme Immobilization by Poly(ethylene glycol) Diglycidyl Ether (PEGDE) for Microelectrode Biosensor Preparation. *Biosens. Bioelectron.* **2011**, *26*, 3993−4000.
(97) Vasylieva, N.; Maucler, C.; Meiller, A.; Viscogliosi, H.; Lieutaud, T.; Barbier, D.; Marinesco, S. Immobilization Method to Preserve Enzyme Specificity in Biosensors: Consequences for Brain Glutamate Detection. *Anal. Chem.* **2013**, *85*, 2507−2515.
(98) Zhang, Z.; Hao, J.; Xiao, T.; Yu, P.; Mao, L. Online Electrochemical Systems for Continuous Neurochemical Measurements with Low-Potential Mediator-Based Electrochemical Biosensors as Selective Detectors. *Analyst* **2015**, *140*, 5039−5047.
(99) Weltin, A.; Kieninger, J.; Urban, G. A. Microfabricated, Amperometric, Enzyme-Based Biosensors for In Vivo Applications. *Anal. Bioanal. Chem.* **2016**, *408*, 4503−4521.
(100) Cheng, H.; Zhang, L.; He, J.; Guo, W.; Zhou, Z.; Zhang, X.; Nie, S.; Wei, H. Integrated Nanozymes with Nanoscale Proximity for In Vivo Neurochemical Monitoring in Living Brains. *Anal. Chem.* **2016**, *88*, 5489−5497.
(101) Wang, X.; Li, Q.; Xu, J.; Wu, S.; Xiao, T.; Hao, J.; Yu, P.; Mao, L. Rational Design of Bioelectrochemically Multifunctional Film with Oxidase, Ferrocene, and Graphene Oxide for Development of In Vivo Electrochemical Biosensors. *Anal. Chem.* **2016**, *88*, 5885−5891.
(102) Su, D.; Feng, B. W.; Xu, P. F.; Zeng, Q.; Shan, B. X.; Song, Y. G. Covalent Organic Frameworks and Electron Mediator-Based Open Circuit Potential Biosensor for In Vivo Electrochemical Measurements. *Anal. Methods* **2018**, *10*, 4320−4328.
(103) Ma, W.; Jiang, Q.; Yu, P.; Yang, L.; Mao, L. Zeolitic Imidazolate Framework-Based Electrochemical Biosensor for In Vivo Electrochemical Measurements. *Anal. Chem.* **2013**, *85*, 7550−7557.
(104) Wu, F.; Yu, P.; Yang, X.; Han, Z.; Wang, M.; Mao, L. Exploring Ferredoxin-Dependent Glutamate Synthase as an Enzymatic Bioelectrocatalyst. *J. Am. Chem. Soc.* **2018**, *140*, 12700−12704.
(105) Wu, J.; Wang, X.; Wang, Q.; Lou, Z.; Li, S.; Zhu, Y.; Qin, L.; Wei, H. Nanomaterials with Enzyme-like Characteristics (Nanozymes): Next-Generation Artificial Enzymes (II). *Chem. Soc. Rev.* **2019**, *48*, 1004−1076.
(106) Wang, Q.; Wei, H.; Zhang, Z.; Wang, E.; Dong, S. Nanozyme: An Emerging Alternative to Natural Enzyme for Biosensing and Immunoassay. *TrAC, Trends Anal. Chem.* **2018**, *105*, 218−224.
(107) Yuce, M.; Ullah, N.; Budak, H. Trends in Aptamer Selection Methods and Applications. *Analyst* **2015**, *140*, 5379−5399.
(108) Kim, Y. S.; Raston, N. H. A.; Gu, M. B. Aptamer-Based Nanobiosensors. *Biosens. Bioelectron.* **2016**, *76*, 2−19.
(109) Dunn, M. R.; Jimenez, R. M.; Chaput, J. C. Analysis of Aptamer Discovery and Technology. *Nat. Rev. Chem.* **2017**, *1*, 76.
(110) Fan, C.; Plaxco, K. W.; Heeger, A. J. Electrochemical Interrogation of Conformational Changes as a Reagentless Method for the Sequence-Specific Detection of DNA. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 9134−9137.
(111) Xiao, Y.; Piorek, B. D.; Plaxco, K. W.; Heeger, A. J. A Reagentless Signal-on Architecture for Electronic, Aptamer-Based Sensors via Target-Induced Strand Displacement. *J. Am. Chem. Soc.* **2005**, *127*, 17990−17991.
(112) Lubin, A. A.; Plaxco, K. W. Folding-Based Electrochemical Biosensors: The Case for Responsive Nucleic Acid Architectures. *Acc. Chem. Res.* **2010**, *43*, 496−505.
(113) Li, H.; Arroyo-Curras, N.; Kang, D.; Ricci, F.; Plaxco, K. W. Dual-Reporter Drift Correction To Enhance the Performance of Electrochemical Aptamer-Based Sensors in Whole Blood. *J. Am. Chem. Soc.* **2016**, *138*, 15809−15812.
(114) Dauphin-Ducharme, P.; Yang, K.; Arroyo-Curras, N.; Ploense, K. L.; Zhang, Y.; Gerson, J.; Kurnik, M.; Kippin, T. E.; Stojanovic, M. N.; Plaxco, K. W. Electrochemical Aptamer-Based Sensors for Improved Therapeutic Drug Monitoring and High-Precision, Feedback-Controlled Drug Delivery. *ACS Sens.* **2019**, *4*, 2832−2837.
(115) Swensen, J. S.; Xiao, Y.; Ferguson, B. S.; Lubin, A. A.; Lai, R. Y.; Heeger, A. J.; Plaxco, K. W.; Soh, H. T. Continuous, Real-Time Monitoring of Cocaine in Undiluted Blood Serum via a Microfluidic, Electrochemical Aptamer-Based Sensor. *J. Am. Chem. Soc.* **2009**, *131*, 4262−4266.
(116) Farjami, E.; Campos, R.; Nielsen, J. S.; Gothelf, K. V.; Kjems, J.; Ferapontova, E. E. RNA Aptamer-Based Electrochemical Biosensor for Selective and Label-Free Analysis of Dopamine. *Anal. Chem.* **2013**, *85*, 121−128.
(117) Chavez, J. L.; Hagen, J. A.; Kelley-Loughnane, N. Fast and Selective Plasmonic Serotonin Detection with Aptamer-Gold Nanoparticle Conjugates. *Sensors* **2017**, *17*, 681.

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

(118) Santos-Cancel, M.; Simpson, L. W.; Leach, J. B.; White, R. J. Direct, Real-Time Detection of Adenosine Triphosphate Release from Astrocytes in Three-Dimensional Culture Using an Integrated Electrochemical Aptamer-Based Sensor. *ACS Chem. Neurosci.* **2019**, *10*, 2070−2079.
(119) Jiang, Y.; Ma, W.; Ji, W.; Wei, H.; Mao, L. Aptamer Superstructure-Based Electrochemical Biosensor for Sensitive Detection of ATP in Rat Brain with In Vivo Microdialysis. *Analyst* **2019**, *144*, 1711−1717.
(120) Nakatsuka, N.; Yang, K.-A.; Abendroth, J. M.; Cheung, K. M.; Xu, X.; Yang, H.; Zhao, C.; Zhu, B.; Rim, Y. S.; Yang, Y.; Weiss, P. S.; Stojanovic, M. N.; Andrews, A. M. Aptamer-Field-Effect Transistors Overcome Debye Length Limitations for Small-Molecule Sensing. *Science* **2018**, *362*, 319−324.
(121) Yu, P.; He, X.; Zhang, L.; Mao, L. Dual Recognition Unit Strategy Improves the Specificity of the Adenosine Triphosphate (ATP) Aptamer Biosensor for Cerebral ATP Assay. *Anal. Chem.* **2015**, *87*, 1373−1380.
(122) He, X.; Zhang, K.; Li, T.; Jiang, Y.; Yu, P.; Mao, L. Micrometer-Scale Ion Current Rectification at Polyelectrolyte Brush-Modified Micropipets. *J. Am. Chem. Soc.* **2017**, *139*, 1396−1399.
(123) Zhang, K.; He, X.; Liu, Y.; Yu, P.; Fei, J.; Mao, L. Highly Selective Cerebral ATP Assay Based on Micrometer Scale Ion Current Rectification at Polyimidazolium-Modified Micropipettes. *Anal. Chem.* **2017**, *89*, 6794−6799.
(124) Idili, A.; Arroyo-Curras, N.; Ploense, K. L.; Csordas, A. T.; Kuwahara, M.; Kippin, T. E.; Plaxco, K. W. Seconds-Resolved Pharmacokinetic Measurements of the Chemotherapeutic Irinotecan *in situ* in the Living Body. *Chem. Sci.* **2019**, *10*, 8164−8170.
(125) Arroyo-Curras, N.; Dauphin-Ducharme, P.; Ortega, G.; Ploense, K. L.; Kippin, T. E.; Plaxco, K. W. Subsecond-Resolved Molecular Measurements in the Living Body Using Chronoamperometrically Interrogated Aptamer-Based Sensors. *ACS Sens.* **2018**, *3*, 360−366.
(126) Canfarotta, F.; Waters, A.; Sadler, R.; McGill, P.; Guerreiro, A.; Papkovsky, D.; Haupt, K.; Piletsky, S. Biocompatibility and Internalization of Molecularly Imprinted Nanoparticles. *Nano Res.* **2016**, *9*, 3463−3477.
(127) Azzouz, A.; Goud, K. Y.; Raza, N.; Ballesteros, E.; Lee, S.-E.; Hong, J.; Deep, A.; Kim, K.-H. Nanomaterial-Based Electrochemical Sensors for the Detection of Neurochemicals in Biological Matrices. *TrAC, Trends Anal. Chem.* **2019**, *110*, 15−34.
(128) Hasanzadeh, M.; Shadjou, N.; de la Guardia, M. Current Advancement in Electrochemical Analysis of Neurotransmitters in Biological Fluids. *TrAC, Trends Anal. Chem.* **2017**, *86*, 107−121.
(129) Gui, R.; Jin, H.; Guo, H.; Wang, Z. Recent Advances and Future Prospects in Molecularly Imprinted Polymers-Based Electrochemical Biosensors. *Biosens. Bioelectron.* **2018**, *100*, 56−70.
(130) Li, B.; Zhou, Y.; Wu, W.; Liu, M.; Mei, S.; Zhou, Y.; Jing, T. Highly Selective and Sensitive Determination of Dopamine by the Novel Molecularly Imprinted Poly(Nicotinamide)/CuO Nanoparticles Modified Electrode. *Biosens. Bioelectron.* **2015**, *67*, 121−128.
(131) Zaidi, S. A. Development of Molecular Imprinted Polymers Based Strategies for the Determination of Dopamine. *Sens. Actuators, B* **2018**, *265*, 488−497.
(132) Saksena, K.; Shrivastava, A.; Kant, R. Chiral Analysis of Ascorbic Acid in Bovine Serum Using Ultrathin Molecular Imprinted Polyaniline/Graphite Electrode. *J. Electroanal. Chem.* **2017**, *795*, 103−109.
(133) Zheng, W.; Zhao, M.; Liu, W.; Yu, S.; Niu, L.; Li, G.; Li, H.; Liu, W. Electrochemical Sensor Based on Molecularly Imprinted Polymer/Reduced Graphene Oxide Composite for Simultaneous Determination of Uric Acid and Tyrosine. *J. Electroanal. Chem.* **2018**, *813*, 75−82.
(134) Xing, X.; Liu, S.; Yu, J.; Lian, W.; Huang, J. Electrochemical Sensor Based on Molecularly Imprinted Film at Polypyrrole-Sulfonated Graphene/Hyaluronic Acid-Multiwalled Carbon Nanotubes Modified Electrode for Determination of Tryptamine. *Biosens. Bioelectron.* **2012**, *31*, 277−283.
(135) Xue, C.; Wang, X.; Zhu, W.; Han, Q.; Zhu, C.; Hong, J.; Zhou, X.; Jiang, H. Electrochemical Serotonin Sensing Interface Based on Double-Layered Membrane of Reduced Graphene Oxide/Polyaniline Nanocomposites and Molecularly Imprinted Polymers Embedded with Gold Nanoparticles. *Sens. Actuators, B* **2014**, *196*, 57−63.
(136) Rosy; Chasta, H.; Goyal, R. N. Molecularly Imprinted Sensor Based on O-aminophenol for the Selective Determination of Norepinephrine in Pharmaceutical and Biological Samples. *Talanta* **2014**, *125*, 167−173.
(137) Chen, J.; Huang, H.; Zeng, Y.; Tang, H.; Li, L. A Novel Composite of Molecularly Imprinted Polymer-Coated PdNPs for Electrochemical Sensing Norepinephrine. *Biosens. Bioelectron.* **2015**, *65*, 366−374.
(138) Li, Y.; Song, H.; Zhang, L.; Zuo, P.; Ye, B.; Yao, J.; Chen, W. Supportless Electrochemical Sensor based on Molecularly Imprinted Polymer Modified Nanoporous Microrod for Determination of Dopamine at Trace Level. *Biosens. Bioelectron.* **2016**, *78*, 308−314.
(139) Tsai, T.; Han, H.; Cheng, C.; Chen, L.; Chang, H.; Chen, J. J. Modification of Platinum Microelectrode with Molecularly Imprinted Over-Oxidized Polypyrrole for Dopamine Measurement in Rat Striatum. *Sens. Actuators, B* **2012**, *171*, 93−101.
(140) Chai, X.; Zhou, X.; Zhu, A.; Zhang, L.; Qin, Y.; Shi, G.; Tian, Y. A Two-Channel Ratiometric Electrochemical Biosensor for In Vivo Monitoring of Copper Ions in a Rat Brain Using Gold Truncated Octahedral Microcages. *Angew. Chem., Int. Ed.* **2013**, *52*, 8129−8133.
(141) Zhao, F.; Zhang, L.; Zhu, A.; Shi, G.; Tian, Y. In Vivo Monitoring of Local pH Values in a Live Rat Brain Based on the Design of a Specific Electroactive Molecule for H^+. *Chem. Commun.* **2016**, *52*, 3717−3720.
(142) Zhou, J.; Zhang, L.; Tian, Y. Micro Electrochemical pH Sensor Applicable for Real-Time Ratiometric Monitoring of pH Values in Rat Brains. *Anal. Chem.* **2016**, *88*, 2113−2118.
(143) Luo, Y.; Zhang, L.; Liu, W.; Yu, Y.; Tian, Y. A Single Biosensor for Evaluating the Levels of Copper Ion and L-Cysteine in a Live Rat Brain with Alzheimer's Disease. *Angew. Chem., Int. Ed.* **2015**, *54*, 14053−14056.
(144) Zhao, L.; Zheng, W.; Mao, L. Recent Advances of Ion-Selective Electrode for In Vivo Analysis in Brain Neurochemistry. *Chin. J. Anal. Chem.* **2019**, *47*, 1480−1491.
(145) Hao, J.; Xiao, T.; Wu, F.; Yu, P.; Mao, L. High Antifouling Property of Ion-Selective Membrane: toward In Vivo Monitoring of pH Change in Live Brain of Rats with Membrane-Coated Carbon Fiber Electrodes. *Anal. Chem.* **2016**, *88*, 11238−11243.
(146) Odijk, M.; van der Wouden, E. J.; Olthuis, W.; Ferrari, M. D.; Tolner, E. A.; van den Maagdenberg, A. M. J. M.; van den Berg, A. Microfabricated Solid-State Ion-Selective Electrode Probe for Measuring Potassium in the Living Rodent Brain: Compatibility with DC-EEG Recordings to Study Spreading Depression. *Sens. Actuators, B* **2015**, *207*, 945−953.
(147) Zhao, L.; Jiang, Y.; Hao, J.; Wei, H.; Zheng, W.; Mao, L. Graphdiyne Oxide Enhances the Stability of Solid Contact-Based Ionselective Electrodes for Excellent In Vivo Analysis. *Sci. China: Chem.* **2019**, *62*, 1414−1420.
(148) Zhao, L.; Jiang, Y.; Wei, H.; Jiang, Y.; Ma, W.; Zheng, W.; Cao, A.-M.; Mao, L. In Vivo Measurement of Calcium Ion with Solid-State Ion-Selective Electrode by Using Shelled Hollow Carbon Nanospheres as a Transducing Layer. *Anal. Chem.* **2019**, *91*, 4421−4428.
(149) Shen, M.; Colombo, M. L. Electrochemical Nanoprobes for the Chemical Detection of Neurotransmitters. *Anal. Methods* **2015**, *7*, 7095−7105.
(150) Colombo, M. L.; Sweedler, J. V.; Shen, M. Nanopipet-Based Liquid-Liquid Interface Probes for the Electrochemical Detection of Acetylcholine, Tryptamine, and Serotonin via Ionic Transfer. *Anal. Chem.* **2015**, *87*, 5095−5100.
(151) Iwai, N. T.; Kramaric, M.; Crabbe, D.; Wei, Y.; Chen, R.; Shen, M. GABA Detection with Nano-ITIES Pipet Electrode: A New

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

Mechanism, Water/DCE-Octanoic Acid Interface. *Anal. Chem.* **2018**, *90*, 3067−3072.
(152) Colombo, M. L.; McNeil, S.; Iwai, N.; Chang, A.; Shen, M. Electrochemical Detection of Dopamine via Assisted Ion Transfer at Nanopipet Electrode Using Cyclic Voltammetry. *J. Electrochem. Soc.* **2016**, *163*, H3072−H3076.
(153) Shen, M.; Qu, Z.; DesLaurier, J.; Welle, T. M.; Sweedler, J. V.; Chen, R. Single Synaptic Observation of Cholinergic Neurotransmission on Living Neurons: Concentration and Dynamics. *J. Am. Chem. Soc.* **2018**, *140*, 7764−7768.
(154) Wisniewski, N.; Moussy, F.; Reichert, W. M. Characterization of Implantable Biosensor Membrane Biofouling. *Fresenius' J. Anal. Chem.* **2000**, *366*, 611−621.
(155) Polikov, V. S.; Tresco, P. A.; Reichert, W. M. Response of Brain Tissue to Chronically Implanted Neural Electrodes. *J. Neurosci. Methods* **2005**, *148*, 1−18.
(156) Frost, M.; Meyerhoff, M. E. In Vivo Chemical Sensors: Tackling Biocompatibility. *Anal. Chem.* **2006**, *78*, 7370−7377.
(157) Stroncek, J. D.; Reichert, W. M. *Indwelling Neural Implants Stratetgies for Contending with the in Vivo Environment*; CRC Press: Boca Raton, FL, USA, 2008.
(158) Burda, J. E.; Sofroniew, M. V. Reactive Gliosis and the Multicellular Response to CNS Damage and Disease. *Neuron* **2014**, *81*, 229−248.
(159) Kozai, T. D. Y.; Jaquins-Gerstl, A. S.; Vazquez, A. L.; Michael, A. C.; Cui, X. T. Brain Tissue Responses to Neural Implants Impact Signal Sensitivity and Intervention Strategies. *ACS Chem. Neurosci.* **2015**, *6*, 48−67.
(160) Salatino, J. W.; Ludwig, K. A.; Kozai, T. D. Y.; Purcell, E. K. Glial Responses to Implanted Electrodes in the Brain. *Nat. Biomed. Eng.* **2017**, *1*, 862−877.
(161) Wellman, S. M.; Kozai, T. D. Y. Understanding the Inflammatory Tissue Reaction to Brain Implants To Improve Neurochemical Sensing Performance. *ACS Chem. Neurosci.* **2017**, *8*, 2578−2582.
(162) Chatard, C.; Sabac, A.; Moreno-Velasquez, L.; Meiller, A.; Marinesco, S. Minimally Invasive Microelectrode Biosensors Based on Platinized Carbon Fibers for in Vivo Brain Monitoring. *ACS Cent. Sci.* **2018**, *4*, 1751−1760.
(163) Lecomte, A.; Descamps, E.; Bergaud, C. A Review on Mechanical Considerations for Chronically-Implanted Neural Probes. *J. Neural. Eng.* **2018**, *15*, 031001.
(164) Clark, J. J.; Sandberg, S. G.; Wanat, M. J.; Gan, J. O.; Horne, E. A.; Hart, A. S.; Akers, C. A.; Parker, J. G.; Willuhn, I.; Martinez, V.; Evans, S. B.; Stella, N.; Phillips, P. E. M. Chronic Microsensors for Longitudinal, Subsecond Dopamine Detection in Behaving Animals. *Nat. Methods* **2010**, *7*, 126−129.
(165) Schwerdt, H. N.; Shimazu, H.; Amemori, K.-i.; Amemori, S.; Tierney, P. L.; Gibson, D. J.; Hong, S.; Yoshida, T.; Langer, R.; Cima, M. J.; Graybiel, A. M. Long-Term Dopamine Neurochemical Monitoring in Primates. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 13260−13265.
(166) Rees, H. R.; Anderson, S. E.; Privman, E.; Bau, H. H.; Venton, B. J. Carbon Nanopipette Electrodes for Dopamine Detection in Drosophila. *Anal. Chem.* **2015**, *87*, 3849−3855.
(167) Yang, C.; Hu, K.; Wang, D.; Zubi, Y.; Lee, S. T.; Puthongkham, P.; Mirkin, M. V.; Venton, B. J. Cavity Carbon-Nanopipette Electrodes for Dopamine Detection. *Anal. Chem.* **2019**, *91*, 4618−4624.
(168) Du, Z. J.; Kolarcik, C. L.; Kozai, T. D. Y.; Luebben, S. D.; Sapp, S. A.; Zheng, X. S.; Nabity, J. A.; Cui, X. T. Ultrasoft Microwire Neural Electrodes Improve Chronic Tissue Integration. *Acta Biomater.* **2017**, *53*, 46−58.
(169) Yang, C.; Cao, Q.; Puthongkham, P.; Lee, S. T.; Ganesana, M.; Lavrik, N. V.; Venton, B. J. 3D-Printed Carbon Electrodes for Neurotransmitter Detection. *Angew. Chem., Int. Ed.* **2018**, *57*, 14255−14259.
(170) Capadona, J. R.; Shanmuganathan, K.; Tyler, D. J.; Rowan, S. J.; Weder, C. Stimuli-Responsive Polymer Nanocomposites Inspired by the Sea Cucumber Dermis. *Science* **2008**, *319*, 1370−1374.
(171) Mendez, J.; Annamalai, P. K.; Eichhorn, S. J.; Rusli, R.; Rowan, S. J.; Foster, E. J.; Weder, C. Bioinspired Mechanically Adaptive Polymer Nanocomposites with Water-Activated Shape-Memory Effect. *Macromolecules* **2011**, *44*, 6827−6835.
(172) Capadona, J. R.; Tyler, D. J.; Zorman, C. A.; Rowan, S. J.; Weder, C. Mechanically Adaptive Nanocomposites for Neural Interfacing. *MRS Bull.* **2012**, *37*, 581−589.
(173) Nguyen, J. K.; Park, D. J.; Skousen, J. L.; Hess-Dunning, A. E.; Tyler, D. J.; Rowan, S. J.; Weder, C.; Capadona, J. R. Mechanically-Compliant Intracortical Implants Reduce the Neuroinflammatory Response. *J. Neural. Eng.* **2014**, *11*, 056014.
(174) Hess-Dunning, A.; Tyler, D. J. A Mechanically-Adaptive Polymer Nanocomposite-Based Intracortical Probe and Package for Chronic Neural Recording. *Micromachines* **2018**, *9*, 583.
(175) Guan, S.; Wang, J.; Gu, X.; Zhao, Y.; Hou, R.; Fan, H.; Zou, L.; Gao, L.; Du, M.; Li, C.; Fang, Y. Elastocapillary Self-Assembled Neurotassels for Stable Neural Activity Recordings. *Sci. Adv.* **2019**, *5*, No. eaav2842.
(176) Fattahi, P.; Yang, G.; Kim, G.; Abidian, M. R. A Review of Organic and Inorganic Biomaterials for Neural Interfaces. *Adv. Mater.* **2014**, *26*, 1846−1885.
(177) Wellman, S. M.; Eles, J. R.; Ludwig, K. A.; Seymour, J. P.; Michelson, N. J.; McFadden, W. E.; Vazquez, A. L.; Kozai, T. D. Y. A Materials Roadmap to Functional Neural Interface Design. *Adv. Funct. Mater.* **2018**, *28*, 1701269.
(178) Feiner, R.; Dvir, T. Tissue-Electronics Interfaces: from Implantable Devices to Engineered Tissues. *Nat. Rev. Mater.* **2018**, *3*, 17076.
(179) Kim, G. H.; Kim, K.; Lee, E.; An, T.; Choi, W.; Lim, G.; Shin, J. H. Recent Progress on Microelectrodes in Neural Interfaces. *Materials* **2018**, *11*, 1995.
(180) Young, A. T.; Cornwell, N.; Daniele, M. A. Neuro-Nano Interfaces: Utilizing Nano-Coatings and Nanoparticles to Enable Next-Generation Electrophysiological Recording, Neural Stimulation, and Biochemical Modulation. *Adv. Funct. Mater.* **2018**, *28*, 1700239.
(181) Wei, Q.; Becherer, T.; Angioletti-Uberti, S.; Dzubiella, J.; Wischke, C.; Neffe, A. T.; Lendlein, A.; Ballauff, M.; Haag, R. Protein Interactions with Polymer Coatings and Biomaterials. *Angew. Chem., Int. Ed.* **2014**, *53*, 8004−8031.
(182) Harreither, W.; Trouillon, R.; Poulin, P.; Neri, W.; Ewing, A. G.; Safina, G. Carbon Nanotube Fiber Microelectrodes Show a Higher Resistance to Dopamine Fouling. *Anal. Chem.* **2013**, *85*, 7447−7453.
(183) Brocenschi, R. F.; Rocha-Filho, R. C.; Duran, B.; Swain, G. M. The Analysis of Estrogenic Compounds by Flow Injection Analysis with Amperometric Detection Using a Boron-Doped Diamond Electrode. *Talanta* **2014**, *126*, 12−19.
(184) Chandra, S.; Miller, A. D.; Bendavid, A.; Martin, P. J.; Wong, D. K. Y. Minimizing Fouling at Hydrogenated Conical-Tip Carbon Electrodes during Dopamine Detection in Vivo. *Anal. Chem.* **2014**, *86*, 2443−2450.
(185) Gui, A. L.; Luais, E.; Peterson, J. R.; Gooding, J. J. Zwitterionic Phenyl Layers: Finally, Stable, Anti-Biofouling Coatings that Do Not Passivate Electrodes. *ACS Appl. Mater. Interfaces* **2013**, *5*, 4827−4835.
(186) Sun, Q.; Yan, F.; Yao, L.; Su, B. Anti-Biofouling Isoporous Silica-Micelle Membrane Enabling Drug Detection in Human Whole Blood. *Anal. Chem.* **2016**, *88*, 8364−8368.
(187) Kang, S.; Lee, M.; Kang, M.; Noh, M.; Jeon, J.; Lee, Y.; Seo, J.-H. Development of Anti-Biofouling Interface on Hydroxyapatite Surface by Coating Zwitterionic MPC Polymer Containing Calcium-Binding Moieties to Prevent Oral Bacterial Adhesion. *Acta Biomater.* **2016**, *40*, 70−77.
(188) Feng, T.; Ji, W.; Tang, A.; Wei, H.; Zhang, S.; Mao, J.; Zhang, Y.; Mao, L.; Zhang, M. Low-Fouling Nanoporous Conductive Polymer-Coated Microelectrode for In Vivo Monitoring of Dopamine in the Rat Brain. *Anal. Chem.* **2019**, *91*, 10786−10791.

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

(189) Liu, X.; Zhang, M.; Xiao, T.; Hao, J.; Li, R.; Mao, L. Protein Pretreatment of Microelectrodes Enables in Vivo Electrochemical Measurements with Easy Precalibration and Interference-Free from Proteins. *Anal. Chem.* **2016**, *88*, 7238−7244.
(190) Kesavan, S.; John, S. A. Simultaneous Determination of Neurotransmitters and a Neuroprotector in Human Blood Serum and Urine Samples Using a Diazonium Grafted Gold Nanoparticle Film Electrode. *Anal. Methods* **2014**, *6*, 9022−9029.
(191) Cassar, I. R.; Yu, C.; Sambangi, J.; Lee, C. D.; Whalen, J. J., III; Petrossians, A.; Grill, W. M. Electrodeposited Platinum-Iridium Coating Improves In Vivo Recording Performance of Chronically Implanted Microelectrode Arrays. *Biomaterials* **2019**, *205*, 120−132.
(192) Shin, D.-S.; Matharu, Z.; You, J.; Siltanen, C.; Tam, V.; Raghunathan, V. K.; Stybayeva, G.; Hill, A. E.; Revzin, A. Sensing Conductive Hydrogels for Rapid Detection of Cytokines in Blood. *Adv. Healthcare Mater.* **2016**, *5*, 659−664.
(193) Chelmowski, R.; Koester, S. D.; Kerstan, A.; Prekelt, A.; Grunwald, C.; Winkler, T.; Metzler-Nolte, N.; Terfort, A.; Woell, C. Peptide-Based SAMs that Resist the Adsorption of Proteins. *J. Am. Chem. Soc.* **2008**, *130*, 14952−14953.
(194) Walker, J. A.; Robinson, K. J.; Munro, C.; Gengenbach, T.; Muller, D. A.; Young, P. R.; Lua, L. H. L.; Corrie, S. R. Antibody-Binding, Antifouling Surface Coatings Based on Recombinant Expression of Zwitterionic EK Peptides. *Langmuir* **2019**, *35*, 1266−1272.
(195) Liu, N. Z.; Xu, Z. Y.; Morrin, A.; Luo, X. L. Low Fouling Strategies for Electrochemical Biosensors Targeting Disease Biomarkers. *Anal. Methods* **2019**, *11*, 702−711.
(196) Wang, B.; Yang, P.; Ding, Y.; Qi, H.; Gao, Q.; Zhang, C. Improvement of the Biocompatibility and Potential Stability of Chronically Implanted Electrodes Incorporating Coating Cell Membranes. *ACS Appl. Mater. Interfaces* **2019**, *11*, 8807−8817.
(197) Patel, J.; Radhakrishnan, L.; Zhao, B.; Uppalapati, B.; Daniels, R. C.; Ward, K. R.; Collinson, M. M. Electrochemical Properties of Nanostructured Porous Gold Electrodes in Biofouling Solutions. *Anal. Chem.* **2013**, *85*, 11610−11618.
(198) Cui, M.; Wang, Y.; Jiao, M.; Jayachandran, S.; Wu, Y.; Fan, X.; Luo, X. Mixed Self-Assembled Aptamer and Newly Designed Zwitterionic Peptide as Antifouling Biosensing Interface for Electrochemical Detection of alpha-Fetoprotein. *ACS Sens.* **2017**, *2*, 490−494.
(199) Dubin, R. A.; Callegari, G. C.; Kohn, J.; Neimark, A. V. Carbon Nanotube Fibers are Compatible with Mammalian Cells and Neurons. *IEEE Trans. Nanobiosci.* **2008**, *7*, 11−14.
(200) Liu, X.; Xiao, T.; Wu, F.; Shen, M.; Zhang, M.; Yu, H.; Mao, L. Ultrathin Cell-Membrane-Mimic Phosphorylcholine Polymer Film Coating Enables Large Improvements for InVivo Electrochemical Detection. *Angew. Chem., Int. Ed.* **2017**, *56*, 11802−11806.
(201) Zhou, L.; Hou, H.; Wei, H.; Yao, L.; Sun, L.; Yu, P.; Su, B.; Mao, L. In Vivo Monitoring of Oxygen in Rat Brain by Carbon Fiber Microelectrode Modified with Antifouling Nanoporous Membrane. *Anal. Chem.* **2019**, *91*, 3645−3651.
(202) Anselme, K.; Davidson, P.; Popa, A. M.; Giazzon, M.; Liley, M.; Ploux, L. The Interaction of Cells and Bacteria with Surfaces Structured at the Nanometre Scale. *Acta Biomater.* **2010**, *6*, 3824−3846.
(203) Ereifej, E. S.; Smith, C. S.; Meade, S. M.; Chen, K.; Feng, H.; Capadona, J. R. The Neuroinflammatory Response to Nanopatterning Parallel Grooves into the Surface Structure of Intracortical Microelectrodes. *Adv. Funct. Mater.* **2018**, *28*, 1704420.
(204) Kim, Y.; Meade, S. M.; Chen, K.; Feng, H.; Rayyan, J.; Hess-Dunning, A.; Ereifej, E. S. Nano-Architectural Approaches for Improved Intracortical Interface Technologies. *Front. Neurosci.* **2018**, *12*, 456.
(205) Cheng, Y.; Feng, G.; Moraru, C. I. Micro- and Nanotopography Sensitive Bacterial Attachment Mechanisms: A Review. *Front. Microbiol.* **2019**, *10*, 1−17.
(206) Chen, Y. C.; Lee, D. C.; Tsai, T. Y.; Hsiao, C. Y.; Liu, J. W.; Kao, C. Y.; Lin, H. K.; Chen, H. C.; Palathinkal, T. J.; Pong, W. F.; Tai, N. H.; Lin, I. N.; Chiu, I. M. Induction and Regulation of Differentiation in Neural Stem Cells on Ultra-Nanocrystalline Diamond Films. *Biomaterials* **2010**, *31*, 5575−5587.
(207) Zang, D.; Yi, H.; Gu, Z.; Chen, L.; Han, D.; Guo, X.; Wang, S.; Liu, M.; Jiang, L. Interfacial Engineering of Hierarchically Porous NiTi/Hydrogels Nanocomposites with Exceptional Antibiofouling Surfaces. *Adv. Mater.* **2017**, *29*, 1602869.
(208) Zhang, P.; Lin, L.; Zang, D.; Guo, X.; Liu, M. Designing Bioinspired Anti-Biofouling Surfaces based on a Superwettability Strategy. *Small* **2017**, *13*, 1503334.
(209) Roberts, J. G.; Toups, J. V.; Eyualem, E.; McCarty, G. S.; Sombers, L. A. In Situ Electrode Calibration Strategy for Voltammetric Measurements In Vivo. *Anal. Chem.* **2013**, *85*, 11568−11575.
(210) Meunier, C. J.; Mitchell, E. C.; Roberts, J. G.; Toups, J. V.; McCarty, G. S.; Sombers, L. A. Electrochemical Selectivity Achieved Using a Double Voltammetric Waveform and Partial Least Squares Regression: Differentiating Endogenous Hydrogen Peroxide Fluctuations from Shifts in pH. *Anal. Chem.* **2018**, *90*, 1767−1776.
(211) Schuweiler, D. R.; Howard, C. D.; Ramsson, E. S.; Garris, P. A. Improving in Situ Electrode Calibration with Principal Component Regression for Fast-Scan Cyclic Voltammetry. *Anal. Chem.* **2018**, *90*, 13434−13442.
(212) Meunier, C. J.; Roberts, J. G.; McCarty, G. S.; Sombers, L. A. Background Signal as an in Situ Predictor of Dopamine Oxidation Potential: Improving Interpretation of Fast-Scan Cyclic Voltammetry Data. *ACS Chem. Neurosci.* **2017**, *8*, 411−419.
(213) Xiao, G.; Song, Y.; Zhang, Y.; Xing, Y.; Zhao, H.; Xie, J.; Xu, S.; Gao, F.; Wang, M.; Xing, G.; Cai, X. Microelectrode Arrays Modified with Nanocomposites for Monitoring Dopamine and Spike Firings under Deep Brain Stimulation in Rat Models of Parkinson's Disease. *ACS Sens.* **2019**, *4*, 1992−2000.
(214) Dash, M. B.; Douglas, C. L.; Vyazovskiy, V. V.; Cirelli, C.; Tononi, G. Long-Term Homeostasis of Extracellular Glutamate in the Rat Cerebral Cortex across Sleep and Waking States. *J. Neurosci.* **2009**, *29*, 580−589.
(215) Owesson-White, C. A.; Ariansen, J.; Stuber, G. D.; Cleaveland, N. A.; Cheer, J. F.; Wightman, R. M.; Carelli, R. M. Neural Encoding of Cocaine-Seeking Behavior is Coincident with Phasic Dopamine Release in the Accumbens Core and Shell. *Eur. J. Neurosci.* **2009**, *30*, 1117−1127.
(216) Belle, A. M.; Owesson-White, C.; Herr, N. R.; Carelli, R. M.; Wightman, R. M. Controlled Iontophoresis Coupled with Fast-Scan Cyclic Voltammetry/Electrophysiology in Awake, Freely Moving Animals. *ACS Chem. Neurosci.* **2013**, *4*, 761−771.
(217) Hefti, F.; Felix, D. Chronoamperometry In Vivo - Does It Interfere with Spontaneous Neuronal-Activity in the Brain. *J. Neurosci. Methods* **1983**, *7*, 151−156.
(218) Crespi, F.; England, T.; Ratti, E.; Trist, D. G. Carbon-Fiber Microelectrodes for Concomitant In Vivo Electrophysiological and Voltammetric Measurements - No Reciprocal Influences. *Neurosci. Lett.* **1995**, *188*, 33−36.
(219) Sreenivas, G.; Ang, S. S.; Fritsch, I.; Brown, W. D.; Gerhardt, G. A.; Woodward, D. J. Fabrication and Characterization of Sputtered-Carbon Microelectrode Arrays. *Anal. Chem.* **1996**, *68*, 1858−1864.
(220) Johnson, M. D.; Franklin, R. K.; Scott, K. A.; Brown, R. B.; Kipke, D. R. Neural Probes for Concurrent Detection of Neurochemical and Electrophysiological Signals In Vivo. *In 2005 27th Annual International Conference of the IEEE Engineering in Medicine and Biology Society* **2006**, 7325−7328.
(221) Johnson, M. D.; Franklin, R. K.; Gibson, M. D.; Brown, R. B.; Kipke, D. R. Implantable Microelectrode Arrays for Simultaneous Electrophysiological and Neurochemical Recordings. *J. Neurosci. Methods* **2008**, *174*, 62−70.
(222) Takmakov, P.; McKinney, C. J.; Carelli, R. M.; Wightman, R. M. Instrumentation for Fast-Scan Cyclic Voltammetry Combined with Electrophysiology for Behavioral Experiments in Freely Moving Animals. *Rev. Sci. Instrum.* **2011**, *82*, 074302.

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118

(223) Stamford, J. A.; Palij, P.; Davidson, C.; Jorm, C. M.; Millar, J. Simultaneous Real-Time Electrochemical and Electrophysiological Recording in Brain-Slices with a Single Carbon-Fiber Microelectrode. *J. Neurosci. Methods* **1993**, *50*, 279−290.
(224) Disney, A. A.; McKinney, C.; Grissom, L.; Lu, X.; Reynolds, J. H. A Multi-Site Array for Combined Local Electrochemistry and Electrophysiology in the Non-Human Primate Brain. *J. Neurosci. Methods* **2015**, *255*, 29−37.
(225) Zhang, H.; Lin, S.; Nicolelis, M. A. L. Acquiring Local Field Potential Information from Amperometric Neurochemical Recordings. *J. Neurosci. Methods* **2009**, *179*, 191−200.
(226) Zhang, H.; Lin, S.; Nicolelis, M. A. L. Spatiotemporal Coupling between Hippocampal Acetylcholine Release and Theta Oscillations In Vivo. *J. Neurosci.* **2010**, *30*, 13431−13440.
(227) Viggiano, A.; Marinesco, S.; Pain, F.; Meiller, A.; Gurden, H. Reconstruction of Field Excitatory Post-Synaptic Potentials in the Dentate Gyrus from Amperometric Biosensor Signals. *J. Neurosci. Methods* **2012**, *206*, 1−6.
(228) Ledo, A.; Lourenco, C. F.; Laranjinha, J.; Brett, C. M. A.; Gerhardt, G. A.; Barbosa, R. M. Ceramic-Based Multisite Platinum Microelectrode Arrays: Morphological Characteristics and Electrochemical Performance for Extracellular Oxygen Measurements in Brain Tissue. *Anal. Chem.* **2017**, *89*, 1674−1683.
(229) Ledo, A.; Lourenco, C. F.; Laranjinha, J.; Gerhardt, G. A.; Barbosa, R. M. Combined In Vivo Amperometric Oximetry and Electrophysiology in a Single Sensor: A Tool for Epilepsy Research. *Anal. Chem.* **2017**, *89*, 12383−12390.
(230) Wu, F.; Cheng, H.; Wei, H.; Xiong, T.; Yu, P.; Mao, L. Galvanic Redox Potentiometry for Self-Driven in Vivo Measurement of Neurochemical Dynamics at Open-Circuit Potential. *Anal. Chem.* **2018**, *90*, 13021−13029.
(231) Xiong, T.; Zhang, K.; Jiang, Y.; Yu, P.; Mao, L. Ion Current Rectification: from Nanoscale to Microscale. *Sci. China: Chem.* **2019**, *62*, 1346−1359.

DOI: 10.1021/acssensors.9b01713
ACS Sens. 2019, 4, 3102−3118