Published in final edited form as:

Anal Lett. 2015 May 3; 48(7): 1044–1069. doi:10.1080/00032719.2014.976867.

RECENT DEVELOPMENTS IN ELECTROCHEMICAL SENSORS FOR THE DETECTION OF NEUROTRANSMITTERS FOR APPLICATIONS IN BIOMEDICINE

Rıfat Emrah Özel^{1,2,†}, Akhtar Hayat^{1,3,†}, and Silvana Andreescu^{1,*}

¹Department of Chemistry and Biomolecular Science, Clarkson University, Potsdam, NY, USA.

Fax: 3152686610; Tel: 3152682394

³Interdisciplinary Research Centre in Biomedical Materials (IRCBM), COMSATS Institute of Information Technology (CIIT), Lahore, Pakistan

Abstract

Neurotransmitters are important biological molecules that are essential to many neurophysiological processes including memory, cognition, and behavioral states. The development of analytical methodologies to accurately detect neurotransmitters is of great importance in neurological and biological research. Specifically designed microelectrodes or microbiosensors have demonstrated potential for rapid, real-time measurements with high spatial resolution. Such devices can facilitate study of the role and mechanism of action of neurotransmitters and can find potential uses in biomedicine. This paper reviews the current status and recent advances in the development and application of electrochemical sensors for the detection of small-molecule neurotransmitters. Measurement challenges and opportunities of electroanalytical methods to advance study and understanding of neurotransmitters in various biological models and disease conditions are discussed.

Keywords

review; electrochemical sensors; neurotransmitters; biomedical; microbiosensors

INTRODUCTION

Neurotransmitters are key molecules controlling behavioral and physiological conditions by regulating communication within the neural network. These molecules are involved in a large variety of neurophysiological processes including sleep, learning, memory, and appetite (Heng 2007). Damage in secretion or uptake of neurotransmitters might result in neurodegenerative diseases, drug addiction, and depressive syndromes (Sheikh 2013). Therefore, analytical methodologies for studying neural function are essential for understanding the relationship between neurotransmitters and organ functions and

*Corresponding author eandrees@clarkson.edu.

²Current address, Department of Biomolecular Engineering, University of California Santa Cruz, Santa Cruz, CA, USA

[†]the authors contributed equally to this work

potentially for early-diagnosis of neurological disorders. Fundamental information on neurotransmitters release can be obtained from *in vitro* studies in controlled biological conditions that mimic physiological conditions such as cell cultures or dissected organs (e.g. brain slices, dissected intestine) maintained in a physiologically active state. These models can provide valuable knowledge on neurotransmitters release and uptake but because of the isolation of cells and tissues from their natural environment, they provide limited information on the functioning and interconnectivity of neurotransmitters in an intact neural system. A more comprehensive evaluation can be obtained from the use of intact whole organisms. In general, development of analytical methodologies for the detection of neurotransmitters is designed toward a particular neurotransmitter tailored for a specific application and biological environment.

Electrochemical measurement devices (e.g. microelectrodes with electrochemical quantification) provide valuable tools for *in vivo* and *in vitro* detection of neurotransmitters due to their cost effectiveness, relatively simple design, miniaturized design, and real-time measurement capability with high spatial resolution. Electrochemical sensors combine the sensitivity and the selectivity within a small analytical device. Compared to conventional analytical methods (e.g. spectroscopy, chromatography) electrochemical sensors can be modified and adapted for the analysis of target neurotransmitters (Perry 2009, Ispas 2012, Wilson 2007).

This review aims to present state-of-the-art methodologies and recent advances in the development of electrochemical methods for monitoring of neural signalling molecules with focus on the translation of electrochemical probes from controlled conditions (e.g., standard or spiked biological solutions and *in vitro* systems) to real time *in vivo* monitoring. The paper highlights developments in electrode design, electrode materials and performance characteristics published within the last five years. Recent examples of application of these methods for the study of neurotransmission in developmental biology (e.g., embryonic study) and whole animals are also highlighted. Examples of electrochemical sensing technologies are critically discussed with examples of biomedical applications. Although there are many types of neurotransmitters within central nervous system, the emphasis is those which are strongly related to the neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and stroke.

STATUS OF ANALYTICAL METHODOLOGIES FOR THE DETECTION OF NEUROTRANSMITTERS

A variety of analytical methodologies have been developed for the qualitative and quantitative detection of neural signaling molecules. Given the dynamically changing levels of neurotransmitters, direct *in situ* detection of these chemicals without sample treatment is desired. This requires the development of probes that can be inserted at precise locations and be able to provide changes of neurotransmitters concentration in real time at the desired monitoring sites. These sensors require miniaturization, full functionality in the detection environment, high sensitivity, and selectivity as well as biocompatibility, and create no or minimum damage or perturbation of the environment. Due to the challenges with direct *in*

situ monitoring of neurotransmitters, especially in complex biological environments, many procedures still rely on the use of microdialysis sampling consisting of insertion of a dialysis probe to tissue location for continuous specimen collection. Usually, microdialysis is followed by the fractionation of the collected sample through liquid chromatography (LC) or capillary electrophoresis (CE) prior to neurotransmitter detection via mass spectrometry (MS), fluorescence spectroscopy, or immunoassays (Perry 2009). In general, these approaches lack spatial and temporal resolution due to the lag time between sample collection and analysis. Therefore, the detection of neurotransmitters with short half-life might result in inaccurate interpretation of the data. Specifically designed microelectrodes can provide real time measurements of neurotransmitters. Redox active neurotransmitters such as dopamine and serotonin can be measured directly by electrochemical means at their oxidation or reduction potential. Electrochemically inactive molecules such as glutamate or lactate have been determined by using specific enzymes to generate redox active molecules. Various configurations of sensors have been reported in literature and some are commercially available: e.g., for the detection of glutamate and lactate. Recent advancements in this field highlight the use of new electrode materials with enhanced conductivity and catalytic properties and the development of microfabricated electrodes coupled with microfluidic systems and automatic operation. Among the many electrochemical sensors for the detection of neurotransmitters reported in literature, relatively few have been used to study neurotransmission and disease conditions in ‘real’ biological models. Current status and recent progress in this direction is reviewed further in this review for the different classes of neurotransmitters.

GENERAL CONSIDERATIONS FOR DESIGNING ELECTROCHEMICAL METHODOLOGIES FOR THE DETECTION OF NEUROTRANSMITTERS

Development of electrochemical probes, especially those designed for *in vivo* real time detection of neurotransmitters requires several strict design characteristics to enable functionality in the complex biological environment. These include: low detection limits that match the physiological concentration range for both normal and pathological conditions, high sensitivity and selectivity, small size to minimize tissue damage, and the use of stable biocompatible coatings. Depending on the application, such sensors can be designed for single point monitoring using techniques like differential pulse voltammetry (DPV) or fast scan cyclic voltammetry (FSCV). Others can be used to monitor dynamic changes of neurotransmitter levels over long periods of time using amperometric techniques at a controlled potential; in this case long time operational stability for several hours or more in the biological fluid or tissue is needed.

For measurements that require implantation, electrodes with small sizes are desired to facilitate easy insertion and reduce tissue damage. The smaller electrode sizes for use in *in vivo* studies are 5 micron carbon fiber microelectrodes (CFMEs) used for direct detection of electrochemically active monoamine neurotransmitters. In general, larger electrodes of larger than 100 microns are used in detection schemes that involve biological recognition such as enzymes that require a large surface area to immobilize biomolecules.

A common requirement for creating electrochemical probes designed to operate in 'real' biological environments is to provide selectivity against electrochemically active species that coexist with neurotransmitters in biological samples. These include ascorbic acid, uric acid, and acetaminophene, among others. A common strategy to eliminate interferences is to cover the electrode with perm-selective membranes to limit access of the interferents to the reactive surface of the sensor. Membranes such as Nafion (Kang 1997, Jeong 2008) or a combination of Nafion and metal tetraaminophthalocyanine (Kang 1997) and hexacyanoferrates (Castro 2008) have been used. Nafion is anionic, and can be used to exclude negatively charged interferences such as ascorbate (Jeong 2008) and nitrite. We have shown that chitosan, a natural biopolymer with excellent film forming properties can also exclude interferences from ascorbic acid through electrostatic repulsion when coated on CFMEs (Özel 2011). Electropolymerized layers that exclude interferences through molecular sieving by modulating pore dimensions constitute another class of membranes. In this case, electrodeposition procedures can provide better control and coverage of the electrode surface. Examples include over-oxidised pyrrole (Walker 2007), polyphenylenediamine (McAteer 1996), and polyion layers of poly-L-lysine and poly-4-styrenesulfonate (Mizutani 1998). Ascorbate oxidase is used in some sensors to eliminate interferences from ascorbate (Goriushkina 2009, Burmeister 2005). In other papers, self-referencing sensors have been used to provide an internal control electrode to differentially assess and subtract the response of interferences. Ceramic, multisite microelectrodes for *in vivo* measurements of glutamate in the central nervous system are an example of this type of sensor (Burmeister 2002, Hascup 2008, Burmeister 2001). The use of electron mediators like $[\text{Os}(\text{bpy})_2\text{ClpyCHO}]^+$ or $\text{PVP}-[\text{Os}(\text{N},\text{N}'\text{-dimethyl-2,2'biimidazole})_3]^{2+/3+}$ (Mano 2005) to decrease the operating potential is also a common practice in sensors that use enzymatic conversion of neurotransmitters such as those for the detection of glutamate and lactate. Potential leaching of the mediators from the electrode surface is a concern when these electrodes are used *in vivo*.

Overlapping signals from multiple neurotransmitters that have relatively close oxidation potentials pose problems with the discrimination of signal among the various neurotransmitters present. Careful selection of the electrode material and electrolyte conditions has been reported to enhance selectivity and sensitivity of electrochemical measurements for dopamine. Pattern recognition methods provide an effective way to differentiate overlapping signals enhancing detection and prediction of concentrations of neurotransmitters measured by voltammetry (Sazonova 2009, Stefan-van Staden 2014).

Common problems encountered when these sensors are used as implantable probes are tissue reaction and electrode fouling when the sensors are placed *in vivo* (Behrend 2009). Mechanical damage of the active surface as the probes are inserted in tissue can also be encountered. The use of biocompatible coatings and small electrode sizes can minimize tissue damage. Electrode fouling has been associated with the non-specific adsorption of proteins on the electrode surface which can decrease sensor response and cause drift in calibration. In most cases, sensor calibration after implantation shows a reduction in sensitivity with values ranging from 40% to 10% decrease (Njagi 2010). A common strategy to reduce nonspecific adsorption is to coat the electrode with bovine serum albumin (BSA)

or other natural proteins or with antifouling polymers such as polyethylene glycol (PEG). While these have reduced considerable the passivation of the electrode, complete prevention of this phenomenon remains an issue (Njagi 2010). Future sensors require development of new materials and innovative engineering design of specific membranes with enhanced antifouling properties.

The following sections provide a description of electrode designs, their performance, applications and limitations for the detection of the main classes of neurotransmitters.

ELECTROCHEMICAL DETECTION OF MONOAMINE NEUROTRANSMITTERS

Dopamine

Dopamine, which belongs to the catecholamine family of neurotransmitters, is synthesized in a sequential reaction in which tyrosine hydroxylase and amino acid decarboxylase convert amino acid tyrosine to L-dihydroxyphenylalanine (L-dopa), followed by decarboxylation of L-dopa to dopamine. Dopamine plays a vital role in the control of movements and loss of dopaminergic neurons containing neuromelanin in the nigrostriatal system and has been associated with the motor symptoms experienced in patients with Parkinson's disease (Munoz 2012). Other studies show that the dopamine oxidation products can inhibit the function of specific proteins (Berman and Hastings 1999) and correlate formation of cysteinyl-dopamine conjugates with dopamine-induced neurotoxicity. As a potent neurotransmitter, changes in the level of dopamine in adrenal glands impact many aspects of brain circuitry. For example, Parkinsonism is associated with a reduced level of dopamine, while schizophrenia is related to increased dopamine activity (Nagy 1999). *In vivo* concentrations of dopamine are in the nanomolar range. Given the wide range of physiological and pathophysiological implications, the development of analytical assays for precise, low level and selective measurement of dopamine are highly desirable.

Dopamine was one of the first neurotransmitters to be determined *in vivo*. Following the original work of Adams and co-workers (Blank 1972, Adams 1972), numerous electrochemical techniques and electrode materials have been investigated for *in vivo* detection of dopamine. Commonly used techniques include constant potential amperometry, differential pulse voltammetry, fast scan cyclic voltammetry and paired pulse voltammetry (Shon 2010, Yoshimi 2011, Suzuki 2007, Jang 2012, Keithley 2011). Carbon and carbon derived microelectrodes and metal (Pt and Au) electrodes have been used as transduction materials (Robinson 2008a, Zachek 2008, Bath 2001). CFMEs provide better detection sensitivity as compared to metal electrodes. The $2e/2H^+$ redox reaction of dopamine under physiological conditions facilitates direct electrochemical detection of this neurotransmitter. Dopamine sensors and fast scan cyclic voltammetry for brain analysis were developed by the pioneering work of Wightman (Cheer 2007, Robinson 2003, Wightman 1988, Robinson 2008b). Direct electrochemical oxidation of dopamine with FSCV at CFME has been the method of choice to measure *in vivo* levels of brain dopamine *in situ* (Cheer 2007, Swamy and Venton 2007). This method requires background subtraction and can suffer from problems with the rapid inactivation of the electrochemically active surface area of the

electrode. Methods for the detection of dopamine using electrochemical techniques have been reviewed recently (Jackowska and Krysinski 2013). Earlier research has shown that dopamine and its oxidation products can strongly adsorb to the electrode surface inactivating the electrochemically active surface. Orientation of adsorbed molecules, concentration and supporting electrolyte all affect the electrochemistry of dopamine (Salaita 1988). Other limitations include the relatively high oxidation potential of dopamine, formation of phenoxy radicals, and interferences from coexisting compounds that can be oxidized at the same operating potential as dopamine (e.g. ascorbic acid, and uric acid) (Jackowska and Krysinski 2013).

An alternative method to the direct electrochemical detection of dopamine is to use enzymes (e.g. polyphenol oxidase, tyrosinase, or laccase) that convert dopamine to dopaquinone, which can then be reduced and measured electrochemically at a relatively low potential of ~ -0.1 V versus the Ag/AgCl reference, thus reducing the risk of interferences. The use of enzymes eliminates problems of consecutive reactions of dopamine oxidation products (Majewska, 2006) and prevents passivation of the electrode. Ascorbic acid has been reported to reduce *o*-dopaquinone (Ronit 2007) and thus specificity of the dopamine signal in the presence of ascorbic acid should be tested when designing such biosensors architectures. An essential step in the development of an enzyme biosensor for the detection of dopamine is in the immobilization of the enzyme on the electrode surface. A review describing the different immobilization methods for tyrosinase has been reported (Duran 2002). Conducting porous and mesoporous materials have been shown to improve binding efficiency of the enzyme. Similarly, a variety of nanoscale materials including metal or oxide nanoparticles, carbon nanowires and nanotubes have been reported to stabilize the enzyme, increase the surface area and enhance catalytic sites and conductivity on electrode surfaces (Maciejewska, 2011, Njagi, Chernov, 2010, Min and Yoo 2009, Njagi, Ispas, and Andreescu 2008, Tang, 2011, Wang, 2010). Few of these bioelectrodes have been adapted for use *in vivo* (Njagi 2008, Cosnier 1997, Tembe 2006). A tyrosinase biosensor based on polypyrrole has been employed for the *in vivo* detection of dopamine and glutamate (Cosnier 1997). Our group has used chitosan and ceria-based metal oxide co-immobilized with tyrosinase on a CFME-type brush to detect dopamine (Njagi 2010). The biosensor was successfully applied to measure *in vivo* levels of dopamine (1 nM detection sensitivity) with broad linear range. The selectivity of sensor was tested against commonly occurring interfering compounds (ascorbic acid and uric acid). The specificity of the signal was demonstrated with nomifensine, a dopamine uptake inhibitor (Figure 1). There was no response to common interference such as ascorbic acid and uric acid when these substances were added to the test solution. The biosensor recorded a response signal in presence and absence of nomifensine. The signal during high frequency stimulation of the median fore brain bundle increased after the nomifensine treatment compared to the control stimulation condition demonstrating the temporal specificity of the dopamine response to stimulation. Histological examination confirmed that sites of stimulation were closely adjacent to the median forebrain bundle, and the dopamine biosensor was in the striatum in each animal (Figure 1B) (Njagi 2010).

Several advances in the electrode material have been made in the past years in the design of electrochemical sensors for the detection of dopamine. More than one hundred publications

for electrochemical detection of dopamine have been reported in the literature during last three years. Among the newly used materials, graphene has found widespread electrochemical application due to its enhanced electrical conductivity, large surface area, and ease of preparation (Wang 2011, Zhu 2011, Si 2011, Wu 2012, Hou 2010, Kim 2010). Several graphene-based sensors have demonstrated functionality for the detection of dopamine in biological samples including in rat striatum (Wang 2011), mouse hippocampus (Kim 2010), and human serum and urine (Henstridge 2010, Hou 2010). Similarly, electrodes modified with ionic liquid composite layer have shown low level detection sensitivity for dopamine that has been attributed to the unique properties of ionic liquids (Li 2011, Li 2011, Chang 2011, Zhu 2010). A molecular sieve/carbon based electrode has been used to monitor the dopamine concentration in human serum (Li 2011). Integration of metal and semiconductor nanoparticles with conductive or catalytic properties have also been shown to enhance detection sensitivity (Reddy 2012, Garcia 2011, Zheng, Wang, and Yang 2011, Celebanska 2011). The best results in term of sensitivity were obtained with a conducting polymer, Poly(3,4-ethylenedioxythiophene) or PEDOT (Stoyanova and Tsakova 2010). Modification of electrodes with polypyrrole and Cu nanoparticles allowed simultaneous detection of dopamine and uric acid in the presence of other interfering compounds (Ulubay and Dursun 2010). Chemical sensors revealed comparable detection limits to enzyme based biosensors and enable simultaneous detection of various compounds based on the difference in the electrochemical potential and long term stability.

Serotonin

Serotonin exhibits regulatory effects in human mood, behavior, alertness, learning, as well as food intake and sleep (Young and Leyton 2002). Malfunctioning in the release and uptake of serotonin has been correlated with neural diseases, such as schizophrenia, depression, migraine, and drug addiction (Mohammad-Zadeh, Moses, and Gwaltney-Brant 2008), and with intestinal diseases, including inflammatory bowel syndrome and celiac disease (Manocha and Khan 2012). Real time *in vivo* and *in vitro* monitoring of serotonin is of interest to understand the quantitative relationship between the physiological serotonin level and its evolution during the various states of these diseases.

Commonly used electrochemical procedures to monitor serotonin concentrations are based on amperometry and voltammetry as detection methods (fast scan cyclic voltammetry and differential pulse voltammetry are the most widely used), and modified CFMEs as working electrodes. A 7 μm diameter Nafion coated CFME enabled detection of serotonin changes within mice brain and assessment of pharmacologically modulated signals (Wood and Hashemi 2013). A similar type of microelectrode was used to monitor alterations of serotonin concentrations at dissected central nervous system of *Drosophila*, fruit fly, after genetic and pharmaceutical alterations. Fast scan cyclic voltammetry measurements were done by using a scan rate of 400 V/s and an oxidation potential of 0.5 V versus Ag/AgCl (Fang, Vickrey, and Venton 2011). We have fabricated a 30 μm carbon fibre-brush type microelectrode modified with carbon nanotubes and Nafion for *in vivo* detection of serotonin within the digestive system of 5dpf (days post fertilization) zebrafish embryos. The microelectrode displayed a linear response between 5 to 200 nM with a detection limit

of 1 nM for serotonin (Njagi 2010). The signal recorded *in vivo* with the implanted electrode enabled quantitative detection of the 5-HT level in embryonic zebrafish, corresponding to a value of 5-HT of 29.9 (± 1.13) nM in normal physiological conditions. Quantitative electrochemical measurements of 5-HT levels were possible at different locations within the intestine and the results obtained by electrochemical methods followed the same trend as those recorded by conventional immunohistochemistry (Figure 2). As compared to immunohistochemistry, electrochemistry has a clear advantage in terms of analysis time and the quantitative information that this method provides. In follow up work, a selective and sensitive serotonin microsensor was fabricated by simply dipping a 5 μ m carbon fiber in 1 % chitosan solution to prevent non-specific and protein adsorption (Özel 2011, Ozel 2014). Direct oxidation of serotonin was monitored at 0.36 V versus Ag/AgCl. The sensor displayed a linear calibration range from 2 to 100 nM with a detection limit of 1.6 nM and provided useful information for the evaluation of serotonin in genetically modified zebrafish embryos (Roach 2013), demonstrating the potential as an analytical tool in developmental biology. In addition to CFMEs, boron-doped diamond microelectrodes have provided serotonin detection capabilities *in vitro* (Singh 2009). Development of new electrochemical methods and devices for serotonin detection and adoption of these probes by biologists and the biomedical community could facilitate further fundamental research in deciphering the important regulatory roles of serotonin within the digestive and central nervous systems.

Epinephrine and Norepinephrine

Epinephrine (also known as adrenaline) is a hormone and a neurotransmitter involved in several neurological and psychiatric disorders (Huynh 2012). It plays an important role as a mediator of the stress-induced development of anxiety and depression disorders (Dazzi 2003). Analytical methods reported for the detection of epinephrine and norepinephrine include colorimetry (Baron 2005), liquid chromatography-mass spectrometry (Thomas 2006), potentiometry with ion-sensitive field effect transistors (Kharitonov 1999), and amperometry.

Electroanalytical techniques are the only methods enabling nanomolar level detection of epinephrine. However, in spite of their potential, electrochemical sensors that have been reported for the detection of epinephrine and norepinephrine have been tested only on standard solutions. A carbon paste electrode modified with carbon nanotubes and 2-(4-oxo-3-phenyl-3,4-dihydroquinazolinyl)-*N'*-phenyl-hydrazinecarbothioamide was used to detect epinephrine in the presence of norepinephrine (Beitollahi 2008). A nanocomposite film of conducting polymers comprising single wall carbon nanotubes, polypyrrole, and gold nanoparticles was used for selective measurement of epinephrine in the presence of ascorbic acid and uric acid (Lu 2011). Similarly, multiwall carbon nanotubes incorporating TiO₂ were employed to detect epinephrine in the presence of p-chloranil as a mediator (Kharian 2012). A composite of over-oxidized polypyrrole–multiwall carbon nanotube was prepared on a glassy carbon electrode that was used for the determination of epinephrine (Shahrokhian 2011). A white rot fungi cell based biosensor on Pt as a working electrode was also reported (Akyilmaz 2011). A thin film of a molecularly imprinted polymer templated with protonated epinephrine as an electrode material for electrochemical transduction with differential pulse voltammetry, capacitive impedometry, and piezoelectric microgravimetry

(Huynh 2012). A gold nanoparticle-doped DNA composite electrode has been used for the detection of norepinephrine in the presence of ascorbic acid (Lu 2004). A nanostructured platinum-gold (Pt–Au) hybrid film modified glassy carbon electrode fabricated by electro-deposition (Thiagarajan 2009) was able to detect both epinephrine and norepinephrine individually and in the presence of ascorbic acid. Simultaneous determination of epinephrine and norepinephrine was investigated by square wave voltammetry using multi-walled carbon nanotubes modified edge plane pyrolytic graphite electrode (Goyal 2011).

The myriad of electrochemical detection schemes for epinephrine and norepinephrine demonstrate that these neurotransmitters can be detected with high sensitivity and selectivity and that it is possible through proper design of the electrode material and interface to successfully discriminate between different neurotransmitters and between neurotransmitters and ascorbic acid. The next step to use these sensors for real time in situ detection and disease monitoring is to miniaturize the sensors (e.g. CFME platforms) and determine analytical performance in tissues and whole organisms in ‘real’ biological samples.

Acetylcholine—Acetylcholine is present in both the peripheral and the central nervous system of many organisms. It is produced by the action of choline acetyltransferase and acetyl coenzyme A on choline in neurons (Brandon 2004). The concentration of acetylcholine in human blood is around 8.66 ± 1.02 nM (Xue 2008a). Plant poisons such as curare and hemlock can cause paralysis due to blocking of acetylcholine receptors sites of muscle cells. Similarly, botulin causes paralysis by preventing the vesicles from releasing acetylcholine. In organisms, acetylcholine is responsible for stimulation of muscles and scheduling rapid eye movement in sleep. Abnormal levels of acetylcholine in the brain has been associated with neuropsychiatric disorders such as Parkinson’s and Alzheimer diseases, as well as with temperature and blood pressure regulation, motor coordination, learning and memory (Khan 2013).

Methods for sensitive determination of acetylcholine in biological samples can contribute to study of many of these conditions. Acetylcholine is difficult to detect because it lacks electroactive, chromophore, and fluorophore groups in addition to the absence of functional groups for conjugation purpose (Wang 2012). Conventional chromatographic methods such as gas chromatography and high performance liquid chromatography have been employed for the detection of acetylcholine (Dunphy 2003). The requirement of large sample volume, tedious sample pretreatment, long separation time, and advanced instrumentation have prevented wide implementation of these techniques for routine analysis of acetylcholine in biological samples. Several types of biological sensors based on enzymes have been developed as alternative methods. The use of implantable microbiosensors, particularly CFMEs, can provide high temporal and spatial resolution that cannot be obtained with conventional methodologies (Khan 2013). Biosensor architectures and detection schemes involve the use of acetyl cholinesterase (AChE) and choline oxidase to convert acetylcholine to form an electrochemically active or a fluorescent product (Larsson 1998, Yao 1995). AChE converts acetylcholine into choline and acetate, while choline oxidase converts choline into betaine and hydrogen peroxide, which is electrochemically active and can be monitored by electrochemical means. This design is relatively complicated as it involves two kinetically controlled enzyme reactions and their corresponding substrates,

acetylcholine and choline. For a compact device the two enzymes need to be co-immobilized on an electrode. HPLC coupled to post-column enzymatic reaction with AChE and choline oxidase has been initially used for the determination of acetylcholine in biological tissue and fluids (Gunaratna 1990). The enzymatically generated H_2O_2 is detected electrochemically at a platinum electrode. Several amperometric biosensors based on the immobilization of AChE/choline oxidase have been reported for the detection of acetylcholine. The most frequently used materials and electrode coatings include discrete membranes, entrapment in photocross-linked polymers, cross-linked redox polymers, and Nafion (Khan 2013). For rapid monitoring of acetylcholine, flow injection analysis methods have been implemented along with immobilized AChE and choline oxidase (Tsafack 2000, Lapp 1996). The use of oxidase reactions in conjunction with the peroxidase enzyme, in a three enzyme configuration, to further convert the H_2O_2 , has also been proposed (Khan 2012). Biosensors based on carbon fibers, platinum/iridium, glassy carbon, mesoporous silica membranes, cadmium sulphide nano crystals, multiwalled carbon nanotubes and many more transducer surfaces have been used for the detection of acetylcholine (Lee 2009, Guerrieri 2006, Shimomura 2009, Bhattachayay 2008, Burmeister 2008, Xue 2008b, Wang 2012), most of which have been developed and tested in standard acetylcholine solutions.

Glutamate—Glutamate is an amino acid neurotransmitter in the central nervous system involved in many normal brain functions such as memory, learning, cognition, and sensation (Danbolt 2001, Featherstone 2009). Abnormalities in the extracellular concentration of glutamate may lead the development of neurodegenerative diseases including epilepsy, stroke, Parkinson's disease, Alzheimer disease, and amyotrophic lateral sclerosis (ALS) (Maragakis and Rothstein 2004). Glutamate is a non-electroactive neurotransmitter; therefore most of the detection methods are based on enzymatic conversion of glutamate to electrochemically active products. This technique requires the immobilization of glutamate oxidase or dehydrogenase on an electrode surface. Glutamate biosensors that are based on glutamate dehydrogenase require the coimmobilization of a coenzyme, $NADP^+$ or NADH, and an electron mediator (Jeffries 1997, Lobo 1997, Doaga 2009, Vahjen 1991). Stable immobilization of the coenzyme and the low stability of this enzyme (Amine 1992) are the main problems associated with the use of dehydrogenase based detection schemes. Carbon paste wax electrodes were made for the amperometric detection of glutamate by flow injection analysis (FIA). To stabilize the enzyme, a thermophilic polymer was incorporated to the electrode design and a linear response was obtained between 0.3 and 5 mM for glutamate. (Pasco 1999). With the introduction of engineered nanomaterials, new strategies were utilized to enhance stability and immobilization of glutamate dehydrogenase. Gholizadeh and co-workers developed a mediator-free voltammetric glutamate biosensor by immobilizing the enzyme to a carbon nanotube modified silicon substrate. Although the developed biosensor was found to be sensitive, the selectivity was not suitable for *in vivo* monitoring (Gholizadeh 2012). An interference-free glutamate sensor was developed by using a bienzymatic system consisting of glutamate dehydrogenase and salicylate hydroxylase. This enzymatic cascade-based biosensor was characterized by a fast response time and a linear range between 10 μ M and 1.5 mM glutamate (Cui 2007). Even though progress has been made for glutamate detection through glutamate dehydrogenase, real time and *in vivo* monitoring with these sensors was not demonstrated.

Alternative glutamate biosensors involve the use of glutamate oxidase (GluOx) to convert glutamate to the electrochemically active H_2O_2 in the presence of oxygen. The advantage of this design is the higher stability of the enzyme (e.g. as compared to dehydrogenases) and independence from a co-enzyme. The electrochemical detection of H_2O_2 requires relatively high operation potential, and has relatively slow electron transfer kinetics at conventional electrodes. Platinum electrodes provide the most sensitive detection for H_2O_2 due to the electrocatalytic effect of Pt on H_2O_2 oxidation (Chen 2012). To prevent interferences from other electroactive compounds (specifically ascorbic acid ($\sim 200 \mu M$), uric acid, dopamine, and serotonin) that are present in the physiological media, electrodes were casted by perm-selective materials such as Nafion (Pan 1996, Govindarajan 2013), *o*-phenylenediamine (Lowry 1998, R. Ryan 1997, Özel 2014), over-oxidized polypyrrole (Walker 2007, Hamdi 2006) and other non-traditional membranes (Mizutani 1998, McMahon 2006). Additionally, size and geometry of the electrode were found to tune the selectivity of biosensors (McMahon 2004). Although polymeric modifications of the electrode surfaces enhance the selectivity, they often decrease the sensitivity and limit diffusion of glutamate to the glutamate oxidase containing surface, thus increasing response time. Some coating materials are toxic and cannot be readily used *in vivo*. Chitosan is a good example of biocompatible material that has been used to impart biocompatibility of glutamate biosensors (Zhang 2005) while also providing a good environment to stabilize the enzyme and electrostatically reject interferences (Özel 2011) (Özel 2014). The sensitivity of the biosensor is strongly dependent on the enzymatic purification and extraction source (Ispas 2010).

Direct real-time *in vivo* measurement in rat brain was achieved by implantable microsensors to study neural activity in various physiological states (Naylor 2011, Oldenziel 2006, Quintero 2011, Pomerleau 2003). The use of nanoparticles and nanocomposites as electrode materials and immobilization matrix improved the sensitivity of glutamate biosensors (Claussen 2011, Batra 2013). Recently, we have used the oxygen storage activity of cerium oxide nanoparticles to develop an enzymatic glutamate oxidase microbiosensor for the detection of glutamate in rat brain to study the evolution of glutamate during brain hypoxia (Özel 2014). This sensor design is particularly useful for monitoring diseases states that involves hypoxic conditions. The nanoparticles work as an oxygen source for the oxidase enzyme enabling biosensor operation under oxygen-free conditions such as ischemic hypoxia which has been associated with several diseases such as cerebral ischemia as well as Alzheimer's and Parkinson's diseases. *In vivo* measurements carried out using Sprague-Dawley rats demonstrates the potential of this method for real time monitoring of glutamate release and provide in situ quantification of minute changes of glutamate during cerebral ischemia and reperfusion (Özel 2014).

Gaseous Neurotransmitters: the Case of Nitric Oxide—Nitric oxide (NO) is a gaseous diatomic molecule possessing cytotoxic and cytoprotective, properties depending upon the physiological concentrations (Park 2010). NO is known to have an important regulatory role as a neurotransmitter in central nervous system (Jo 2011). Concentrations of NO in peripheral media were found to be changed in neural degenerative diseases. However, quantification of NO remains a challenge due to its nanomolar concentration range in the physiological environment and its short half-life. Commonly used analytical techniques for

the detection of NO are spectroscopic (fluorescence, ultraviolet-visible, and chemiluminescence) and electrochemical methods. Spectroscopic approaches are based on indirect measurement of NO through monitoring of secondary species produced from interaction of dyes with NO. Electrochemical techniques provide direct detection of NO within complex media (Bedioui 2013, Chen 2013, Opländer 2012, Hawkins 2013, Kumar 2012). Advantages of electrochemical methods are the *in situ* detection ability and the low detection limits of these methods. Miniaturized 5 micron diameter electrode probes can be conveniently used for *in vivo* NO measurements.

NO is electrochemically active, yet surface modifications to increase selectivity and sensitivity of microelectrodes within complex biological media are required. Major progress has been achieved in the development of NO ultramicroelectrodes, through engineering catalytic and permeable membranes to enhance selectivity and detection sensitivity. Most NO sensors use permselective membranes to limit the diffusion of interfering biomolecules (ascorbic acid, uric acid, nitrate, dopamine, *etc.*) by electrostatic repulsion and/or size exclusion filters (Özel 2013, Ho 2010, Gutierrez 2009, Dang 2011, Vinu 2014). Nafion and *o*-phenylenediamine are the most extensively used polymeric membranes. While Nafion, a cationic exchange polymer, prevents negatively charged interfering species by electrostatic repulsion; *o*-PD blocks the access of larger molecules to the electrode surface. We have used a combination of Nafion and *o*-PD to fabricate a 5 μm CFME that was used to evaluate NO levels within the intestine of embryonic zebrafish. Nitric oxide levels were measured by differential pulse voltammetry at ~ 0.75 V versus Ag/AgCl. Microelectrodes exhibited a linear range spanning from 0.25 to 50 μM and successfully prevented interferences from serotonin and ascorbic acid (Özel 2013). Finnerty and co-workers used Nafion modified Pt/Ir microelectrodes for the continuous real-time amperometric detection of NO (~ 0.9 V versus Ag/AgCl) in extracellular cerebral fluid (Finnerty 2012) and in different brain regions of freely moving rats (Finnerty 2012). Nafion casted Pt/Ir electrodes showed high stability for over 8 days. Fabrication of a selective and sensitive NO sensor through electrodeposition of Meldola's Blue, *o*-PD on a 125 μm Pt wire was reported. To achieve biocompatibility, the microelectrode was modified by immersion of the wire in a 1 % chitosan solution. The sensitivity and selectivity of these electrodes were evaluated by amperometry at 0.9 V versus Ag/AgCl. Linear range for NO was found to be from 0.01 to 600 μM . The reported response time was less than 8 s and the selectivity was demonstrated versus ascorbic acid, dopamine, and nitrite (Njagi 2010).

Another class of NO microsensors are those based on heme proteins and metalloporphyrins that can catalytically oxidize NO. The use of metalloporphyrins has been found to enhance the electron transfer kinetics for the oxidation of NO due to the high affinity of NO for metals (Oni 2005). The selectivity of the sensor can be tuned by addition of permselective coatings (Prakash 2012, Santos 2013). Santos and co-workers reported the development of a NO microsensor made from carbon fibers modified by hemin, multi-wall carbon nanotubes, and chitosan, and showed *in vivo* monitoring capability of the NO reduction at -0.762 V versus Ag/AgCl by square wave voltammetry. The microsensor was found to respond linearly between 0.25 and 1 μM NO with a detection limit of 25 nM. The authors evaluated the effect of interfering species present in the extracellular brain fluid. The microsensor was

selective towards NO (Santos 2013). Metallophthalocyanines are another group of metal-ligand complex displaying similar catalytic properties for NO oxidation/reduction to porphyrins (Bedioui and Griveau 2013). An amperometric NO sensor was produced by electropolymerization of metallo 4, 4, 4, 4 tetra-amine phthalocyanine (MTAPc) on Pt-modified nanoporous anodised alumina oxide template (Yap 2012). To achieve selectivity, the electrode surface was coated with Nafion. Various metal centers containing MTAPcs were evaluated and the highest sensitivity was observed using platinum with a linear range from 10 to 100 nM for NO.

In addition to the use of perm-selective membranes and metal-ligand coatings, various other surface modification material such as organic dyes (Peng 2009) and glycoproteins (Trouillon 2010) have been used as electrode materials for *in vivo* and *in vitro* NO detection. Several comprehensive reviews on analytical methodologies of NO detection are available in the literature (Bedioui and Griveau 2013, Coneski 2012, Privett 2010, Chen 2011).

CHALLENGES, OPPORTUNITIES, AND FUTURE PERSPECTIVE

It is increasingly clear that electrochemistry is a promising technique for *in situ* real-time detection of neurotransmitters that can be used to answer important questions on the timing, dynamics, quantity, and spatial localization of these important signaling agents. Such devices have shown excellent performance for the detection of neurotransmitters with high sensitivity and selectivity, and can be particularly suitable for measuring disease conditions in which neurotransmitters play critical roles. Most work in the past five years concentrated on the development of new electrode coatings and the integration of nanotechnology in order to improve analytical characteristics: e.g. selectivity, sensitivity, and biocompatibility. Many types of nanoparticles and nanostructures (e.g. carbon nanotubes, metal, and metal oxide nanoparticless) as well as several types of polymers or hybrid combinations of organic-inorganic nanostructures have been used to fabricate membranes with enhanced biocompatibility and redox properties. In spite of progress, the application of electrochemical probes is still restricted mostly to measurements in standard conditions with some examples of functionality in *in vitro* systems that still require sample preparation. Progress has been slow in the translation of this technology from biosensor labs to biological or biomedical settings.

In the past five years, we see an increase in the use of these sensors to measure neurotransmitters released in individual tissues such as the hippocampus (Suzuki 2013, Njagi 2010, Ganesana 2012). Recently developed multielectrode arrays of planar carbon nanotubes on indium tin oxide microelectrodes enabled long term recording of electrophysiological activity of the neurotransmitter dopamine at nM concentrations and changes in the extracellular chemical microenvironment in mouse striatal brain slices (Suzuki 2013). Such microelectrode chips can be developed in the future for the detection of other neurotransmitters involved in brain function as well as for pharmacological evaluation for drug screening and toxicity assessment neurotransmitters

From the large body of literature that has been reported on the design of myriad of electrode configurations for the detection of neurotransmitters, very few papers deal with *in vivo*

measurements with electrodes inserted in tissue as diagnostic probes or to monitor disease status in whole organisms. Adoption of these sensors by the biomedical community to studies of neurotransmitter release *in vivo* has been hampered by the limited availability of commercially available biosensors and electrodes, their relatively high price, and the complexity of some of the detection methods. There is a need for better sensors and improved processing methods to extract the maximum information possible from these sensors, and there is a need to improve selectivity and sensitivity of these probes *in vivo*. Successful translation of this technology also relies on collaboration of electroanalytical chemists with neuroscientists. To improve these probes, several substantial challenges need to be overcome before electrochemical probes can be fully implemented in the biomedical practice for long term *in vivo* assessment of neurotransmitters where continuous readings are required. The issue of non-specific adsorption and reduced tissue reaction remains problematic and requires engineering of innovative membranes to prevent non-specific adsorption of proteins while also enabling effective electron transfer and biocompatibility. Elimination of interferences and overlapping signals from the many electroactive molecules in the physiological environment is another issue that requires better design of the interface and development of novel approaches and materials for selective membranes. It might require interfacing of electrochemistry with chromatographic separation, or, the use of pattern recognition techniques to discriminate signals. Similarly, variations in the oxygen level, temperature, or pH may result in spurious signals. Careful evaluation of these variables, testing of interferences in realistic conditions, and validation of sensor response with conventional methods must be performed to define the nature of the molecule, or molecules, that are responsible for the observed signals. Moreover, sensors can also lose their sensitivity during operation in biological system. In most cases (when reported), calibration post *in vivo* measurements is significantly reduced as compared to the pre-measurement calibration. This loss of sensitivity is in general attributed to electrode fouling or mechanical damage of the active area. Several novel biosensor designs including the use of self-referencing probes and appropriate calibration methods have been proposed to solve these problems. However, increasing the number of electrodes (e.g. adding a self-reference electrode) also increases the size of the electrode probe which can produce further tissue damage. Many electrodes are still too bulky to be implanted in tissue (~ 150 microns especially those that use enzymes) and require further miniaturization. The use of nanostructures to enlarge the surface area can be useful to further reduce the size of electrodes and stabilize enzymes, in sensing designs non-electrochemically active neurotransmitters that require biomolecular recognition. Better electronic communication between the redox active site of enzymes and the electrode (e.g. wiring of redox site of enzymes) is still needed. While several advances have been made in the use of redox polymers, the operation of these devices *in vivo* still requires further evaluation. Other catalytic materials or redox active particles can be used in the future to enhance electrical communication and prevent leaching of the mediator.

In the light of the recent developments in the electroanalytical and biosensing fields, fabrication of nanoscale sensors will be of further interest for the detection of neurotransmitters at the single cell level and for brain activity mapping. Recent reports on the use of nanopipettes and nanosensors technology for the detection of biomolecules in

confined environments such as single cells and neurons (Actis 2013, Clausmeyer 2014, Schrlau 2009). These nano-enabled devices open doors for the real-time investigation of physiological processes at the single-cell level with high spatial resolution and minimal tissue damage. With the increased availability of more sensitive electronic sensors and signal amplifiers, it might be possible in the future to fabricate an array of nanoelectrodes that can facilitate the investigation of brain physiology and study of neurodegenerative diseases. We envision that in the future, analytical chemists, biochemists, engineers and neuroscientists will work in close collaboration to fully explore applications of electroanalytical methods to address biomedical questions and fully demonstrate the value of this technology to the biomedical community.

ACKNOWLEDGMENTS

This work was supported by NIH #R21NS078738-01 and NSF #1200180. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the funding agencies.

REFERENCES

- Actis, Paolo; Maalouf, Michelle M.; Kim, Hyunsung John; Lohith, Akshar; Vilozy, Boaz; Adam Seger, R.; Pourmand, Nader. Compartmental Genomics in Living Cells Revealed by Single-Cell Nanobiopsy. *ACS Nano*. 2013; 8(1):546–553. [PubMed: 24279711]
- Adams RN, Murrill E, McCreery R, Blank L, Karolczak M. “6-Hydroxydopamine, a new oxidation mechanism. *Eur J Pharmacol*. 1972; 17(2):287–292. [PubMed: 4337349]
- Akyilmaz E, Turemis M, Yasa I. Voltammetric determination of epinephrine by White rot fungi (*Phanerochaete chrysosporium* ME446) cells based microbial biosensor. *Biosensors Bioelectronics*. 2011; 26(5):2590–2594. [PubMed: 21115238]
- Amine A, Kauffmann JM. Preparation and characterization of a fragile enzyme immobilized carbon paste electrode. *Bioelectrochemistry Bioenergetics*. 1992; 28(1–2):117–125.
- Baron R, Zayats M, Willner I. Dopamine-, L-DOPA-, adrenaline-, and noradrenaline-induced growth of Au nanoparticles: assays for the detection of neurotransmitters and of tyrosinase activity. *Anal. Chem*. 2005; 77(6):1566–1571. [PubMed: 15762558]
- Bath BD, Martin HB, Wightman RM, Anderson MR. Dopamine adsorption at surface modified carbon-fiber electrodes. *Langmuir*. 2001; 17(22):7032–7039.
- Batra, Bhawna; Pundir, CS. An amperometric glutamate biosensor based on immobilization of glutamate oxidase onto carboxylated multiwalled carbon nanotubes/gold nanoparticles/chitosan composite film modified Au electrode. *Biosensors Bioelectronics*. 2013; 47:496–501. [PubMed: 23628843]
- Bedioui, Fethi; Griveau, Sophie. Electrochemical Detection of Nitric Oxide: Assesment of Twenty Years of Strategies. *Electroanalysis*. 2013; 25(3):587–600.
- Behrend CE, Cassim SM, Pallone MJ, Daubenspeck JA, Hartov A, Roberts DW, Leiter JC. Toward Feedback Controlled Deep Brain Stimulation: Dynamics of Glutamate Release in the Subthalamic Nucleus in rats. *J. Neurosci. Meth*. 2009
- Beitollahi H, Karimi-Maleh H, Khabazzadeh H. Nanomolar and Selective Determination of Epinephrine in the Presence of Norepinephrine Using Carbon Paste Electrode Modified with Carbon Nanotubes and Novel 2-(4-Oxo-3-phenyl-3,4-dihydroquinazolinyl)-N ‘-phenyl-hydrazinecarbothioamide. *Anal. Chem*. 2008; 80(24):9848–9851. [PubMed: 19072278]
- Berman SB, Hastings TG. Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: implications for Parkinson’s disease. *J. Neurochemistry*. 1999; 73(3):1127–1137.
- Bhattachayay D, Pal P, Banerjee S, Sanyal SK, Turner APF, Sarkar P. Electrochemical acetylcholine chloride biosensor using an acetylcholine esterase biomimic. *Anal. Lett*. 2008; 41(8):1387–1397.

- Blank CL, Murrill E, Adams RN. Central nervous system effects of 6-aminodopamine and 6-hydroxydopamine. *Brain Res.* 1972; 45(2):635–637. [PubMed: 5085708]
- Brandon EP, Mellott T, Pizzo DP, Coufal N, D'Amour KA, Gobeske K, Lortie M, Lopez-Coviella I, Berse B, Thal LJ, Gage FH, Blusztajn JK. Choline transporter 1 maintains cholinergic function in choline acetyltransferase haploinsufficiency. *J. Neuroscience.* 2004; 24(24):5459–5466. [PubMed: 15201317]
- Burmeister JJ, Gerhardt GA. 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(5):1037–1042. [PubMed: 11289414]
- Burmeister JJ, Palmer M, Gerhardt GA. L-lactate measures in brain tissue with ceramic-based multisite microelectrodes. *Biosensors Bioelectronics.* 2005; 20(9):1772–1779. [PubMed: 15681193]
- Burmeister JJ, Pomerleau F, Huettl P, Gash CR, Wemer CE, Bruno JP, Gerhardt GA. Ceramic-based multisite microelectrode arrays for simultaneous measures of choline and acetylcholine in CNS. *Biosensors Bioelectronics.* 2008; 23(9):1382–1389. [PubMed: 18243683]
- Burmeister JJ, Pomerleau F, Palmer MJ, Day BK, Huettl P, Gerhardt GA. Improved ceramic-based multisite microelectrode for rapid measurements of L-glutamate in the CNS. *J. Neurosci. Meth.* 2002; 119:163–171.
- Castro SSL, Mortimer RJ, de Oliveira MF, Stradiotto NR. Electrooxidation and determination of dopamine using a Nafion (R)-cobalt hexacyanoferrate film modified electrode. *Sensors.* 2008; 8(3):1950–1959.
- Celebanska A, Tomaszewska D, Lesniewski A, Opallo M. Film electrode prepared from oppositely charged silicate submicroparticles and carbon nanoparticles for selective dopamine sensing. *Biosensors Bioelectronics.* 2011; 26(11):4417–4422.
- Chang JL, Wei GT, Zen JM. Screen-printed ionic liquid/preanodized carbon electrode: Effective detection of dopamine in the presence of high concentration of ascorbic acid. *Electrochemistry Comm.* 2011; 13(2):174–177.
- Cheer, Joseph F.; Wassum, Kate M.; Sombers, Leslie A.; Heien, Michael LAV.; Ariansen, Jennifer L.; Aragona, Brandon J.; Phillips, Paul EM.; Wightman, R Mark. Phasic Dopamine Release Evoked by Abused Substances Requires Cannabinoid Receptor Activation. *J. Neurosci.* 2007; 27(4):791–795. [PubMed: 17251418]
- Chen, Wei; Cai, Shu; Ren, Qiong-Qiong; Wen, Wei; Zhao, Yuan-Di. Recent advances in electrochemical sensing for hydrogen peroxide: a review. *Analyst.* 2012; 137(1):49–58. [PubMed: 22081036]
- Chen, Xiaoqiang; Tian, Xizhe; Shin, Injae; Yoon, Juyoung. Fluorescent and luminescent probes for detection of reactive oxygen and nitrogen species. *Chemical Society Rev.* 2011; 40(9):4783–4804.
- Chen, Yunfeng; Song, Huiting; Mao, Jiawei; Liu, Ming; Ding, Chao; Pan, Zhiquan. Design and synthesis of two macrocyclic dinuclear copper(II) complexes with reversible binding of nitric oxide. *Inorganic Chemistry Comm.* 2013; 27:131–137.
- Clausmeyer, Jan; Actis, Paolo; Córdoba, Ainara López; Korchev, Yuri; Schuhmann, Wolfgang. Nanosensors for the detection of hydrogen peroxide. *Electrochemistry Comm.* 2014; 40:28–30.
- Claussen, Jonathan C.; Artilles, Mayra S.; McLamore, Eric S.; Mohanty, Subhashree; Shi, Jin; Rickus, Jenna L.; Fisher, Timothy S.; Porterfield, D. Marshall Electrochemical glutamate biosensing with nanocube and nanosphere augmented single-walled carbon nanotube networks: a comparative study. *J. Materials Chem.* 2011; 21(30):11224–11231.
- Coneski, Peter N.; Schoenfisch, Mark H. Nitric oxide release: Part III. Measurement and reporting. *Chemical Society Rev.* 2012; 41(10):3753–3758.
- Cosnier S, Innocent C, Allien L, Poitry S, Tsacopoulos M. An electrochemical method for making enzyme microsenors. Application to the detection of dopamine and glutamate. *Anal. Chem.* 1997; 69(5):968–971. [PubMed: 21639234]
- Cui, Yue; Barford, John P.; Renneberg, Reinhard. Development of an interference-free biosensor for l-glutamate using a bienzyme salicylate hydroxylase/l-glutamate dehydrogenase system. *Enzyme Microbial Technol.* 2007; 41(6–7):689–693.
- Danbolt, Niels C. Glutamate uptake. *Progress Neurobiology.* 2001; 65(1):1–105.

- Dang, Xueping; Hu, Chengguo; Wang, Yingkai; Hu, Shengshui. Gold nanoparticle film grown on quartz fiber and its application as a microsensor of nitric oxide. *Sensors Actuators B: Chemical*. 2011; 160(1):260–265.
- Dazzi L, Seu E, Cherchi G, Biggio G. Antagonism of the stress-induced increase in cortical norepinephrine output by the selective norepinephrine reuptake inhibitor reboxetine. *Eur J Pharmacol*. 2003; 476(1–2):55–61. [PubMed: 12969749]
- Doaga, Rodica; McCormac, Timothy; Dempsey, Eithne. Electrochemical Sensing of NADH and Glutamate Based on Meldola Blue in 1,2-Diaminobenzene and 3,4-Ethylenedioxythiophene Polymer Films. *Electroanalysis*. 2009; 21(19):2099–2108.
- Dunphy R, Burinsky DJ. Detection of choline and acetylcholine in a pharmaceutical preparation using high-performance liquid chromatography/electrospray ionization mass spectrometry. *J. Pharmaceutical Biomedical Analysis*. 2003; 31(5):905–915.
- Duran N, Rosa MA, D'Annibale A, Gianfreda L. Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: a review. *Enzyme and Microbial Technology*. 2002; 31(7):907–931.
- Fang, Huaifang; Vickrey, Trisha L.; Venton, B Jill. Analysis of Biogenic Amines in a Single Drosophila Larva Brain by Capillary Electrophoresis with Fast-Scan Cyclic Voltammetry Detection. *Anal. Chem*. 2011; 83(6):2258–2264. [PubMed: 21322586]
- Featherstone, David E. Intercellular Glutamate Signaling in the Nervous System and Beyond. *ACS Chemical Neuroscience*. 2009; 1(1):4–12. [PubMed: 22778802]
- Finnerty, Niall J.; O'Riordan, Saidhbhe L.; Brown, Finbar O.; Serra, Pier A.; O'Neill, Robert D.; Lowry, John P. In vivo characterisation of a Nafion[registered sign]-modified Pt electrode for real-time nitric oxide monitoring in brain extracellular fluid. *Anal. Methods*. 2012; 4(2):550–557.
- Finnerty, Niall J.; O'Riordan, Saidhbhe L.; Palsson, Erik; Lowry, John P. Brain nitric oxide: Regional characterisation of a real-time microelectrochemical sensor. *J. Neuroscience Methods*. 2012; 209(1):13–21.
- Ganesana M, Erlichman JS, Andreescu S. Real-time monitoring of superoxide accumulation and antioxidant activity in a brain slice model using an electrochemical cytochrome c biosensor. *Free Radical Biology Medicine*. 2012; 53(12):2240–2249. [PubMed: 23085519]
- Garcia MG, Armendariz GME, Godinez LA, Torres J, Sepulveda-Guzman S, Bustos E. Detection of dopamine in non-treated urine samples using glassy carbon electrodes modified with PAMAM dendrimer-Pt composites. *Electrochim. Acta*. 2011; 56(22):7712–7717.
- Gholizadeh, Azam; Shahrokhian, Saeed; zad, Azam Iraj; Mohajerzadeh, Shamsoddin; Vosoughi, Manouchehr; Darbari, Sara; Sanaee, Zeinab. Mediator-less highly sensitive voltammetric detection of glutamate using glutamate dehydrogenase/vertically aligned CNTs grown on silicon substrate. *Biosensors Bioelectronics*. 2012; 31(1):110–115. [PubMed: 22040749]
- Goriushkina TB, Soldatkin AP, Dzyadevych SV. Application of Amperometric Biosensors for Analysis of Ethanol, Glucose, and Lactate in Wine. *J. Agricult. Food Chem*. 2009; 57(15):6528–6535.
- Govindarajan, Sridhar; McNeil, Calum J.; Lowry, John P.; McMahon, Colm P.; O'Neill, Robert D. Highly selective and stable microdisc biosensors for l-glutamate monitoring. *Sensors Actuators B: Chemical*. 2013; 178:606–614.
- Goyal RN, Bishnoi S. Simultaneous determination of epinephrine and norepinephrine in human blood plasma and urine samples using nanotubes modified edge plane pyrolytic graphite electrode. *Talanta*. 2011; 84(1):78–83. [PubMed: 21315901]
- Guerrieri A, Lattanzio V, Palmisano F, Zambonin PG. Electrosynthesized poly(pyrrole)/poly(2-naphthol) bilayer membrane as an effective anti-interference layer for simultaneous determination of acetylcholine and choline by a dual electrode amperometric biosensor. *Biosensors Bioelectronics*. 2006; 21(9):1710–1718. [PubMed: 16169212]
- Gunaratna PC, Wilson GS. Optimization of multienzyme flow reactors for determination of acetylcholine. *Anal. Chem*. 1990; 62(4):402–407. [PubMed: 2310017]
- Hamdi, Naser; Wang, Jianjun; Walker, Eric; Maidment, Nigel T.; Monbouquette, Harold G. An electroenzymatic l-glutamate microbiosensor selective against dopamine. *J. Electroanalytical Chem*. 2006; 591(1):33–40.

- Hascup KN, Hascup ER, Pomerleau F, Huettl P, Gerhardt GA. Second-by-second measures of L-glutamate in the prefrontal cortex and striatum of freely moving mice. *Journal of Pharmacology Experimental Therapeutics*. 2008; 324(2):725–731. [PubMed: 18024788]
- Hawkins, Clare L.; Davies, Michael J. Detection and characterisation of radicals in biological materials using EPR methodology. *Biochimica et Biophysica Acta (BBA)*.
- Heng, Julian Ik-Tsen; Moonen, Gustave; Nguyen, Laurent. Neurotransmitters regulate cell migration in the telencephalon. *European J. Neuroscience*. 2007; 26(3):537–546.
- Henstridge MC, Dickinson EJF, Aslanoglu M, Batchelor-McAuley C, Compton RG. Voltammetric selectivity conferred by the modification of electrodes using conductive porous layers or films: The oxidation of dopamine on glassy carbon electrodes modified with multiwalled carbon nanotubes. *Sensors Actuators B-Chemical*. 2010; 145(1):417–427.
- Ho Shim, Jun; Do, Hyunkyung; Lee, Youngmi. Simple Fabrication of Amperometric Nitric Oxide Microsensors Based on Electropolymerized Membrane Films. *Electroanalysis*. 2010; 22(3):359–366.
- Hou SF, Kasner ML, Su SJ, Patel K, Cuellari R. Highly Sensitive and Selective Dopamine Biosensor Fabricated with Silanized Graphene. *J. Phys. Chem. C*. 2010; 114(35):14915–14921.
- Huynh TP, Bikram KCC, Lisowski W, D'Souza F, Kutner W. Molecularly imprinted polymer of bis(2,2'-bithienyl)methanes for selective determination of adrenaline. *Bioelectrochemistry*. 2012
- Ispas, CR. Biosensors Based on Inorganic Nanoparticles with Biometric Properties : Biomedical Applications and in Vivo Cytotoxicity Measurements: A Thesis. Clarkson University; 2010.
- Ispas, Cristina R.; Crivat, Georgeta; Andreescu, Silvana. Review: Recent Developments in Enzyme-Based Biosensors for Biomedical Analysis. *Anal. Lett.* 2012; 45(2–3):168–186.
- Jackowska K, Krysinski P. New trends in the electrochemical sensing of dopamine. *Anal. Bioanal. Chem.* 2013; 405(11):3753–3771. [PubMed: 23241816]
- Jang DP, Kim I, Chang SY, Min HK, Arora K, Marsh MP, Hwang SC, Kimble CJ, Bennet KE, Lee KH. Paired pulse voltammetry for differentiating complex analytes. *Analyst*. 2012; 137(6):1428–1435. [PubMed: 22299131]
- Jeffries, Cy; Pasco, Neil; Baronian, Keith; Gorton, Lo. Evaluation of a thermophile enzyme for a carbon paste amperometric biosensor: L-glutamate dehydrogenase. *Biosensors Bioelectronics*. 1997; 12(3):225–232.
- Jeong H, Jeon S. Determination of Dopamine in the Presence of Ascorbic Acid by Nafion and Single-Walled Carbon Nanotube Film Modified on Carbon Fiber Microelectrode. *Sensors*. 2008; 8(11):6924–6935.
- Jo, Areum; Do, Hyunkyung; Jhon, Gil-Ja; Suh, Minah; Lee, Youngmi. Electrochemical Nanosensor for Real-Time Direct Imaging of Nitric Oxide in Living Brain. *Anal. Chem.* 2011; 83(21):8314–8319. [PubMed: 21942337]
- Kang, Tian-Fang; Shen, Guo-Li; Yu, Ru-Qin. Voltammetric behaviour of dopamine at nickel phthalocyanine polymer modified electrodes and analytical applications. *Anal. Chim. Acta*. 1997; 354(1–3):343–349.
- Keithley RB, Takmakov P, Bucher ES, Belle AM, Owesson-White CA, Park J, Wightman RM. Higher Sensitivity Dopamine Measurements with Faster-Scan Cyclic Voltammetry. *Anal. Chem.* 2011; 83(9):3563–3571. [PubMed: 21473572]
- Khan A, Ghani SAB. Multienzyme microbiosensor based on electropolymerized o-phenylenediamine for simultaneous in vitro determination of acetylcholine and choline. *Biosensors Bioelectronics*. 2012; 31(1):433–438. [PubMed: 22154168]
- Khan A, Khan AA, Asiri AM, Rub MA, Azum N, Rahman MM, Khan SB, Ghani SA. A new trend on biosensor for neurotransmitter choline/acetylcholine--an overview. *Appl Biochem. Biotechnol.* 2013; 169(6):1927–1939. [PubMed: 23354504]
- Kharian S, Teymoori N, Khalilzadeh MA. Multi-wall carbon nanotubes and TiO₂ as a sensor for electrocatalytic determination of epinephrine in the presence of p-chloranil as a mediator. *J. Solid State Electrochemistry*. 2012; 16(2):563–568.
- Kharitonov AB, Shipway AN, Willner I. An Au nanoparticle/bisbipyridinium cyclophane-functionalized ion sensitive field-effect transistor for the sensing of adrenaline. *Anal. Chem.* 1999; 71(23):5441–5443. [PubMed: 10596219]

- Kim YR, Bong S, Kang YJ, Yang Y, Mahajan RK, Kim JS, Kim H. Electrochemical detection of dopamine in the presence of ascorbic acid using graphene modified electrodes. *Biosensors Bioelectronics*. 2010; 25(10):2366–2369. [PubMed: 20307965]
- Kumar, Pankaj; Kalita, Apurba; Mondal, Biplab. Copper(ii) complexes as turn on fluorescent sensors for nitric oxide. *Dalton Transactions*. 2012; 41(35):10543–10548. [PubMed: 22825656]
- Lapp H, Spohn U, Janasek D. An enzymatic chemiluminescence optrode for choline detection under flow injection conditions. *Anal. Lett.* 1996; 29(1):1–17.
- Larsson N, Ruzgas T, Gorton L, Kokaia M, Kissinger P, Csoregi E. Design and development of an amperometric biosensor for acetylcholine determination in brain microdialysates. *Electrochimica Acta*. 1998; 43(23):3541–3554.
- Lee SR, Rahman MM, Ishida M, Sawada K. Development of a highly-sensitive acetylcholine sensor using a charge-transfer technique on a smart biochip. *Trac-Trends Anal. Chem.* 2009; 28(2):196–203.
- Li YH, Liu XS, Wei WZ. Square Wave Voltammetry for Selective Detection of Dopamine Using Polyglycine Modified Carbon Ionic Liquid Electrode. *Electroanalysis*. 2011; 23(12):2832–2838.
- Li YH, Liu XS, Zeng XD, Liu XY, Kong B, Wei WZ, Luo SL. Selective and sensitive detection of dopamine in the presence of ascorbic acid by molecular sieve/ionic liquids composite electrode. *Electrochimica Acta*. 2011; 56(6):2730–2734.
- Lobo, Maria Jesús; Miranda, Arturo J.; Tuñón, Paulino. Amperometric biosensors based on NAD(P)-dependent dehydrogenase enzymes. *Electroanalysis*. 1997; 9(3):191–202.
- Lu LP, Wang SQ, Lin XQ. Fabrication of layer-by-layer deposited multilayer films containing DNA and gold nanoparticle for norepinephrine biosensor. *Anal. Chim. Acta*. 2004; 519(2):161–166.
- Lu XQ, Li YY, Du J, Zhou XB, Xue ZH, Liu XH, Wang ZH. A novel nanocomposites sensor for epinephrine detection in the presence of uric acids and ascorbic acids. *Electrochimica Acta*. 2011; 56(21):7261–7266.
- Maciejewska J, Pisarek K, Bartosiewicz I, Kryszinski P, Jackowska K, Bieganski AT. Selective detection of dopamine on poly(indole-5-carboxylic acid)/tyrosinase electrode. *Electrochimica Acta*. 2011; 56(10):3700–3706.
- Majewska UE, Chmurski K, Biesiada K, Olszyna AR, Bilewicz R. Dopamine oxidation at per(6-deoxy-6-thio)-alpha-cyclodextrin monolayer modified gold electrodes. *Electroanalysis*. 2006; 18(15):1463–1470.
- Mano N, Heller A. Detection of glucose at 2 fM concentration. *Anal. Chem.* 2005; 77(2):729–732. [PubMed: 15649080]
- Manocha, Marcus; Khan, Waliul I. Serotonin and GI Disorders: An Update on Clinical and Experimental Studies. *Clin Trans Gastroenterol*. 2012; 3(13):6.
- Maragakis, Nicholas J.; Rothstein, Jeffrey D. Glutamate transporters: animal models to neurologic disease. *Neurobiology Disease*. 2004; 15(3):461–473.
- McAteer K, O'Neill RD. Strategies for decreasing ascorbate interference at glucose oxidase-modified poly(o-phenylenediamine)-coated electrodes. 1996
- McMahon, Colm P.; Killoran, Sarah J.; Kirwan, Sarah M.; O'Neill, Robert D. The selectivity of electrosynthesised polymer membranes depends on the electrode dimensions: implications for biosensor applications. *Chem. Comm.* 2004:2128–2130. [PubMed: 15368008]
- McMahon, Colm P.; Rocchitta, Gaia; Serra, Pier A.; Kirwan, Sarah M.; Lowry, John P.; O'Neill, Robert D. The efficiency of immobilised glutamate oxidase decreases with surface enzyme loading: an electrostatic effect, and reversal by a polycation significantly enhances biosensor sensitivity. *Analyst*. 2006; 131(1):68–72. [PubMed: 16365665]
- Min K, Yoo YJ. Amperometric detection of dopamine based on tyrosinase-SWNTs-Ppy composite electrode. *Talanta*. 2009; 80(2):1007–1011. [PubMed: 19836587]
- Mizutani, Fumio; Sato, Yukari; Hirata, Yoshiki; Sawaguchi, Takahiro; Yabuki, Soichi. Glucose oxidase/polyion complex-bilayer membrane for elimination of electroactive interferents in amperometric glucose sensor. *Anal. Chim. Acta*. 1998; 364(1–3):173–179.
- Mizutani, Fumio; Sato, Yukari; Sawaguchi, Takahiro; Yabuki, Soichi; Iijima, Seiichiro. Rapid measurement of transaminase activities using an amperometric l-glutamate-sensing electrode

based on a glutamate oxidase-polyion complex-bilayer membrane. *Sensors Actuators B: Chemical*. 1998; 52(1–2):23–29.

Mohammad-Zadeh LF, Moses L, Gwaltney-Brant SM. Serotonin: a review. *J. Veterinary Pharmacology Therapeutics*. 2008; 31(3):187–199.

Munoz P, Huenchuguala S, Paris I, Segura-Aguilar J. Dopamine oxidation and autophagy. *Parkinsons Dis*. 2012; 2012:920–953.

Nagy PI, Alagona G, Ghio C. Theoretical studies on the conformation of protonated dopamine in the gas phase and in aqueous solution. *J. Am. Chem. Soc*. 1999; 121(20):4804–4815.

Naylor, Erik; Aillon, Daniel V.; Gabbert, Seth; Harmon, Hans; Johnson, David A.; Wilson, George S.; Petillo, Peter A. Simultaneous real-time measurement of EEG/EMG and l-glutamate in mice: A biosensor study of neuronal activity during sleep. *J. Electroanalytical Chem*. 2011; 656(1–2):106–113.

Njagi J, Chernov MM, Leiter JC, Andreescu S. Amperometric Detection of Dopamine in Vivo with an Enzyme Based Carbon Fiber Microbiosensor. *Anal. Chem*. 2010; 82(3):989–996. [PubMed: 20055419]

Njagi J, Ispas C, Andreescu S. Mixed ceria-based metal oxides biosensor for operation in oxygen restrictive environments. *Anal. Chem*. 2008; 80(19):7266–7274. [PubMed: 18720950]

Njagi, John; Ball, Michael; Best, Marc; Wallace, Kenneth N.; Andreescu, Silvana. Electrochemical Quantification of Serotonin in the Live Embryonic Zebrafish Intestine. *Analytical Chemistry*. 2010; 82(5):1822–1830. [PubMed: 20148518]

Njagi, John; Erlichman, Joseph S.; Aston, Jeffrey W.; Leiter, JC.; Andreescu, Silvana. A sensitive electrochemical sensor based on chitosan and electropolymerized Meldola blue for monitoring NO in brain slices. *Sensors Actuators B: Chemical*. 2010; 143(2):673–680.

Oldenziel WH, Dijkstra G, Cremers TIFH, Westerink BHC. In vivo monitoring of extracellular glutamate in the brain with a microsensor. *Brain Research*. 2006; 1118(1):34–42. [PubMed: 16956598]

Oni, Joshua; Diab, Nizam; Reiter, Sabine; Schuhmann, Wolfgang. Metallophthalocyanine-modified glassy carbon electrodes: effects of film formation conditions on electrocatalytic activity towards the oxidation of nitric oxide. *Sensors Actuators B: Chemical*. 2005; 105(2):208–213.

Opländer, Christian; Volkmar, Christine M.; Paunel-Görgülü, Adnana; Fritsch, Thomas; van Faassen, Ernst E.; Mürtz, Manfred; Grieb, Gerrit; Bozkurt, Ahmet; Hemmrich, Karsten; Windolf, Joachim; Suschek, Christoph V. Dermal application of nitric oxide releasing acidified nitrite-containing liniments significantly reduces blood pressure in humans. *Nitric Oxide*. 2012; 26(2):132–140. [PubMed: 22306967]

Ozel, Rfat Emrah; Wallace, Kenneth N.; Andreescu, Silvana. Alterations of intestinal serotonin following nanoparticle exposure in embryonic zebrafish. *Environmental Science: Nano*. 2014; 1(1):27–36. [PubMed: 24639893]

Özel, Rıfat Emrah; Alkasir, Ramiz SJ.; Ray, Kayla; Wallace, Kenneth N.; Andreescu, Silvana. Comparative Evaluation of Intestinal Nitric Oxide in Embryonic Zebrafish Exposed to Metal Oxide Nanoparticles. *Small*. 2013

Özel, Rıfat Emrah; Ispas, Cristina; Ganesana, Mallikarjunarao; Leiter, JC.; Andreescu, Silvana. Glutamate oxidase biosensor based on mixed ceria and titania nanoparticles for the detection of glutamate in hypoxic environments. *Biosensors Bioelectronics*. 2014; 52:397–402. [PubMed: 24090755]

Özel, Rıfat Emrah; Wallace, Kenneth N.; Andreescu, Silvana. Chitosan coated carbon fiber microelectrode for selective in vivo detection of neurotransmitters in live zebrafish embryos. *Anal. Chim. Acta*. 2011; 695(1–2):89–95. [PubMed: 21601035]

P Lowry, John; Ryan, Michael R.; O'Neill, Robert D. Behaviourally induced changes in extracellular levels of brain glutamate monitored at 1 s resolution with an implanted biosensor. *Anal. Comm*. 1998; 35(3):87–89.

Pan, Shengtian; Arnold, Mark A. Selectivity enhancement for glutamate with a Nafion/glutamate oxidase biosensor. *Talanta*. 1996; 43(7):1157–1162. [PubMed: 18966594]

- Park, Sarah S.; Hong, Minyoung; Song, Cha-Kyong; Jhon, Gil-Ja; Lee, Youngmi; Suh, Minah. Real-Time in Vivo Simultaneous Measurements of Nitric Oxide and Oxygen Using an Amperometric Dual Microsensor. *Anal. Chem.* 2010; 82(18):7618–7624. [PubMed: 20715758]
- Pasco N, Jeffries C, Davies Q, Downard AJ, Roddick-Lanzilotta AD, Gorton L. Characterisation of a thermophilic L-glutamate dehydrogenase biosensor for amperometric determination of L-glutamate by flow injection analysis. *Biosensors Bioelectronics.* 1999; 14(2):171–178. [PubMed: 10101839]
- Peng, Yanfen; Ji, Yiping; Zheng, Dongyun; Hu, Shengshui. In situ monitoring of nitric oxide release from rat kidney at poly(eosin b)-ionic liquid composite-based electrochemical sensors. *Sensors Actuators B: Chemical.* 2009; 137(2):656–661.
- Perry, Maura; Li, Qiang; Kennedy, Robert T. Review of recent advances in analytical techniques for the determination of neurotransmitters. *Anal. Chim. Acta.* 2009; 653(1):1–22. [PubMed: 19800472]
- Pomerleau F, Day BK, Huettl P, Burmeister JJ, Gerhardt GA. Real Time in Vivo Measures of l-Glutamate in the Rat Central Nervous System Using Ceramic-Based Multisite Microelectrode Arrays. *Annals New York Academy Sciences.* 2003; 1003(1):454–457.
- Porras-Gutierrez, Ana; Griveau, Sophie; Richard, Cyrille; Pailleret, Alain; Gutierrez-Granados, Silvia; Bedioui, Fethi. Hybrid Materials from Carbon Nanotubes, Nickel Tetrasulfonated Phthalocyanine and Thin Polymer Layers for the Selective Electrochemical Activation of Nitric Oxide in Solution. *Electroanalysis.* 2009; 21(21):2303–2310.
- Prakash, Subash; Rajesh, Seenivasan; Singh, Sarkkarai Raja; Karunakaran, Chandran; Vasu, Veerapandy. Electrochemical incorporation of hemin in a ZnO-PPy nanocomposite on a Pt electrode as NOx sensor. *Analyst.* 2012; 137(24):5874–5880. [PubMed: 23113320]
- Privett, Benjamin J.; Shin, Jae Ho; Schoenfish, Mark H. Electrochemical nitric oxide sensors for physiological measurements. *Chem. Soc. Rev.* 2010; 39(6):1925–1935. [PubMed: 20502795]
- Quintero, Jorge E.; Pomerleau, François; Huettl, Peter; Johnson, Kirk W.; Offord, James; Gerhardt, Greg A. Methodology for rapid measures of glutamate release in rat brain slices using ceramic-based microelectrode arrays: Basic characterization and drug pharmacology. *Brain Research.* 2011; 1401:1–9. [PubMed: 21664606]
- R Ryan, Michael; Lowry, John P.; O'Neill, Robert D. Biosensor for Neurotransmitter L-Glutamic Acid Designed for Efficient Use of L-Glutamate Oxidase and Effective Rejection of Interference. *Analyst.* 1997; 122(11):1419–1424. [PubMed: 9474818]
- Reddy S, Swamy BEK, Jayadevappa H. CuO nanoparticle sensor for the electrochemical determination of dopamine. *Electrochimica Acta.* 2012; 61:78–86.
- Roach, Gillian; Wallace, Rachel Heath; Cameron, Amy; Ozel, Rifat Emrah; Hongay, Cintia F.; Baral, Reshica; Andreescu, Silvana; Wallace, Kenneth N. Loss of ascl1a prevents secretory cell differentiation within the zebrafish intestinal epithelium resulting in a loss of distal intestinal motility. *Developmental Biology.* 2013; 376(2):171–186. [PubMed: 23353550]
- Robinson DL, Hermans A, Seipel AT, Wightman RM. Monitoring rapid chemical communication in the brain. *Chem. Rev.* 2008a; 108(7):2554–2584. [PubMed: 18576692]
- Robinson DL, Hermans A, Seipel AT, Wightman RM. Monitoring rapid chemical communication in the brain. *Chem. Rev.* 2008b; 108:2554–2584. [PubMed: 18576692]
- Robinson, Donita L.; Venton, B Jill; Heien, Michael LAV.; Wightman, R Mark. Detecting Subsecond Dopamine Release with Fast-Scan Cyclic Voltammetry in Vivo. *Clin. Chem.* 2003; 49(10):1763–1773. [PubMed: 14500617]
- Ronit, Freeman; Johann, Elbaz; Ron, Gill; Maya, Zayats; Itamar, Willner. Analysis of Dopamine and Tyrosinase Activity on Ion-Sensitive Field-Effect Transistor (ISFET) Devices. *Chemistry - A European J.* 2007; 13(26):7288–7293.
- Salaita, Ghaleb N.; Laguren-Davidson, Laarni; Lu, Frank; Walton, Nicholas; Wellner, Edna; Stern, Donald A.; Batina, Nikola; Frank, Douglas G.; Lin, Chiu-Hsun; Benton, Clifford S.; Hubbard, Arthur T. Electrochemical reactivity of 2,2',5,5'-tetrahydroxybiphenyl and related compounds adsorbed at pt (111) surfaces: studies by eels, leed, auger spectroscopy and cyclic voltammetry. *J. Electroanalytical Chem. Interfacial Electrochemistry.* 1988; 245(1–2):253–273.

- Santos, Ricardo M.; Rodrigues, Marcelo S.; Laranjinha, João; Barbosa, Rui M. Biomimetic sensor based on hemin/carbon nanotubes/chitosan modified microelectrode for nitric oxide measurement in the brain. *Biosensors Bioelectronics*. 2013; 44:152–159. [PubMed: 23419387]
- Santos, Vanessa N.; Mendonça, Glaydson LF.; Freire, Valder N.; Holanda, Alda KM.; Sousa, Jackson R.; Lopes, Luiz GF.; Ellena, Javier; Correia, Adriana N.; de Lima-Neto, Pedro. Electrochemical and Monte Carlo studies of self-assembled trans-[Fe(cyclam)(NCS)₂]⁺ complex ion on gold surface as electrochemical sensor for nitric oxide. *Electrochimica Acta*. 2013; 91:1–10.
- Sazonova N, Njagi JI, Marchese ZS, Ball MS, Andreescu S, Schuckers S. Detection and prediction of concentrations of neurotransmitters using voltammetry and pattern recognition. 2009 Annual International Conference of the Ieee Engineering in Medicine and Biology Society, Vols. 2009; 1–20:3493–3496.
- Schrlau, Michael G.; Dun, Nae J.; Bau, Haim H. Cell Electrophysiology with Carbon Nanopipettes. *ACS Nano*. 2009; 3(3):563–568. [PubMed: 19309170]
- Shahrokhi S, Saberi RS. Electrochemical preparation of over-oxidized polypyrrole/multi-walled carbon nanotube composite on glassy carbon electrode and its application in epinephrine determination. *Electrochimica Acta*. 2011; 57:132–138.
- Sheikh, Saba; Safia; Haque, Ejazul; Mir, Snobar S. Neurodegenerative Diseases: Multifactorial Conformational Diseases and Their Therapeutic Interventions. *J. Neurodegenerative Diseases*. 2013; 2013:8.
- Shimomura T, Itoh T, Sumiya T, Mizukami F, Ono M. Amperometric biosensor based on enzymes immobilized in hybrid mesoporous membranes for the determination of acetylcholine. *Enzyme Microbial Technol*. 2009; 45(6–7):443–448.
- Shon YM, Lee KH, Goerss SJ, Kim IY, Kimble C, Van Gompel JJ, Bennet K, Blaha CD, Chang SY. High frequency stimulation of the subthalamic nucleus evokes striatal dopamine release in a large animal model of human DBS neurosurgery. *Neurosci Lett*. 2010; 475(3):136–40. [PubMed: 20347936]
- Si P, Chen HL, Kannan P, Kim DH. Selective and sensitive determination of dopamine by composites of polypyrrole and graphene modified electrodes. *Analyst*. 2011; 136(24):5134–5138. [PubMed: 22010122]
- Singh, Yogesh S.; Sawarynski, Lauren E.; Michael, Heather M.; Ferrell, Robert E.; Murphey-Corb, Michael A.; Swain, Greg M.; Patel, Bhavik A.; Andrews, Anne M. Boron-Doped Diamond Microelectrodes Reveal Reduced Serotonin Uptake Rates in Lymphocytes from Adult Rhesus Monkeys Carrying the Short Allele of the 5-HTTLPR. *ACS Chemical Neuroscience*. 2009; 1(1): 49–64. [PubMed: 20352073]
- Stefan-van Staden RI, Moldoveanu I, van Staden JF. Pattern recognition of neurotransmitters using multimode sensing. *J. Neuroscience Methods*. 2014; 229:1–7.
- Stoyanova, Aneliya; Tsakova, Vessela. Copper-modified poly(3,4-ethylenedioxythiophene) layers for selective determination of dopamine in the presence of ascorbic acid: II Role of the characteristics of the metal deposit. *J. Solid State Electrochemistry*. 2010; 14(11):1957–1965.
- Suzuki A, Ivandini TA, Yoshimi K, Fujishima A, Oyama G, Nakazato T, Hattori N, Kitazawa S, Einaga Y. Fabrication, characterization, and application of boron-doped diamond microelectrodes for in vivo dopamine detection. *Anal. Chem*. 2007; 79(22):8608–8615. [PubMed: 17918970]
- Suzuki I, Fukuda M, Shirakawa K, Jiko H, Gotoh M. Carbon nanotube multi-electrode array chips for noninvasive real-time measurement of dopamine, action potentials, and postsynaptic potentials. *Biosensors Bioelectronics*. 2013; 49:270–275. [PubMed: 23774164]
- Swamy, BE Kumara; Venton, B. Jill Carbon nanotube-modified microelectrodes for simultaneous detection of dopamine and serotonin in vivo. *Analyst*. 2007; 132:876–884. [PubMed: 17710262]
- Tang H, Lin P, Chan HLW, Yan F. Highly sensitive dopamine biosensors based on organic electrochemical transistors. *Biosensors Bioelectronics*. 2011; 26(11):4559–4563. [PubMed: 21652201]
- Tembe S, Karve M, Inamdar S, Haram S, Melo J, D'Souza SF. Development of electrochemical biosensor based on tyrosinase immobilized in composite biopolymeric film. *Anal. Biochem*. 2006; 349(1):72–77. [PubMed: 16360108]

- Thiagarajan S, Chen SM. Applications of nanostructured Pt-Au hybrid film for the simultaneous determination of catecholamines in the presence of ascorbic acid. *J. Solid State Electrochemistry*. 2009; 13(3):445–453.
- Thomas A, Geyer H, Mester HJ, Schanzer W, Zimmermann E, Thevis M. Quantitative determination of adrenaline and noradrenaline in urine using liquid chromatography-tandem mass spectrometry. *Chromatographia*. 2006; 64(9–10):587–591.
- Trouillon, Raphaël; Cheung, Christine; Patel, Bhavik Anil; O'Hare, Danny. Electrochemical study of the intracellular transduction of vascular endothelial growth factor induced nitric oxide synthase activity using a multi-channel biocompatible microelectrode array. *Biochimica et Biophysica Acta (BBA) - General Subjects*. 2010; 1800(9):929–936. [PubMed: 20435094]
- Tsafack VC, Marquette CA, Pizzolato F, Blum LJ. Chemiluminescent choline biosensor using histidine-modified peroxidase immobilised on metal-chelate substituted beads and choline oxidase immobilised on anion-exchanger beads co-entrapped in a photocrosslinkable polymer. *Biosensors Bioelectronics*. 2000; 15(3–4):125–133. [PubMed: 11286329]
- Ulubay S, Dursun Z. Cu nanoparticles incorporated polypyrrole modified GCE for sensitive simultaneous determination of dopamine and uric acid. *Talanta*. 2010; 80(3):1461–1466. [PubMed: 20006114]
- Vahjen W, Bradley J, Bilitewski U, Schmid RD. Mediated Enzyme Electrode for the Determination of L-Glutamate. *Anal. Lett.* 1991; 24(8):1445–1452.
- Vinu Mohan AM, Aswini KK, Biju VM. Electrochemical codeposition of gold particle-poly(2-(2-pyridyl) benzimidazole) hybrid film on glassy carbon electrode for the electrocatalytic oxidation of nitric oxide. *Sensors Actuators B: Chemical*. 2014; 196:406–412.
- Walker E, Wang J, Hamdi N, Monbouquette HG, Maidment NT. Selective detection of extracellular glutamate in brain tissue using microelectrode arrays coated with over-oxidized polypyrrole. *Analyst*. 2007; 132(11):1107–1111. [PubMed: 17955144]
- Wang CI, Chen WT, Chang HT. Enzyme Mimics of Au/Ag Nanoparticles for Fluorescent Detection of Acetylcholine. *Anal. Chem.* 2012; 84(22):9706–9712. [PubMed: 23101755]
- Wang, Yan; Zhang, Xiuhua; Chen, Yang; Xu, Hui; Tan, Yumei; Wang, Shengfu. Detection of dopamine based on tyrosinase-Fe₃O₄ nanoparticles-chitosan nanocomposite biosensor. *Am J Biomed Sci*. 2010; 2:209–216.
- Wang ZH, Xia JF, Zhu LY, Chen XY, Zhang FF, Yao SY, Li YH, Xia YZ. A Selective Voltammetric Method for Detecting Dopamine at Quercetin Modified Electrode Incorporating Graphene. *Electroanalysis*. 2011; 23(10):2463–2471.
- Wightman, R Mark; May, Leslie J.; Michael, Adrian C. Detection of dopamine dynamics in the brain. *Anal. Chem.* 1988; 60(13):769A–779A.
- Wilson, George S.; Ammam, Malika. In vivo biosensors. *FEBS Journal*. 2007; 274(21):5452–5461. [PubMed: 17937773]
- Wood, Kevin M.; Hashemi, Parastoo. Fast-Scan Cyclic Voltammetry Analysis of Dynamic Serotonin Responses to Acute Escitalopram. *ACS Chemical Neuroscience*. 2013; 4(5):715–720. [PubMed: 23597074]
- Wu L, Feng LY, Ren JS, Qu XG. Electrochemical detection of dopamine using porphyrin-functionalized graphene. *Biosensors Bioelectronics*. 2012; 34(1):57–62. [PubMed: 22341756]
- Xue W, Cui TH. A High-Resolution Amperometric Acetylcholine Sensor Based on Nano-Assembled Carbon Nanotube and Acetylcholinesterase Thin Films. *J. Nano Res.* 2008a; 1:1–9.
- Xue W, Cui TH. A thin-film transistor based acetylcholine sensor using self-assembled carbon nanotubes and SiO₂ nanoparticles. *Sensors Actuators B-Chemical*. 2008b; 134(2):981–987.
- Yao T, Suzuki S, Nishino H, Nakahara T. On-line amperometric assay of glucose, L-glutamate, and acetylcholine using microdialysis probes and immobilized enzyme reactors. *Electroanalysis*. 1995; 7(12):1114–1117.
- Yap CM, Xu GQ, Ang SG. Amperometric Nitric Oxide Sensor Based on Nanoporous Platinum Phthalocyanine Modified Electrodes. *Anal. Chem.* 2012; 85(1):107–113. [PubMed: 23173722]
- Yoshimi K, Naya Y, Mitani N, Kato T, Inoue M, Natori S, Takahashi T, Weitemier A, Nishikawa N, McHugh T, Einaga Y, Kitazawa S. Phasic reward responses in the monkey striatum as detected by voltammetry with diamond microelectrodes. *Neuroscience Res*. 2011; 71(1):49–62.

- Young, Simon N.; Leyton, Marco. The role of serotonin in human mood and social interaction: Insight from altered tryptophan levels. *Pharmacology Biochemistry Behavior*. 2002; 71(4):857–865.
- Zachek MK, Hermans A, Wightman RM, McCarty GS. Electrochemical dopamine detection: Comparing gold and carbon fiber microelectrodes using background subtracted fast scan cyclic voltammetry. *J. Electroanalytical Chem*. 2008; 614(1–2):113–120.
- Zhang, Maogen; Mullens, Conor; Gorski, Waldemar. Chitosan-Glutamate Oxidase Gels: Synthesis, Characterization, and Glutamate Determination. *Electroanalysis*. 2005; 17(23):2114–2120.
- Zheng Y, Wang Y, Yang XR. Aptamer-based colorimetric biosensing of dopamine using unmodified gold nanoparticles. *Sensors Actuators B-Chemical*. 2011; 156(1):95–99.
- Zhu MF, Zeng CQ, Ye JS. Graphene-Modified Carbon Fiber Microelectrode for the Detection of Dopamine in Mice Hippocampus Tissue. *Electroanalysis*. 2011; 23(4):907–914.
- Zhu ZH, Qu LN, Guo YQ, Zeng Y, Sun W, Huang XT. Electrochemical detection of dopamine on a Ni/Al layered double hydroxide modified carbon ionic liquid electrode. *Sensors Actuators B-Chemical*. 2010; 151(1):146–152.

Figure 1.
(A) Amperometric detection of dopamine with a metal oxide/tyrosinase modified microelectrode at rat striatum. *In vivo* recordings were obtained at an applied potential of -150 mV (vs. Ag/AgCl reference electrode). No amperometric signal was observed at enzyme-free sensor (green line). (B) Histochemical images of the brain slice showing the stimulation site (left) and measurement site (right) (Njagi 2010).

Figure 2.

A: Schematic representation depicting insertion of the microelectrode in the intestine of 5dpf zebrafish embryos and the anterior (blue-B), mid (green – C) and posterior (red – D) segments of the 5 dpf embryonic intestine. The colors match the line color of the voltammetric profiles measured with the microsensor *in vivo* at the respective locations within the intestine. **B, C, D:** Anti-5-HT immunohistochemistry (fluorescence images on the left panel) and differential pulse voltammograms (diagrams on the right panel) measured at

the anterior (B), mid (C) and posterior (D) parts of the intestine. The supporting electrolyte was E3 medium. From reference (Njagi 2010) with permission.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript