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Perspective

Recent Advances in the Development and Characterization of Electrochemical and Electrical Biosensors for Small Molecule Neurotransmitters

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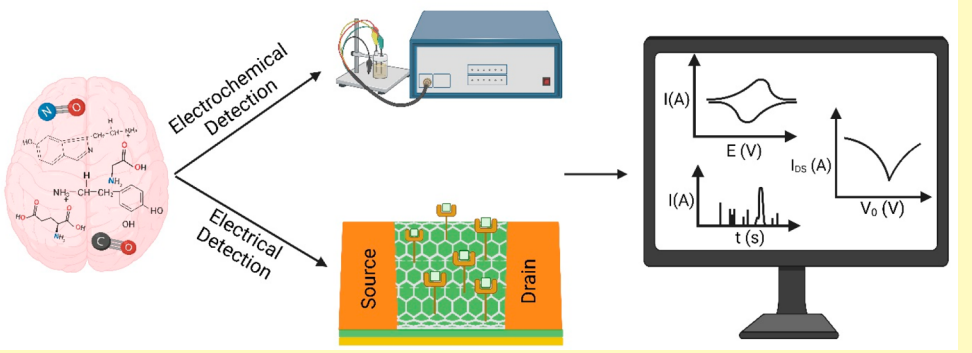

ABSTRACT: Neurotransmitters act as chemical messengers, determining human physiological and psychological function, and abnormal levels of neurotransmitters are related to conditions such as Parkinson's and Alzheimer's disease. Biologically and clinically relevant concentrations of neurotransmitters are usually very low (nM), so electrochemical and electronic sensors for neurotransmitter detection play an important role in achieving sensitive and selective detection. Additionally, these sensors have the distinct advantage to potentially be wireless, miniaturized, and multichannel, providing remarkable opportunities for implantable, long-term sensing capabilities unachievable by spectroscopic or chromatographic detection methods. In this article, we will focus on advances in the development and characterization of electrochemical and electronic sensors for neurotransmitters during the last five years, identifying how the field is progressing as well as critical knowledge gaps for sensor researchers.

KEYWORDS: *electrochemistry, biosensors, biorecognition, neurotransmitters, small molecules*

Neurotransmitters are chemicals released by neurons, glial cells, and platelets that play an important role in cell communication. There are more than a hundred kinds of neurotransmitters including amino acids (e.g., glutamate, GABA, glycine), monoamines (e.g., dopamine, histamine, serotonin), peptides (e.g., oxytocin), and gasotransmitters (e.g., NO, CO). The levels of neurotransmitters in humans are related to many diseases and disorders; for example, an abnormal level of dopamine can influence brain function and cause symptoms of Parkinson's or attention deficit hyperactivity disorder (ADHD).[1] Serotonin acts as an important biomarker for a variety of diseases such as diabetes and cancer.[2−4] Therefore, it is very important to detect and monitor any imbalance in neurotransmitters.

Because many biological processes and diseases are influenced by neurotransmitters, several analytical techniques have been used to detect and quantify them, such as chromatography, mass spectrometry (MS), fluorescence, surface-enhanced Raman spectroscopy (SERS), and electrochemical and electronic detection. Previously, several reviews[5,6] have been published that summarized the advances in detection methods for different neurotransmitters including the use of positron emission tomography (PET) and genetically encoded fluorescent sensors for in vivo imaging.[7,8] Although these reviews showcase the wide range of advancements in neurotransmitter detection technology, few have focused specifically on advancements in electronic and electrochemical sensors. Herein electronic sensors are defined as devices that detect the analyte of interest through a change in voltage or current without changing the analyte oxidation state (e.g., FETs), and electrochemical sensors are defined as systems of electrodes that respond to a change in

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redox activity due to the interaction between the analyte of interest and the electrode membrane; both types can utilize biorecognition elements. These categories of sensors are particularly advantageous due to their (1) cost-effectiveness, (2) mass-producibility, (3) ease of operation, (4) good temporal resolution (with μs detection capabilities), (5) wireless capabilities, and (6) good spatial resolution (with single-cell, single vesicle, and even intracellular measurements).[9,10]

To fill this literature gap, this perspective focuses on progress made within the last five years in the electronic and electrochemical detection of neurochemicals, including monoamines and amino acids. Due to the size and impact of this field, it is not possible to include all advances; however, the aim of this perspective is to provide a succinct overview of advances in sensor design published in high impact journals, such as ACS and RSC journals, and to identify major research gaps that the field should focus on in the future. Typical electrochemical and electronic detection systems often contain biorecognition elements, a signal transducer, and a mechanism for signal detection. Electroactive neurotransmitters can be reduced/oxidized directly on an electrode surface at specific potentials, producing a current or potential change, allowing for their quantitative detection. For nonelectroactive analyte detection, a biorecognition element is typically used for their capture. Advances in biorecognition elements for neurotransmitters (including antibodies, aptamers, enzymes, and polymers) and three affinity characterization methods are summarized herein. Improvements in sensor development are considered, including: (a) field effect transducer (FET) multiplex sensing; (b) stretchable/flexible electrodes, (c) carbon-fiber microelectrode (CFME) multiplex sensing; (d) antifouling strategies; (e) nanopipette and nanowire electrodes; and (f) microelectrode arrays. In each section, we present an overview of research progress from the past five years and elaborate on their novelty and importance. A brief review of current challenges and outlooks in electronic and electrochemical detection of neurotransmitters is provided to conclude.

BIORECOGNITION

It is impossible to directly detect the reduction/oxidation signal from neurotransmitters that do not undergo these processes within a potential window relevant to physiological conditions. Biorecognition using aptamers, antibodies, enzymes, or polymers has been widely employed to capture target analytes with high specificity and emit a measurable signal. Characterizing binding affinity between an affinity agent and a target molecule is essential to subsequently design a sensitive and selective sensor. In this section, we introduce common biorecognition elements and summarize three binding affinity characterization methods including surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), and fluorescence, which are commonly used for neurotransmitters and their biorecognition elements, relevant to many sensing platforms beyond those discussed here.

Biorecognition Elements. Biorecognition elements are essential parts of biosensors; they are used to provide bioanalyte specificity and are coupled to a transducer to form a biosensor.[11] The primary types of biorecognition elements for neurotransmitters are enzymes, antibodies, aptamers, and polymers. When considering the detection of small organic molecules like neurotransmitters, the ability to distinguish between targets with similar structures, sizes, and functional groups remains a challenging yet crucial aspect of biosensing. Though some neurotransmitters are intrinsically electroactive and can thus be directly detected using electrochemical methods,[12−14] biorecognition elements are necessary to detect those neurotransmitters which do not have electroactive properties in physiologically relevant conditions. Additionally, it is important to design a detection scheme that is effective at the biologically relevant concentration of an analyte in a complex sample matrix; this relevant concentration is generally at the nanomolar level for neurotransmitters.[15] Thus, the utility of biorecognition elements is apparent in lowering the limit of detection by exploiting biological models of specificity through structural and chemical interactions.

From the development of the first enzymatic biosensor in 1962 by Clark and Lyons,[16] use of biorecognition elements in sensing has been widely clinically adopted and commercialized for purposes such as continuous glucose monitoring. While glucose is not a neurotransmitter, it set an important precedent in biosensing. Enzyme−ligand interactions allow for specific detection of the species by enzyme-coupled electrochemical biosensors.[17−19] Enzymes target species with complementary binding sites and may subsequently catalyze a reaction (e.g., redox) for detection. Careful design for the immobilization of enzymes while maintaining their biological activity is essential to enzymatic biosensor development and innovation. Boulmedais and colleagues[20] used a new immobilization strategy of ferrocene methanol-facilitated electrodeposition of glucose oxidase and cross-linker on a gold electrode to detect glucose at millimolar levels by amperometry. Fenoy et al.[21] utilized a copolymer in the immobilization of the acetylcholine-specific enzyme for their graphene-based FET and were able to obtain a reproducible and selective sensor with improved pH sensitivity and an LOD of 2.3 μM. These novel immobilization methods will lend themselves to miniaturization of sensors through targeted functionalization or improved sensitivity, which is a clear direction for the field. Advantages of using enzymes include their chemical stability and regeneration. Enzymes may also be applied to complex systems by introducing multiple enzymes in conjunction to develop a multiplex sensing platform.[22]

Another protein-based and biologically abundant source for potential biorecognition elements is antibodies.[23−25] Very high specificity can be achieved by exploiting the paratope−epitope interface for antigen targets. However, due to the size of antibodies, especially as compared to neurotransmitters, alternatives such as antibody fragments, nanobodies, and short peptide mimetics may be more conducive to sensing on the nanoscale.[26] As more components of biosensing are being miniaturized or are utilizing nanotechnology, full antibodies being in this same size range presents a problem for the arrangement of these biorecognition elements on sensor surfaces. Additionally, artificial antibodies can be engineered to have better binding affinity and stability compared to their natural counterparts. Although antibodies are important in the field of biosensing and have been used, for example, to detect protein biomarkers via electrochemical impedance spectroscopy,[27] they have gained little popularity in the electronic detection of neurotransmitters, typically due to size constraints. Their miniaturized counterparts also have yet to be widely adopted as a preferred method for neurotransmitter detection and have been used primarily in other fields of biosensing (i.e., for other bioanalytes) but may hold promise for future applications.[28−30]

Though protein-based biorecognition platforms have their advantages, nucleic acid-based sensing is on the rise,[31] especially

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Table 1. Summary of Recent Literature Utilizing Biorecognition Elements for the Electrochemical Detection of Neurotransmitters[a]

Biorecognition element	Analyte	K_d (M)	LOD (M)	Technique	ref
antibody	serotonin	[b]	3.2×10^{-9}	DPV	23
	GABA	[b]	9.8×10^{-8}	FET	24
	histamine	[b]	3.0×10^{-9}	EIS	25
aptamer	dopamine	1.0×10^{-6}	6.7×10^{-8}	Amperometry	63
	dopamine	1.5×10^{-7}	1.0×10^{-14}	FET	34
	dopamine	5.0×10^{-9}	1.0×10^{-10}	CV, EIS	31
	serotonin	3.0×10^{-8}	1.0×10^{-11}	FET	34
	serotonin	[b]	$\sim 10^{-15}$	FET	49
enzyme	epinephrine	4.0×10^{-8}	8.3×10^{-8}	CV	60
	epinephrine	[b]	6.0×10^{-8}	Amperometry	61
	acetylcholine	[b]	1.0×10^{-9}	FET	17
	acetylcholine	7.4×10^{-5}	2.3×10^{-6}	FET	21
	dopamine	5.4×10^{-5}	3.0×10^{-12}	CV	18
	dopamine	9.0×10^{-6}	4.3×10^{-7}	DPV	19
	glutamate	[b]	[b]	Amperometry	66

[a]For neurotransmitters listed multiple times under the same biorecognition category, the element used is not necessarily identical. [b]Signifies that information was not given in the article.

due to their superior stability. Aptamers, which are single-stranded DNA or RNA, are generally chosen based on their analyte specificity through the sequential evolution of ligands by exponential enrichment (SELEX) process. They lend their selectivity to biosensors based on a combination of intermolecular interactions and resultant conformational changes.[26] Xu et al.[32] demonstrated that once the appropriate aptamer has been determined, which is often via database, it can be simply incorporated into a sensor. The incorporation can be achieved through thiolation, taking advantage of gold−sulfur bond strength for attachment to an electrode or chip for electrochemical impedance spectroscopy detection to characterize the reproducibility of aptamer-functionalized sensors. Aptamer-facilitated detection is on the frontiers of neurotransmitter sensing, as there are significant demands to detect neurotransmitters at extremely low concentrations. Zhao et al.[33] have demonstrated that field-effect transistors (FETs) can detect serotonin at a femtomolar level via an implantable detection scheme, which can be operated in mouse brain tissue. Nakatsuka et al.[34] have recently shown that aptamer FETs can be used to detect small molecules such as dopamine and serotonin based on their conformational changes in the challenging, yet biologically relevant, matrices of high ionic strength solutions, thereby circumventing the fundamental Debye limitation. Despite their high stability, selectivity, and binding affinity, aptamers cannot be used for every application; it is not always possible to design an appropriate aptamer, and the sensor may not be robust in the presence of nucleases, which often exist in real-world samples.[26] Luckily, some progress has been made in developing methods for designing nuclease-resistant aptamers, which will be especially important for future in vivo applications.[35]

The final classification of biorecognition elements to be discussed is polymers. Similar to the aforementioned affinity agents, polymers may be derived from or include naturally occurring molecules. Polymers have not been broadly exploited in the field of electronic biosensing; however, robust detection schemes for small biomolecules using optical sensing platforms have been developed with polymer affinity agents.[36] Unlike antibodies, enzymes, and aptamers, polymers tend to be much less specific to their target analyte, which can allow for multiplex detection, though can make differentiation of different analytes difficult.[37] The principles of these polymer-based sensors are transferrable to electronic sensing methods and lead to a promising new direction for neurotransmitter detection. Though not a true biorecognition element, conducting polymers have shown utility in electrochemical sensing of neurotransmitters.[38−40] Examples of biorecognition elements used in electrochemical or electronic sensors for neurotransmitters are given in Table 1, along with the corresponding LOD and K_d when available.

As the field moves toward in vivo sensor design, the biocompatibility of these biorecognition elements will be increasingly important. Though most of the discussed ligands are naturally derived, studying cytotoxicity, immunogenicity, and other concomitant effects will be an essential step in sensor development, especially for synthetic polymers. Chemical stability and sensor surface reusability for long-term implantation will become equally important figures of merit to the desired selectivity and sensitivity. Added complexity notwithstanding, each biorecognition element category possesses its own advantages and disadvantages for consideration. Progress has been made toward implantable electronic biosensing using enzyme-,[41] antibody-,[42] and aptamer-based sensors,[43] but has yet to be demonstrated for most analytes of interest, leaving a gap in the field that is ripe for development.

Characterization of Binding Affinity. After determining the appropriate biorecognition element for the analyte in question, it is essential to characterize both its incorporation into the sensor and its interaction with the neurotransmitter with one of many techniques. Fluorescence quenching is a common and relatively simple technique for determining dissociation constants.[44,45] It is used when the biorecognition element is fluorescent or can be fluorescently labeled, and binding to the analyte causes emission quenching. This property can be exploited to evaluate the relative amount of binding at different analyte concentrations through changes in fluorescence intensity.[46] The resulting data can be fitted to a binding model to find the K_d.[47,48] This technique was used by Nakatsuka et al.[34] and by Wang et al.[49] for neurotransmitter−aptamer interactions.

Alternatively, isothermal titration calorimetry (ITC) can be used to investigate the thermodynamics of an interaction.[50]

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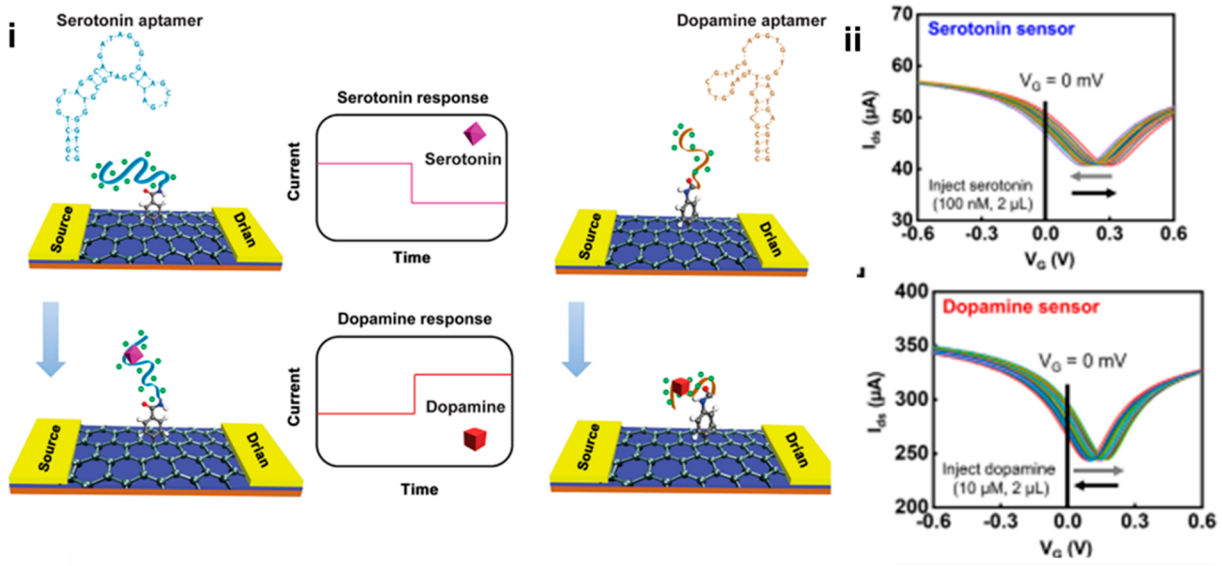

Figure 1. Multiplex detection with FETs. G-FET based serotonin and dopamine dual detection. (i) Schematic illustration of the multiplex detection principle. When target serotonin and dopamine analytes bind to the aptamers, the conformation of the aptamers will change, inducing a change in the source−drain current. (ii) Continuous monitoring of transfer curves when injecting serotonin and dopamine solutions. Adapted with permission from ref 70, Copyright 2022 American Chemical Society.

Sample and reference cells are kept at the same temperature while a small amount of one partner in the interaction is repeatedly injected into the sample cell containing the other interaction partner. The required flow of heat into or out of the sample cell relative to the reference cell to maintain a steady temperature can be used to find the change in enthalpy and free energy as well as the dissociation constant for the interaction.[47,48,51,52] This technique is highly sensitive but requires very accurate concentrations of the interactants. Micro-ITC instruments are also now commercially available, which allow for much lower sample consumption and required concentrations than traditional ITC. Liu et al.[53] used ITC to determine the affinity between dopamine and an aptamer as well as to evaluate aptamer selectivity. ITC and fluorescence have also been increasingly used in tandem to improve confidence in results.[47,48,53]

While fluorescence and ITC characterize interactions in solution, surface plasmon resonance (SPR) is able to more closely replicate the surface-bound conditions in most sensors.[54,55] Most commonly, a biorecognition element will be immobilized on the metal surface, and the analyte is flowed over the surface to monitor signal changes which can reveal the interaction. Signals are measured by shining monochromatic light onto the back of the metal surface, where it is totally internally reflected, and collecting the light reflected back at different angles. At a certain angle which acts as the measured quantity, the collected light intensity is lowered due to absorption by a surface plasmon in the metal, and this angle is dependent on the solution refractive index. When the target analyte binds to the biorecognition element, the refractive index of the solution near the surface changes, thus causing a change in signal. SPR suffers from low intensity signals for small analytes like most neurotransmitters since changes in solution refractive index are proportional to the increase in mass, though some progress has been made.[56−58] Zeynaloo et al.[59] used SPR to characterize the affinity between glutamate and a glutamate-binding protein for use in a cyclic voltammetry sensor by using a very sensitive instrument and optimizing buffer conditions.

Often, sensors are developed using biorecognition elements such as enzymes that are characterized with the previously discussed methods or others.[60−62] However, ensuring that the elements retain binding activity during sensing can be difficult, and these prior characterizations do not account for changes due to the sensing environment. To address this problem, it is possible to characterize binding affinity from measurements made with electrochemical sensors if the signal can be fit to a binding model. One such example is from Park et al.,[39] where FET signal responses were used to find an equilibrium binding constant (K_d) of 2 fM between dopamine and a dopamine-receptor-modified membrane, allowing for an extremely low LOD of 100 fM. Álvarez-Martos et al.[63] similarly used chronoamperometry signals to find a K_d for dopamine and an RNA aptamer in PBS buffer and in human serum samples, which explained some of the difference in LOD between sensing in buffer (67 nM) and serum (114 nM). This characterization method is becoming more common,[21,64,65] as it allows for the study of binding in the same environment as detection, making results more relevant to development of the sensor.

ELECTRONIC AND ELECTROCHEMICAL BIOSENSORS

Field Effect Transistor (FET) Multiplex Sensing. The first field effect transistor-based (FET) biosensor was invented by Piet Bergveld in the 1970s for electrochemical and biological application.[67] The basic principles of FETs are well summarized in previous literature.[84,85] The advantages of FET-based sensors include low cost of mass production, large dynamic range, quick response to signal, and easy integration with microfluidic systems and large-scale circuitry. The organic electronic material-based FETs show great biocompatibility and flexible shapes, making them innovative candidates for implantable devices.[68] Many different types of nanomaterials are used as the conducting layers of FET biosensors, such as silicon nanowires (SiNWs), graphene, metal oxides, and carbon nanotubes (CNTs). Some recent, innovative examples include a reduced-

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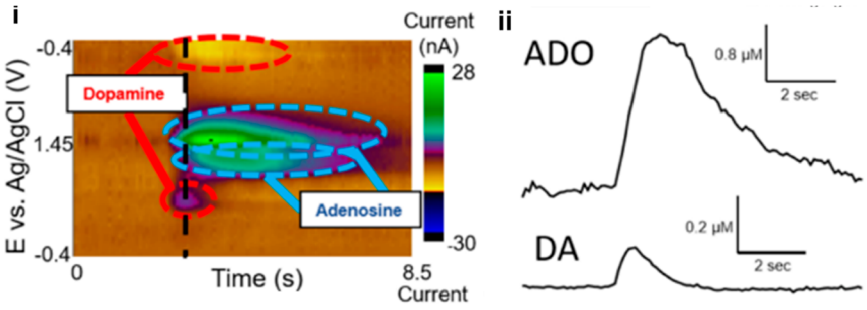

Figure 2. CFME multiplex sensing. (i) Representative false-color plot of adenosine and dopamine codetection. The dopamine oxidation peak is different from adenosine's primary and secondary oxidation peaks. (ii) Amperometric trace of adenosine and dopamine detection. Adapted with permission from ref 86, Copyright 2021 American Chemical Society.

graphene-oxide FET-based biosensor for the detection of acetylcholine through immobilized acetylcholinesterase by Fenoy et al.[18] Park et al.[39] developed aptamer-conjugated carboxylated polypyrrole nanotubes (CPNTs) for exogenous dopamine detection from PC12 cells. Zhao et al.[33] developed aptamer-modified In_2O_3-based FET neuroprobes for in vivo serotonin detection in mouse brain tissue. Dopamine was also detected by Kim et al.[68] using ultrathin In_2O_3 films.

FETs are one of the most-used methods for spatial multiplexing, a major area of interest for biosensors.[69] The common challenges for spatial multiplexing are that (1) each sensing unit needs to have suitable selectivity toward specific chemicals and (2) each analyte's signal needs to be identifiable to enable simultaneous measurements. FETs are overcoming these challenges by being modified with different biorecognition elements on their conducting channels separately, allowing for each transistor to record information for a single analyte, achieving multiplex detection. For example, Gao et al.[70] functionalized serotonin and dopamine aptamers separately on two side-by-side graphene FETs (Figure 1). The two aptamers have different conformational changes upon binding, causing different effects on the graphene-conducting channel and shifts of transfer curves. They subsequently used this dual gate graphene FET soft probe to detect serotonin and dopamine in murine brain tissues ex vivo. Liu et al.[71] also demonstrated the simultaneous detection of serotonin and dopamine through different resulting current shift directions which originated from different aptamer conformational changes. Multiplex sensing enables the detection of multiple targets at the same time and helps with advancing our understanding of the brain and other biological systems which usually involves multiple neurotransmitters being released at the same time.

Stretchable/Flexible Electrodes. Recent advances in nanomaterials, microfabrication, bioengineering, and measurement methods have built the foundation for flexible/stretchable biosensor innovations. Perhaps the most obvious advancement over the last few decades, scientists have been devoted to developing sensors that can perform in more realistic, in vivo environments. Stretchable electrodes are well-suited for detection in tissues and organs which undergo movement (e.g., heart beating, blood flow, etc.). To fabricate a stretchable electrode/biosensor, one of two strategies are typically employed: adapting special geometric structures like mesh,[72] waves/wrinkles,[73] serpentine shapes,[74] or using new nanomaterials like nanostructured gold/PDMS films,[75] PEDOT-coated carbon nanotubes,[76] et cetera. The fabrication and application of the stretchable sensors have been well-summarized in previous literature.[77−79] The most common stretchable sensors are wearable electrodes for glucose detection.[79]

One challenge in developing biocompatible stretchable sensors is the material's resistance to tensile stress during the stretch process, impacting measurement stability.[77] To overcome this challenge, Liu et al.[77] fabricated a stretchable sensor based on gold nanotubes, TiO_2 nanoparticles, and carbon nanotubes, which have great antifouling and decontamination properties. The stretchable electrochemical sensor achieved dynamic serotonin concentration detection after being inserted into rats' ileum segments, revealing that the serotonin overflow was correlated with intestinal peristalsis. Recently, Li et al.[80] developed a "NeuroString" stretchable biosensor, consisting of a graphene/iron oxide nanoparticle network within an elastomer. The NeuroString sensor is as soft as biological tissues. Because of the advantageous tissue-mimicking structure, softness, and stretchability, the NeuroString was used to monitor neurotransmitter dynamics in vivo in mouse brains as well as intestinal tissues. These sensors achieved multichannel and multiplexed sensing of monoamines, including dopamine, serotonin, norepinephrine, and epinephrine with detection limits of 5.6 nM, 7.2 nM, 3.5 nM, and 6.6 nM, respectively. The high sensitivity of these sensors, coupled with recent advancements in achievable tensile strength, make flexible sensors a promising solution for implantable and wearable technology. Future efforts to modify flexible sensors with biorecognition elements will continue to push this promising sensor field forward.

Carbon-Fiber Microelectrode Multiplex Sensing. Electrochemical techniques provide good sensitivity, specificity, and temporal resolution, yielding quantitative insight on neurotransmitter levels. Traditional electrochemical neurotransmitter detection methods such as cyclic voltammetry (CV), differential pulse voltammetry (DPV), fast scan cyclic voltammetry (FSCV), and amperometry have remained popular over the last five years. Major developments in this field have primarily been due to innovative electrode modifications over advancements in detection methodology. Thus, this section focuses on innovative electrode modifications that have filled gaps in neurotransmitter selectivity and sensitivity.

Carbon-fiber microelectrodes (CFMEs) are favored as an electrochemical biosensor due to their sub-10 μm size and surface composition.[81] The carbon-fiber surface has numerous oxygen-containing functional groups that have high affinity for cationic neurotransmitter adsorption, allowing CFMEs to achieve detection limits down to nM levels. Additionally, the small size of the electrodes contributes to high temporal resolution as the smaller the electrode, the less current signal it detects; therefore, CFMEs can handle higher capacitive currents

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that come with improved temporal resolution from faster scan rates. The currents produced at these microelectrodes are extremely small and can be detected in highly resistive solutions. At slow scan rates, radial diffusion effects are observed at these electrodes; therefore it is common to use techniques with faster scan rates, like FSCV, or techniques with no potential scan, like amperometry.[81−84]

Within the last five years, FSCV using bare CFMEs with disk and cylindrical shapes remains common for neurotransmitter detection (Figure 2).[69,71,73] Although there are many examples of researchers detecting only one neurotransmitter at a time,[12,14,39,40,65,85] major advancements have been achieved in multiplex sensing, a historically challenging problem, especially for in vivo measurements. Upon stimulation, neurons and other neurotransmitter-containing cells release multiple molecules of interest. Multiplex detection of neurotransmitters can reduce cost, time, and reagent consumption by achieving one-time detection of multiple species with a smaller sample size. This, in turn, yields important insight about coincident biological processes that continues to drive the field forward. For example, Borgus et al.[86] detected transient dopamine and adenosine simultaneously with FSCV and a bare CFME in vivo (Figure 2).[86] This is the first example of dual detection of spontaneous adenosine and dopamine, providing valuable insight on how adenosine can impact dopamine concentrations. This example of multiplex detection was possible due to the different oxidation potentials of dopamine and adenosine and due to the unique CV shape of adenosine, making it distinguishable from other neurotransmitter signals. Wilson et al.[87] also took advantage of differing oxidation potentials to demonstrate in vivo, simultaneous detection of dopamine and hydrogen peroxide within awake, moving rats using FSCV and bare CFMEs.

In some cases, multiplex detection requires new algorithms and data analysis methods. Movassaghi et al.[88] achieved the simultaneous detection of dopamine and serotonin using rapid pulse voltammetry (RPV) combined with partial least-squares regression methods to identify two components in a complex system. With the development of machine learning and AI, multiplex sensing has the potential for automation and standardization of multianalyte detection in a complex biological matrix.[88]

Antifouling Strategies. In addition to multiplex detection capabilities, major advancements within electrochemical sensing are seen in electrode modifications that achieve lower limits of detection, enhance analyte selectivity, improve performance, and prevent electrode fouling. Fouling of the electrode surface is a major setback for the field. It reduces the longevity and sensitivity of the electrode, preventing longer-term in vivo studies and requiring researchers to exchange their electrodes often, increasing the amount of time it takes to perform an experiment. There are several examples of modifications that tackle this problem.

One method to prevent fouling is the chemical modification of CFMEs with previously discussed biocompatible coatings. These coatings increase the hydrophilicity of the electrode surface and decrease the adhesion of interfering proteins, therefore slowing down the rate of electrode fouling and extending the life of the electrode. For example, Wei et al.[89] used a leukocyte membrane to coat a carbon-fiber electrode surface. Because of the very hydrophilic character of the electrode, it was highly resistant to nonspecific protein adsorption, allowing long-term in vivo analysis of neurochemical signals. Another example of both an antifouling and quantification innovation was modifying CFMEs with gold nanoparticles and glutamate oxidase by Wang et al.[66] This created an ultrafast glutamate sensor that can quantify the number of glutamate molecules released from single synaptic vesicles. Other commonly used antifouling agents and immobilization methods are also summarized in previous literature.[90]

Adding physical nanostructures is another promising antifouling strategy. For example, nanopore structures or filtration elements enable the separation of interfering proteins based on size differences. Feng et al.[91] used a polytannic acid (PTA)-doped nanoporous conducting membrane to coat CFMEs. Due to the hydrophilicity of its surface, it reduced the fouling and achieved sensitive dopamine detection. Other promising nanostructures include gold nanocones,[12] iron oxide-capped graphene sheets,[92] and nanodiamonds[93] to prevent fouling from common agents like serotonin and 5-hydroxyindoleacetic acid. The cutoff size of target-interfering proteins can usually be adjusted through tuning the nanostructure, offering more flexibility to control fouling. Biological antifouling strategies[90] usually involve the adoption of enzymes which can degrade[94] or control adsorption of[95] the interfering biomolecules, or antibody-modified magnetic beads[96] which can deplete interfering molecules from the sensors. Notedly, these antifouling strategies cannot completely avoid the influence of fouling chemicals or proteins, though they can greatly decrease the noise from interfering ions and increase the signal of the target analyte with enhanced conductivity or increased electroactive sites.

NANOPIPETTE AND NANOWIRE ELECTRODES

Although CFMEs remain the most popular electrode choice for neurotransmitter electrochemical detection, their size and selectivity limitations have pushed the use of smaller electrodes, such as nanopipette and nanowire electrodes. The design of nanopipette electrodes has gained increasing interest for electrochemical sensing since their first introduction in 1977 by Brown and Fleming for electrophysiology recording.[97] Compared to conventional micro- and nanoelectrodes, nanopipettes contain electrode nanopores that assist in achieving highly selective detection of small molecule neurotransmitters while avoiding interference from larger molecules. Due to the unique needle-like geometry and versatility (robust chemical modification of the inner wall material), the nanopipette electrodes are advantageous in small volume detection with simple and reproducible fabrication at low costs. Several literature articles have summarized fabrication methods, electrode materials, and geometric design of nanopipette biosensors.[98−100] Among a variety of nanopipette biosensors, conical glass pipettes have been widely used because of their low cost, simplicity, and reproducibility. Typically, sensing mechanisms used with nanopipettes can be classified into two groups: (1) resistive pulse sensing, where introduction of the target analyte influences the solution's ionic strength, leading to a sudden current change, or (2) ion current rectification (ICR) sensing, where the target analyte movement affects the electrical double layer on the electrode surface, causing a change in the current at opposite potentials.[100] Coupling these electrodes with other detection methods has come about in recent years. Yang et al.[101] used a cavity-based carbon-nanopipette in combination with FSCV to detect dopamine. This was the first example of testing nanopipette performance with FSCV. Their model was capable of achieving spatial resolution on the scale of hundreds of nanometers, much smaller than what can be achieved with a

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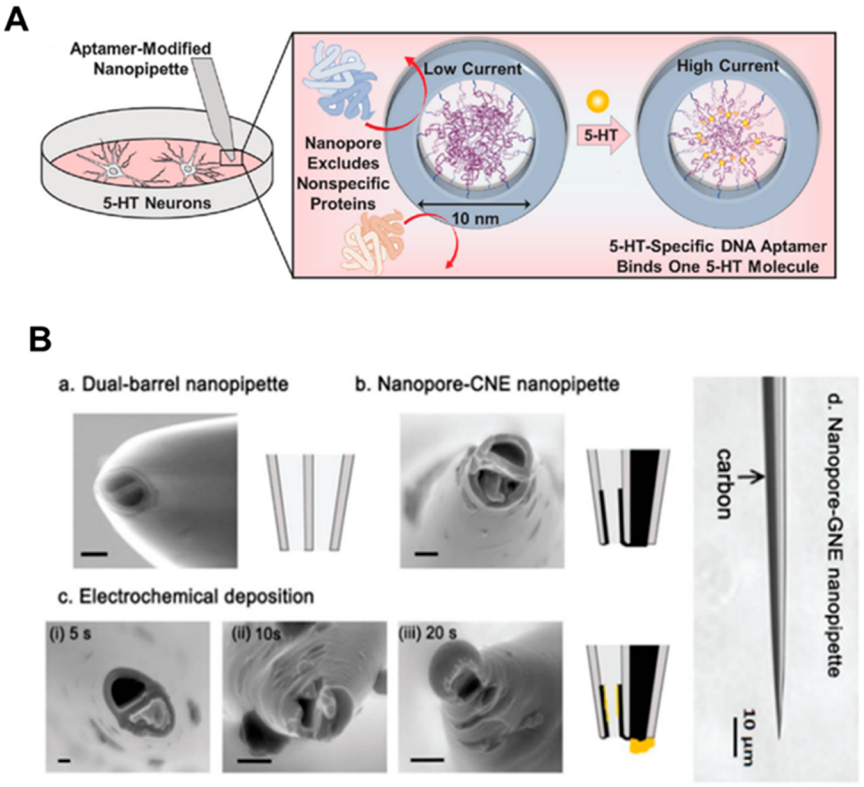

Figure 3. Nanopipette electrochemical sensors for neurotransmitter detection. (A) Schematic of an aptamer-modified single-barrel nanopipette for the detection of serotonin secreted from human serotonergic neurons. The diameter of the nanopipette is less than 10 nm diameter which blocks nonspecific proteins from interfering. Reprinted by permission from Springer Nature Molecular Psychiatry, ref 102, Copyright 2021 Nature. (B) The dual-channel nanopipettes for dopamine detection. SEM image of (a) dual-barrel nanopipette without any modification, (b) nanopore carbon nanoelectrode pipettes, (c) nanopore-gold nanoelectrode nanopipettes with different deposition times; scale bars for SEM images are all 100 nm. (d) Optical microscopy image of a nanopore-gold nanoelectrode nanopipette with 10 μM scale bar. Reprinted by permission of the Royal Society of Chemistry Analyst, ref 107, Copyright 2020 RSC.

microelectrode and providing the field with an improved method for investigating neurotransmitters within smaller organisms or smaller biological domains. Nanopipettes are often categorized into either single-barrel or multibarrel models, based on the number of compartments. In a single-barrel nanopipette sensor, the material of the inside wall can be coated with different affinity agents like aptamers or coated with conducting materials like gold and carbon through various fabrication or coating approaches to achieve the nonlabeled detection of the target analyte. An example of using a single-barrel nanopipette electrode for neurotransmitter detection was conducted by Nakatsuka et al.[102,103] In this work (Figure 3A), they developed a serotonin-specific aptamer-modified quartz nanopore electrode for detecting serotonin secreted from neurons. The aptamer-modified nanopore structure enabled selective and sensitive detection of serotonin with picomolar detection limits, an order of magnitude lower than is typically achieved with CFMEs. The serotonin aptamer experiences a conformational change upon serotonin binding, which induced a current−voltage curve shift while interfering proteins were physically excluded.[103] Polyimidazolium-brush-modified nanopipettes were also deployed by Zhang et al.[104] for the real-time detection of intracellular ATP in chromaffin cells through the interaction between polyimidazolium and ATP. For redox-inactive neurotransmitter species like γ-aminobutyric acid (GABA), which have no charge in neutral pH solutions, Iwai et al.[105] developed a nanopipette electrode to modulate pH by incorporating an organic acid into the oil phase of the orifice of the nanopipettes, achieving the detection of GABA with a highly sensitive 22.4 μM LOD. Additionally, dual-barrel nanopipette-based sensors have been developed for the multifunctional detection of neurotransmitters. Yang et al.[106] utilized a dual-barrel nanopipette electrode by combining functional nanopore and gold nanoelectrodes (GNE) (Figure 3B). Both barrels are functional electrodes, with varying capabilities for neurotransmitter detection. The dual-channel nanopipette enabled the label-free detection of dopamine in a dopamine/ascorbic acid mixed system with 10 nM LOD. The GNE barrel traps dopamine, permitting dopamine detection by surface enhanced-Raman spectroscopy.

The small size, selectivity, and the robust and customizable nature of nanopipettes showcases how these electrodes are facilitating highly sensitive, in vivo neurotransmitter detection.

Nanowire-based (NW) electrodes have been widely used in the detection of neurotransmitters, primarily at intracellular levels. The unique nanoconical structure and small tip sizes allow nanowire-based electrodes to accomplish intracellular-level detection and detection within specific organelles, providing insights into the biological activity of organelles, such as mitochondria.[108] The fabrication of the nanowire electrodes often relies on carbon, semiconductor materials, or both, with advanced nanofabrication techniques. Historically, using electrodes for intracellular detection is difficult because they must be stiff enough to penetrate the cells without altering

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the cell bioactivity during the penetration process. The field has been actively moving past this limitation by modifying NW electrodes. Yang et al.[109] developed novel NW electrodes for detection of various intracellular neurotransmitters. For example, GluOX-immobilized-single SiC@C cylindrical-shaped nanowire biosensors enabled real-time single neuron exocytotic glutamate detection and intravascular detection, which revealed the glutamate release mode in living hippocampal neurons. In addition, the same group developed biomimetic molecular catalysts (BMCs)-modified NWs, which can be synthesized at a large-scale, achieving real-time intracellular glutathione monitoring with a detection limit of 8.3 μM.[110] A similar strategy of depositing Au-PEDOT nanocomposites onto nonconductive nanowires was also developed for real-time intracellular NO amperometry detection in a single living cell.[111]

With significant advances in nanoparticles and various nanosynthetic methods, there are an increasing number of novel nanowire electronics. Zhao et al.[112] recently developed a U-shaped nanowire probe sensor with a controllable tip size and geometry to record intracellular neuron signals. Zhang et al.[113] developed a novel, flexible synaptic transistor using p-type P3HT/PEO NWs and n-type ZnO NWs as the semiconductor channel, applicable for electrocardiograms. They tested the synaptic transistor's transfer curves under various conditions to emulate different neurotransmission signals, revealing the neuroactivities during drug withdrawal, satiety, and depression. Although this Perspective is unable to provide a comprehensive summary of this exciting field, multiple reviews have summarized various nanowire-based electronics.[47,114]

Microelectrode Arrays. Single cell amperometry, FSCV, or other electrochemical measurements can provide insights into an individual cell's neurotransmitter exocytosis process within a short time window, helping reveal many neurological disease mechanisms and cell responses under various physiological conditions. However, those approaches are time-consuming and labor-intensive, especially when detecting exocytosis from a large number of individual cells to gain insight into the heterogeneity among cells. The microelectrode array (MEA)-based electrode can achieve the collection of multiple neurotransmitter electrical signals through the integration of arrays of electrodes or electronic structures. The electrode spacing is usually smaller than the cell size; therefore, each individual cell's exocytosis signal can be recorded with the consideration of heterogeneity among cells in the same tissue.[81] Microelectrode arrays have also been widely used in the recording of extracellular electrophysiology at a large scale.[115]

With the rapid development of microelectromechanical systems (MEMS) technology, researchers now can fabricate MEAs with a great number of electrode arrays and electronic units. For example, White et al.[116] developed a novel silicon-based 1024-on-chip electrode array using complementary metal-oxide-semiconductor (CMOS) technology (Figure 4A). The chip-integrated amplifiers and electronic circuits facilitated single-vesicle amperometric detection from PC12 cells. They correlated the high-throughput simultaneous measurement results to explain the chemical influence for Parkinson's disease treatment (L-Dopa). Gao et al.[117] cultured neurons on an MEA biosensor and achieved in vitro, multisite, and long-term detection of two different neurotransmitters. They observed concentration-dependent signals from glutamate and GABA with LODs around 100 nM and 50 nM, respectively.

Carbon is a widely used material for MEA fabrication due to its compatibility with CV, FSCV, amperometry, and other

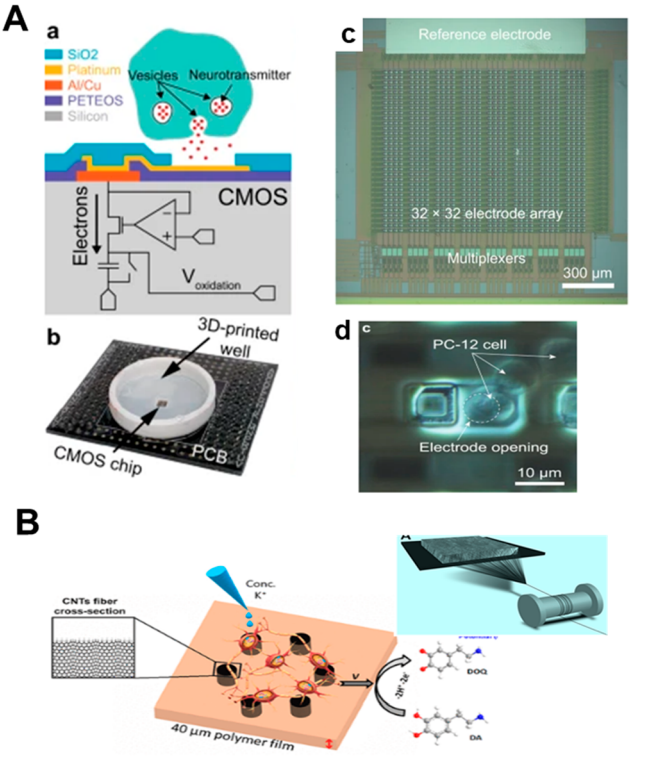

Figure 4. Microelectrode arrays for neurotransmitter detection. (A) (a) Schematic diagram of individual CMOS structure. The neurotransmitters released by vesicles will result in electrical current change at the amplifier. (b) Each CMOS chip has a well for electrolytic solutions and connects with external electronics. (c) Optical image of the device with 32 × 32 electrode array integrated to the chip. (d) Optical image of a PC12 cell settled on top of the electrode. Reproduced from ref 120, Copyright 2021 Nature under a Creative Commons Attribution 4.0 License. (B) Schematic diagram of carbon nanotube fiber cross section, rods made from dry spinning process. Adapted with permission from ref 121, Copyright 2020 American Chemical Society.

electrochemical detection methods. In recent work, Gupta et al.[121] reported a carbon nanotube fiber rod-based microelectrode array for dopamine, serotonin, epinephrine, and norepinephrine detection with CV and square-wave voltammetry (SWV) techniques (Figure 4B).[121] The high density of the microelectrode arrays demonstrated detection limits at a picomolar level and selective dopamine detection from PC12 cells after K^+ stimulation. Castagnola et al.[118] developed glassy carbon microelectrode arrays with MEMS nanofabrication techniques to achieve the simultaneous detection of dopamine and serotonin with FSCV. The electrodes were also used for in vivo detection of dopamine and serotonin in rat brains.

The highlighted examples above showcase only a small portion of the work being done on MEAs over the last five years. In general, there is a need to obtain information on multiple exocytosis events at the same time; a major challenge for MEAs is to distinguish and analyze multiple signals from different electrodes, which may occur at the same time, and correlate it to cell heterogeneity. In terms of sensor design, scientists and engineers working on microelectrode arrays must continue efforts to (1) improve the signal-to-noise ratio and (2) enhance their biocompatibility with biological samples, which usually suffers from the stiffness of the electrode array materials

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compared to biological tissues to achieve better performance in vivo.[119]

CHALLENGES, OPPORTUNITIES, AND OUTLOOK

The development of electrochemical and electronic biosensors for neurotransmitter detection has advanced significantly in recent years, driven by the development of nanotechnology, nanomaterials, and new detection and data analysis methods. This perspective discussed and summarized recent progress in the sensor design of nanopipettes, nanowires, microelectrode arrays, stretchable electrodes, and innovative approaches for multiplex sensing. Based on advances achieved so far, there are several areas ripe for future technological developments focused on neurotransmitter detection:

(1) Combining electrochemical detection with other analytical tools. The integration of complementary analytical tools can help provide more information on neurotransmitter-related biological processes. Several examples of this already exist, such as coupling electrochemical and confocal microscopy.[122] Coupling with additional techniques such as fluorescence imaging can help visualize the neurotransmitter distribution in different tissues, while electrochemical detection can achieve highly time-resolved detection. The combination of electrochemistry with Raman spectroscopy or mass spectrometry can provide molecular fingerprint information, aiding in multiplex detection of a wide range of neurotransmitters by helping to differentiate between signals from different analytes.

(2) Innovation in electrode fabrication. There is a need to develop stable and reproducible electrodes for sensing to improve the long-term utility of electrodes for robust recordings, especially in vivo. The glassy CFME tips can lack consistency in stability and reproducibility and are not well-suited to implantable sensing because they are fragile. The introduction of new technology may improve this—one example in this spirit is the recent use of 3D printing techniques for carbon electrode fabrication for neurotransmitter detection by Yang et al.[123] where they achieved customizable and reproducible geometries.

(3) Signal analysis. For in vivo and multiplex neurotransmitter detection, it is very challenging to simultaneously identify and quantify the signal from various neurotransmitters because of their different release dynamics and interference among different species. Recently, deep learning-based algorithms were adapted by Xue et al.[124] for voltammogram analysis of different neurotransmitters automatically. Machine learning, AI, and other data analysis methods can be used to simplify the data analysis process and reveal otherwise hidden information about neurotransmitter release phenomena.

(4) Wireless sensing. Integrating flexible sensors or electronics with phones for health management is becoming increasingly popular. To achieve effective monitoring of neurotransmitter changes in live, free-moving animals, wireless sensors can be adapted as well. A battery-free wireless device was developed recently by Wu et al.,[125] providing insights into the future design of wireless neurotransmitter biosensors.

With the continued improvement of sensitivity, selectivity, portability, reliability, and compatibility of electrochemical and electronic neurotransmitter detection methods, neurotransmitter sensors can support point-of-care detection of disease biomarkers, reveal physiological properties behind many disease mechanisms, and improve our understanding of cell-brain communications.

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Notes

The authors declare no competing financial interest.

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