GOLD NANOPARTICLE-MODIFIED CARBON FIBER MICROELECTRODES FOR ENHANCED NEUROTRANSMITTER DETECTION

By

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ABSTRACT

Carbon fiber microelectrodes (CFMEs) are the standard biosensors for monitoring neurotransmitters due to their biocompatibility, size, and electrochemical properties. However, unmodified-CFMEs have been known to have several limitations for neurotransmitter detection with fast scan cyclic voltammetry (FSCV). Because carbon fiber microelectrodes are mostly composed of basal plane carbon, unmodified-electrodes have relatively low electroactive surface areas that lead to poor sensitivities. Additionally, surface fouling may occur with certain neurochemicals that could potentially obstruct further neurotransmitter adsorption onto the electrode surface. Recently, there has been an emphasis on improving the sensitivity of CFMEs, and the most widespread method of enhancing electrode functionality is achieved by coating the fiber surface with a conductive polymer or another form of carbon. In this study, the electrochemical detection of neurotransmitters is enhanced by electrodepositing gold nanoparticles (AuNPs) onto the fiber surface using electrodeposition via cyclic voltammetry. Gold is an ideal electrode material for neurochemical detection as it is a stable, conductive, and a relatively inert metal.

The modified-electrodes improved sensitivity of dopamine detection with respect to unmodified electrodes. The thin and uniform layer of gold nanoparticles on the fiber-surface increased electroactive surface area for dopamine adsorption, which lead to considerably higher peak oxidative currents and lower limits of detection. The potential separation of the oxidation

and reduction peaks (ΔE_p) of the AuNP-CFMEs was smaller, suggesting higher conductivity and faster electron transfer kinetics which is ideal for measuring the fast-phasic firing of dopaminergic neurons and fast neurochemical fluctuations in the brain. Furthermore, the AuNP-modified electrodes also possessed uniform stability for dopamine detection for over a four-hour period and potentially beyond which is the typical time period for *in vivo* experiments. This is significant as it denoted the potential applicability of the AuNP-CFMEs for *in vivo* dopamine dynamics testing. Additionally, a linear rise in peak oxidative current readings was observed with the AuNP-modified electrodes with respect to both dopamine concentration and scan rate, indicative of dopamine adsorption control and kinetic control to the electrode surface.

This study has a myriad of applications for enhanced neurochemical detection and is crucial for the development of novel electrode sensors for *in vivo* neurotransmitter measurements to analyze the neurochemical effects of drug abuse and other psychostimulants, Parkinson's disease, depression, and other behavioral states and pharmacological effects.

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Besides my advisor, I would also like to thank the rest of my thesis committee: Dr. Shouzhong Zou and Dr. Matthew Hartings alongside the professors of the Chemistry Department here at American University, for their insightful comments, continuous support and encouragement. My sincere thanks also goes to the rest of the Zestos lab members (Pauline Wonnenberg and Alex Mendoza) for all of the thought-provoking discussions and emotional support throughout my time here at American University. To this list I would also like to add Casey Culhane and Raquel Lara: members of the Experimental Biochemistry Lab II group, both whom started this research with me as a class project.

Last but not least, I would like to thank my friends and family: my parents Shanthi Devi and Mohanaraj Thangaveloo, my sister Kayshana Mohanaraj, my dearest friend India Gregory, for all of their love and support throughout my life.

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LIST OF ILLUSTRATIONS

LIST OF ABBREVIATIONS

CFME	Carbon Fiber-Microelectrode
FSCV	Fast-Scan Cyclic Voltammetry
AuNP	Gold Nanoparticle
AuNP-CFME	Gold Nanoparticle-Carbon Fiber Micro-Electrode
SEM	Scanning Electron Microscopy
EDS/EDX	Energy-Dispersive X-ray Spectroscopy
LOD	Limit of Detection
FRET	Fluorescence/Forster Resonance Energy Transfer
PET	Photoinduced Electron Transfer
MMPs	Magnetic Microparticles
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
DA	Dopamine
PD	Parkinson's Disease
PEI	Polyethyleneimine
DOQ	Dopamine-Ortho-Quinone
DOPAC	3,4-Dihydroxyphenylacetic
HDCV	High-Definition Cyclic Voltammetry
PEDOT	Poly(3,4-Ethylenedioxythiophene)
CNS	Central Nervous System

CHAPTER 1

PROJECT GOALS

The aim of this study is to enhance the use of carbon-fiber microelectrodes for neurochemical measurements through the electrodeposition of gold nanoparticles onto the carbon-fiber surface. This study will be divided into the objectives listed below and conducted in the following order:

1. Simultaneous formation of gold nanoparticles using cyclic voltammetry and modification of fiber surface through the electrodeposition of gold nanoparticles onto the carbon fiber surfaces (Figure 2).
    a. The size and morphology of the gold nanoparticles is been proven to significantly influence catalytic efficiency; hence, 20 cycles, and an electrodeposition time of ~1 minute per scan cycle is optimal.[1]
    b. A bare carbon fiber will be imaged using scanning electron microscopy (SEM) to not only analyze surface features, but to also ensure a direct comparison to the modified electrodes.
    c. Following electrodeposition, each AuNP-CFME will again be imaged using SEM to analyze surface features such as the size, uniformity, and distribution of the gold-nanoparticles.
    d. Energy-dispersive x-ray spectroscopy (EDS/EDX) measurements will be done in conjunction with SEM to further confirm the presence of AuNPs.
2. *In-vitro* dopamine oxidation detection via fast-scan cyclic voltammetry (FSCV).
    a. Each unmodified electrode will be characterized through a series of experiments prior to surface modification as listed below:

i. Stability Experiment (0 hours, 1 hour, 2 hours, 3 hours, 4 hours)
    1. The unmodified carbon fiber microelectrode will be placed in the flow cell for four hours. Measurements will be taken at every hour for the detection of 1μM dopamine.
    2. A stable response to dopamine up to four hours without water oxidation is important to test its viability to conduct *in vivo* neurochemical measurements.

ii. Scan Rate Experiment (50V/s, 100V/s, 200V/s, 500V/s, 750V/s, 1000V/s)
    1. The scan rate will be varied from 50V/s to 1000V/s for 1μM dopamine to evaluate dopamine adsorption control to the electrode surface.

iii. Concentration Experiment (50nM, 100nM, 500nM, 1μM, 5μM, 10μM, 25μM, 50μM, 100μM)
    1. The dopamine concentration will be varied from 50nM to 100μM to evaluate dopamine adsorption control to the electrode surface for physiologically relevant dopamine concentrations in the brain.

b. Following the electrodeposition of the gold nanoparticles and optical and chemical characterizations, each AuNP-CFME will be characterized through a series of experiments identical to the ones previously mentioned.

3. Electrochemical performance evaluation of unmodified- and AuNP-CFMEs for the detection of dopamine oxidation

a. The changes in peak oxidative current, stability, and the potential peak separation (ΔE_p) will be examined to compare the overall electrochemical performance of both unmodified- and AuNP-CFMEs

After a direct comparison between the unmodified and gold-nanoparticle modified carbon fiber-microelectrodes, the AuNP-CFMEs are hypothesized to possess higher sensitivity for neurochemical measurements due to several factors. First, the thin and uniform coat of gold nanoparticles on the electrode surface should render a higher electroactive surface area relative to the unmodified electrode; thus, increasing the capability of neurotransmitter adsorption onto the electrode (modified) surface, which would yield higher peak oxidative currents at lower limits of detection (LOD). Furthermore, the peak-to-peak separation (ΔE_p) in the cyclic voltammograms of the AuNP-CFMEs could potentially be smaller, suggesting faster electron transfer kinetics due to the higher conductivity of the metallic gold electrode surface. Finally, the modified electrodes are also thought to possess uniform stability to dopamine oxidation for over four hours, which is the typical duration of *in vivo* experiments utilizing fast scan cyclic voltammetry and carbon fiber microelectrodes.

CHAPTER 2

INTRODUCTION

Introduced nearly forty years ago, carbon fiber microelectrodes (CFMEs) are the standard biosensors for neurochemical measurements.[2] The biocompatibility and size (<10μm in diameter) of carbon fibers make them optimal for implantation because there is reduced tissue damage and they are minimally invasive with respect to larger standard electrodes.[2] Furthermore, CFMEs are known to possess compatible electrochemical properties and are able of making quick measurements when used with fast electrochemical techniques, most commonly fast-scan cyclic voltammetry (FSCV).[2]

Initially, coupling electrochemistry and electrophysiology to measure electrically-induced dopamine release and neuronal activity in freely-moving animal models was a challenge.[3] However, the Wightman group successfully developed software and technology that were capable of performing such measurements.[4] The large charging current produced by scanning at fast rates is relatively stable at the carbon fiber working electrode, and this current can also be subtracted to yield a background-subtracted cyclic voltammogram (separating the faradaic and non-faradaic current), which is helpful in identifying and quantifying neurotransmitter release.[2] By varying the electrode to voltage measurement between cycles, the electrode can be used for electrophysiological purposes such as measuring firing patterns of neurons adjacent to the electrode.[2]

However, unmodified electrodes have been known to have several limitations. Because carbon fibers are largely comprised of basal plane carbon, unmodified electrodes have relatively low surface areas, leading to relatively low sensitivities.[5] Additionally, surface fouling may occur with certain neurochemicals and macromolecules that may form polymers at the electrode

surface, coating it in non-conductive materials; thus, diminishing its applicability as an electrode sensor by obstructing further neurotransmitter adsorption.[5] In recent years, there has been an emphasis on improving electrode functionality for neurochemical measurements and the most widespread method of enhancing electrode sensitivity is by modifying the fiber surface.[2] One of the earliest reported efforts on electrode modification focuses on enhancing electrode performance for dopamine detection over interferents such as ascorbic acid.[6, 7] This study utilized an anionic cation exchange polymer known as Nafion, which electrostatically repels the similar negatively-charged ascorbate and prevented it from adsorbing onto the electrode surface.[6, 7] However, electrode response time was lower with Nafion-coated electrodes. Alternatively, the Wightman group was able to enhance electrode sensitivity and selectivity for catecholamines while maintaining electrode response time by modifying their electrodes with 4-sulfobenzene.[8] However, there was still measurable ascorbic acid current as 4-sulfobenzene is not as impermeable to anions.[8]

Properties of Gold Nanoparticles

Limited research has been performed on using nanomaterials for diagnostic purposes. In particular, gold nanoparticles (AuNPs) have been a topic of interest in the bionanotechnology field due to their distinct characteristics and surface functionalities.[9] Properties such as size-[10] and shape-related optoelectronic features,[11] low toxicity,[12] and biocompatibility are just a few features that make AuNPs bio-nanotechnologically appealing.[9] Additionally, spherical AuNPs in an aqueous solution display an array of colors with increasing size and absorb at ~525nm as a result of oscillating conduction electrons.[13, 14] However, the adsorption peak does not appear with small nanoparticles ($d < 2$nm).[9] Size, shape, surface ligand, temperature and even the presence of other nanomaterials are factors that could account for this phenomenon.[15, 16, 17]

Bionanotechnology Applications of Gold-Nanoparticles

A popular application of AuNP-modification is detecting target biomolecules.[18] Conjugating AuNPs with antibodies or oligonucleotides leads to *in vitro* detection and diagnostics measures for diseases such as cancer.[19] For example, conjugating AuNPs with oligonucleotides and target-specific antibodies, and magnetic microparticles (MMPs) functionalized with monoclonal antibodies amplifies sensitivity for detecting nucleic acids and target proteins.[20] This method is most commonly known as bio-barcode assay.[20] Once the target molecule is detected, a large amount of barcode oligonucleotides is released and as a result identifies and quantifies the target molecule.[20] Mirkin *et al.* demonstrated the level of sensitivity of this technique by detecting a prostate specific antigen with a 330fg/mL LOD.[21]

Alternatively, non-covalent conjugation of AuNP and fluorophores increase sensitivity towards biomolecular targets.[9] This is accomplished by employing an array of selective receptors to create a configuration that distinguishes analytes.[22] This technique was not only able to classify twelve distinctive species/strains of bacteria with 95% accuracy, but it was also capable of rapidly and accurately recognizing normal, cancerous, and metastatic cells.[23] Additionally, replacing the polymer transducer with green fluorescent protein increases sensitivity in mammalian cancer cells sensing.[24] Sensitivity can also be amplified by enzyme catalysis through the binding (competitive) event of the analyte protein and AuNP, which releases ß-galactosidase and restores its activity.[25]

AuNPs are also commonly used as delivery systems for therapeutic agents to cells. Studies have shown that the interaction between functionalized AuNPs and cell membranes can be used to improve delivery efficiency.[26] Stellacci *et al.* investigated the regulation of cell membrane penetration through the surface ligand arrangement on AuNPs and discovered that the

ordered arrangement of functionalized AuNPs were able to penetrate the cell membrane, while the random arrangement of the same functionalized AuNPs were trapped in vesicular bodies.[27]

In an attempt to treat cancer and gene disorders, AuNPs have also been employed as attractive scaffolds to create transfection agents in gene therapy.[9] Mirkin *et al.* also explored the potential of AuNP-oligonucleotide complexes to control the protein expressions of cells by acting as gene regulating agents.[28] The knockdown of luciferase expression by RNA-AuNP conjugates indicated that the half-life of the conjugates was six times longer than that of free (double stranded) dsRNA, and also suggested a high gene knockdown potential.[29] Alternatively, the role of amino acid-based AuNPs in DNA transfection was investigated by Rotello *et al.*, and it was established that AuNPs coated with lysine provided non-toxic transfection vectors for DNA delivery.[30]

To summarize, AuNPs have distinct properties that make them extremely useful in the bionanotechnology industry. By manufacturing their surface monolayer, a diagnostic analyte targeting system of high sensitivity and selectivity can be created.[9] Furthermore, AuNPs can be employed for imaging purposes due to their exceptional physical properties and their wide range of surface functionality.[9] Because of their efficacy in surface loading of drug and gene coupled with its controllable release, AuNP-based delivery systems have also shown potential in therapeutics.[9] The versatility of AuNPs make them extremely valuable for next-generation biomedical purposes such as the use of electrochemical sensors for neurotransmitter detection with fast-scan cyclic voltammetry, which will be discussed in this thesis.

In Vivo Applications of Carbon-Fiber Microelectrodes

The primary areas of study involving carbon fiber-microelectrodes include neurotransmission mechanisms, effects of pharmacological agents, and regulating naturally-

evoked neurotransmitter release. The ability of carbon fiber-microelectrodes (CFMEs) to make fast electrochemical measurements is essential to better understanding monoamine neurotransmission. In particular, anesthetized mice have been used to study a vesicular group that is not released during standard activity by electrically-stimulating dopamine release; also known as the vesicular reserve pool.[31] It was determined that mice lacking synapsin, a protein that secures vesicles to the reserve pool, have amplified dopamine release as a larger fragment of vesicles is released.[31] The effects of brain injury on dopamine transmission have also been analyzed where both dopamine surplus levels and dopamine clearance levels were lower and slower, respectively, following an injury.[32] This shows that dopamine agonists can be useful in remedying brain injury by elevating dopamine levels that have been lowered through trauma.

Additionally, carbon fiber-microelectrodes have also been utilized in a variety of pharmacological studies. In particular, the effect of addictive drugs such as cocaine and amphetamine on dopamine release has lately become of interest among neuroscientists. An increase in dopamine transients as well as a gradual elevation in basal dopamine levels are observed with cocaine.[33, 34] Cheer *et al.* discovered an instant spike in dopamine levels in the brain after intravenously administering an acute dose of cocaine, ethanol and nicotine.[35] Meanwhile, dopamine release is reduced when cannabinoid antagonists are administered, suggesting that the dopamine transients were facilitated by cannabinoid receptors.[2] The administration of nomifensine (DA uptake inhibitor) and haloperidol (D2 DA receptor antagonist) in anesthetized mice are additional examples in investigating dopamine transients.[36]

Measuring behaviorally-induced dopamine transients is now one of the latest applications of carbon fiber microelectrodes (CFMEs). With the recent progression in instrumentation and electrode performance,[37] measuring short-term dopamine release can now be done in the

nanomolar range.[2] Correlating synchronized dopamine release with certain behavioral traits to analyze operant conditioning has also become increasingly significant, particularly, the role of dopamine in self-administered cocaine.[2] Phillips *et al.* reported that dopamine transients were observed in rats directly before and after pressing a lever to deliver a dose of cocaine.[38] The dynamics of dopamine transients were then further investigated by Stuber *et al.* where dopamine-release was still observed even before pressing the lever when the cocaine pump was turned off, and significantly declined after releasing the lever when the dose of cocaine was not delivered.[39] This indicated that spontaneous dopamine-release is controlled by varying mechanisms. If a reward can be predicted, the dopamine transients observed as a result of predictive stimulus has been shown through voltammetry at microelectrodes.[40]

Measuring *in vivo* neurotransmission using CFMEs has become increasingly important and, although the current technology and electrochemical methods are sufficiently well developed, there is still a need for improvements in sensor design and novel method development to offer new ways for neurochemical measurements for biomedical purposes.

Fast Scan Cyclic Voltammetry (FSCV) & Analytical Method Development

Cyclic voltammetry, potential pulse techniques, amperometry are a few common methods for detecting neurochemical changes in biological tissues.[41, 42, 43] These methods identify neurotransmitters by measuring the reduction and oxidation reactions at the working electrode. With FSCV, a triangular waveform is applied which scans at a high rate to oxidize and reduce an analyte at the electrode surface.[44] The large charging current produced by scanning at fast rates (>100 V/sec) is relatively stable at the carbon fiber working electrode, and this current can also be subtracted from the non-faradaic current to yield a background-subtracted cyclic voltammogram helpful in identifying and quantifying neurotransmitter release.[44] Now, FSCV is

extensively used both *in vitro* and *in vivo* to detect various electroactive analytes such as norepinephrine, adenosine, serotonin, dissolved oxygen (O_2), dopamine, and many other neurotransmitters. [45, 46, 47]

Due to its optimal electrochemistry and neurobiological importance, dopamine continues to be the key neurotransmitter for electrochemical detection using FSCV. Dopamine is a crucial chemical messenger that plays a pivotal role in the control of movement, learning, memory, cognition, and emotion within the nervous system of the human body.[2] The deficiency or surplus of dopamine can cause numerous neurological and psychological interference; among these are Parkinson's Disease (PD), schizophrenia, and amphetamine and cocaine addiction.[48] Today, PD continues to be a prevalent disorder as a result of dopamine deficiency caused by degeneration of midbrain neurons involved in dopamine synthesis.[48] PD symptoms include tremor, slowness of movement, stiffness, and problems in maintaining balance.[48] On the other hand, psychostimulants such as amphetamine and cocaine cause euphoric effects by increasing extracellular dopamine. Drug abuse eventually substitutes the regular flow of dopamine, conditioning the brain to require a surplus of dopamine, eventually leading to addictive behavior.[49] Measuring neurochemical changes in freely-behaving animal models using cyclic voltammetry has been one of the major breakthroughs of understanding the central dopamine system and how it contributes to operant behavior [50, 51] and drug addiction.[52, 53, 54]

Dopamine neurotransmission has been the topic of focus so far because it is one of the most frequently characterized molecules for electrochemical detection.[44] However, there is still a wide range of diseases and behavioral disorders where dopaminergic neurotransmission is not as well understood. Certain electroactive brain molecules are not oxidized by the dopamine voltammetric sweep (-0.4V to +1.3V, 400V/s); hence, analytical method development is

important to improve neurotransmitter and metabolite detection.[44] For instance, waveform modification and the alteration of the physical and chemical properties of electrode materials can enhance electrochemical sensitivity for neurotransmitter selectivity and detection.[55] Waveform modification strategies have been proven to electrochemically detect neurotransmitters including but not limited to dopamine,[56, 57] serotonin,[58] adenosine,[59, 60] histamine,[61] octopamine (primarily studied in invertebrates), norepinephrine, and others.[62] Additionally, electrode surface-modification using polymers and other coatings such as Nafion,[63, 64] overoxidized-polypyrrole,[65] functionalized carbon nanotubes,[66] and others have been reported to improve neurotransmitter detection by increasing conductivity and reducing surface fouling.[67]

Zestos *et al.* has successfully tested for the improvement of 3,4-dihydroxyphenylacetic (DOPAC) selectivity and discrimination from dopamine using a combination of polymer coatings and waveform modifications.[55] This was accomplished by functionalizing the electrode surface using polymers such as Nafion and polyethyleneimine (PEI) to distinguish both dopamine and DOPAC.[55] A more positive charge is applied to the electrode surface using PEI coatings as a result of the protonation of the nitrogen functionalized groups; thus, electrostatically attracting the negatively-charged DOPAC species.[55] Alternatively, the negatively-charged Nafion-coated CFMEs were able to electrostatically attract dopamine, but instead repel DOPAC.[55] Similar to the conventional "dopamine waveform,[57]" Zestos *et al.* developed a novel "DOPAC waveform" with a holding potential of 0V instead of -0.4V.[55] As a result, the electrostatic repulsion of DOPAC from the electrode surface is hindered at the negative potential.[55]

Over the last several decades, the field of *in vivo* electrochemistry has progressed tremendously and undergone a great deal of standardization. Today, electrochemistry is

frequently used for high-speed, spatially resolved *in vivo* neurochemical measurements.[44] Though current assays are versatile and robust, there is still a need for innovations in sensor design and the development of analytical methods to create new possibilities for monitoring neurotransmitter dynamics. Future efforts may provide insight into the neurochemical basis of certain behavior disorders and diseases, in addition to monitoring neurotransmitter at even lower concentrations and increasing the number of identifiable analytes.[44] This thesis will discuss the surface-modification of CFMEs using gold nanoparticles for the enhancement of dopamine detection that could potentially allow for the development of novel electrode sensors for *in vivo* neurotransmitter detection and measurements.

CHAPTER 3

EXPERIMENTAL

Preparation of Carbon Fiber Microelectrodes

The fabrication of CFMEs was performed according to procedures previously reported literature. Briefly, the carbon-fibers (7 microns in diameter) are separated into single strands and aspirated into individual capillaries using a vacuum pump (single-barrel borosilicate capillary without microfilament, 1.2mm outer diameter, 0.68mm inner diameter). Each prepared capillary is analyzed under a microscope before being pulled using the PC-100 Narishige puller into two separate electrodes. Each electrode is again analyzed under a microscope to ensure that the carbon fiber is pulled through the tapered end of the electrode and cut with surgical scissors or a razor blade at an optimal length of ~100 microns in length, and that the carbon fiber is at least three-quarters of the way through each electrode to make an electrical connection. The tapered end of each electrode (glass-carbon fiber interface) is sealed by immersion in a mixture of diethylenetriamine (DETA) hardener/curing agent and an epoxy resin (EPON 828), followed by acetone.

Characterization of Unmodified Carbon Fiber Microelectrodes via FSCV

Prior to the electrodeposition of gold nanoparticles, three unmodified electrodes are characterized through a series of experiments (i.e. stability, scan-rate, and concentration). To test the stability of each unmodified electrode, each electrode is placed in the flow cell for four hours scanning at a waveform of -0.4V to +1.3V at a 400V/s scan rate. Measurements are taken at every hour for the detection of 1μM dopamine. A uniform electrode response for over a four-hour period, which is the typical time period for *in vivo* experiments is important to denote the

potential applicability for *in vivo* testing. Moving on, each unmodified electrode is tested with 1μM dopamine at varying scan rates from 50V/s to 1,000V/s at 10Hz and a waveform of -0.4V to +1.3V. A linear rise in peak oxidative current is expected as the scan rate increases, denoting dopamine adsorption control to the electrode surface. Lastly, each unmodified electrode is then exposed to dopamine concentrations varying from 50nM to 100μM and at a waveform of -0.4V to +1.3V with a 400V/s scan rate. A linear rise in peak oxidative current is expected at low dopamine concentrations, which is indicative of dopamine adsorption control at the electrode surface. However, an asymptotic curve is anticipated at higher concentrations which would suggest that dopamine is saturated at the electrode surface with more diffusion control taking place.

Preparation and Imaging of AuNP-CFMEs

An unmodified carbon fiber is imaged via scanning electron microscopy (SEM), to ensure a direct comparison to the AuNP-CFME prior to electrodeposition. The formation of gold nanoparticles on the carbon fiber surface is carried out by electrodeposition with cyclic voltammetry, scanning in the negative potential from +0.8V to -0.8V in a 0.5mM auric chloride ($HAuCl_4$) solution containing 0.1M KCl for 20 cycles at a scan rate of 50 mV/s.[1] It should also be noted that electrodeposition time can also influence size and amount of gold nanoparticles formed on the fiber surface; thus, the duration of electrodeposition of each electrode is maintained at 64 seconds (approximately one minute) per scan cycle. Following electrodeposition, each AuNP-CFME is again imaged using SEM to analyze surface features such as size, uniformity and distribution of gold nanoparticles. The disappearance of the cylindrical carbon fiber ridges should suggest the presence of gold nanoparticles. Additionally,

energy-dispersive x-ray spectroscopy (EDX/EDS) measurements are conducted in conjunction with SEM to further confirm the presence of AuNPs.

Characterization of AuNP-CFMEs via Fast Scan Cyclic Voltammetry

Following electrodeposition of the gold nanoparticles, the three AuNP-CFMEs are characterized through a series of experiments (i.e. stability, scan-rate, and concentration). To test the stability of each AuNP-CFME, each modified electrode is placed in the flow cell for four hours scanning at a waveform of -0.4V to +1.3V at a 400V/s scan rate. Measurements are taken at every hour for the detection of 1μM dopamine. A uniform electrode response for over a four-hour period, which is the typical time period for *in vivo* experiments is important to denote the potential applicability to conduct *in vivo* neurochemical measurements. Moreover, each AuNP-CFME is tested with 1μM dopamine at varying scan rates from 50V/s to 1,000V/s and a waveform of -0.4V to +1.3V. A linear rise in peak oxidative current is expected as the scan rate increases, denoting dopamine adsorption control to the electrode surface. Lastly, each AuNP-CFME is then exposed to dopamine concentrations varying from 50nM to 100μM and at a waveform of -0.4V to +1.3V with a 400V/s scan rate. A linear rise in peak oxidative currents is significant to indicate dopamine adsorption control at the electrode surface, while a deviation from linearity would suggest that dopamine is saturated at the electrode surface showing diffusion control instead.

The changes in oxidative currents, and the potential separation of the redox peaks are examined and plotted using Microsoft Excel and Graph Pad Prism to compare sensitivity and electrocatalytic behavior as well as dopamine adsorption control between the unmodified- and AuNP-CFMEs. Statistical significance is determined with a t-test and a one-way analysis of variance (ANOVA).

CHAPTER 4

REPRESENTATIVE RESULTS

Schematic of Fast Scan Cyclic Voltammetry

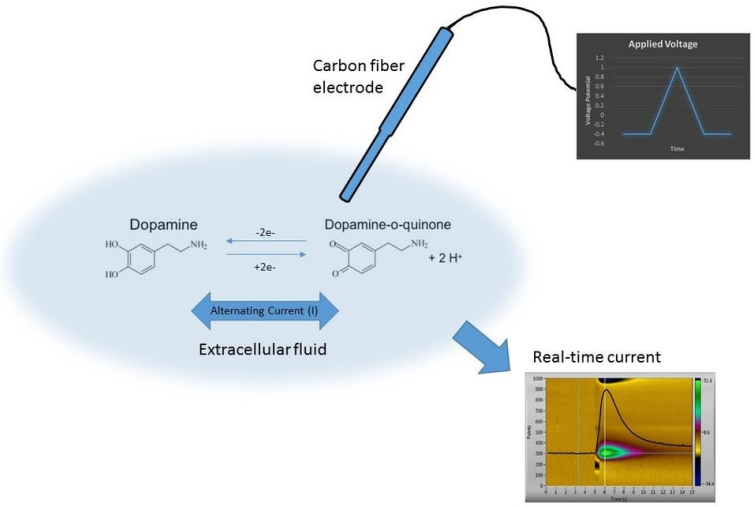

Figure 1: Schematic of Fast-Scan Cyclic Voltammetry Testing to Measure Dopamine Oxidation In Vitro.[5] (https://en.wikipedia.org/wiki/Fast-scan_cyclic_voltammetry#/media/File:Fast-scan_cyclic_voltammetry_to_measure_dopamine.jpg)

The triangular waveform plot of Figure 1 represents the applied dopamine waveform, scanning from -0.4V to +1.3V at a 400V/s scan rate where dopamine oxidizes into dopamine-ortho-quinone (DOQ). DOQ is then reduced back down to dopamine in the reverse (negative) scan. A voltage is applied to the carbon-fiber working electrode from the potentiostat to facilitate the two-electron transfer process from the analyte to the electrode surface. Finally, the HDCV software then yields a three-dimensional false color plot that is overlaid with a current versus time plot. The latter represents the dopamine oxidation reaction; the current is negligible in the presence of ACSF or PBS buffer, and then rises vertically when dopamine oxidizes into dopamine-ortho-quinone (adsorption onto electrode surface) and reduces back to dopamine (desorption from electrode surface). On the other hand, the color plot is a false three-dimensional

(3D) representation of current where the yellow plot represents the relatively neutral background current. Dopamine oxidation is represented by the green plot (positive current) whereas the reduction of dopamine-ortho-quinone back to dopamine is seen in the blue plot (negative current).

SEM Images (Bare- and AuNP-CFMEs)

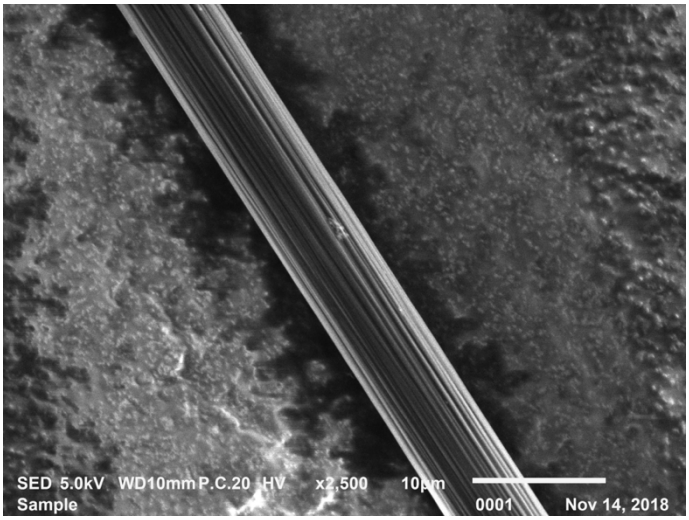

Figure 2(a): SEM Image of Bare Carbon-Fiber at x2,500 Magnification.[5]

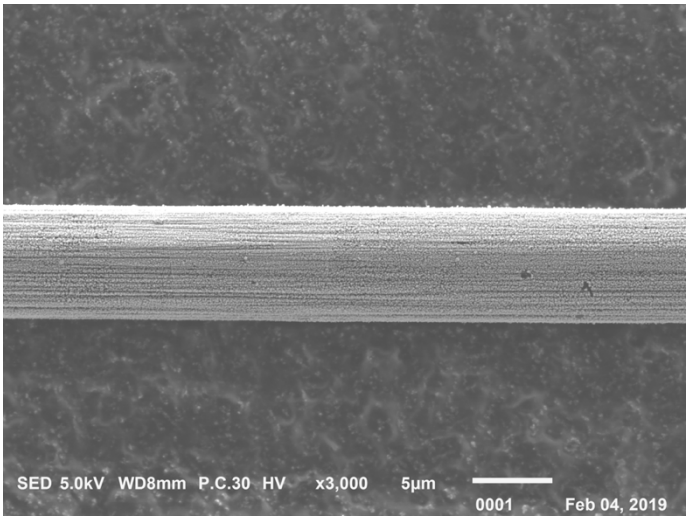

Figure 2(b): SEM Image of Gold Nanoparticle-Modified Carbon Fiber with an Electrodeposition Time of ~ 1 min at x3,000 Magnification.

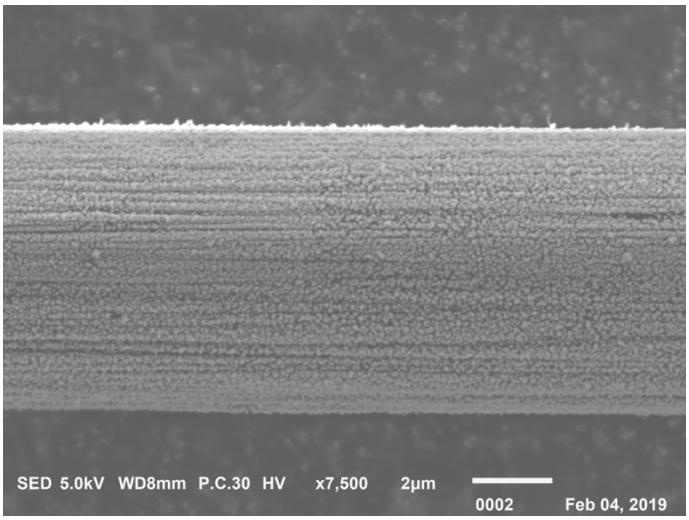

Figure 2(c): SEM Image of Gold Nanoparticle-Modified Carbon Fiber with an Electrodeposition Time of ~1 min at x7,500 Magnification.

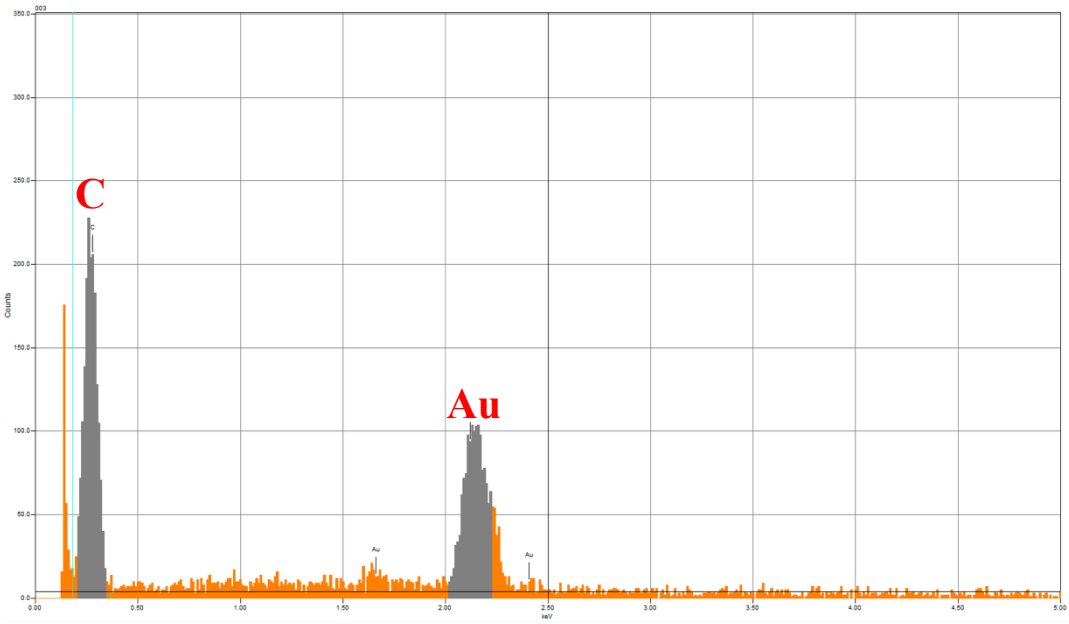

Figure 2(d): EDS/EDX Spectrum of the Gold Nanoparticle-Modified Electrode to Positively Identify the Presence and Relative Abundance of Gold.

Both unmodified- and AuNP modified-electrodes were imaged using scanning electron microscopy (SEM) to analyze differences in surface features such as size, uniformity and distribution of gold nanoparticles. Figure 2a shows a bare carbon-fiber with external cylindrical ridges and a diameter of ~7 micrometers (microns). Figures 2b and 2c depict the gold

nanoparticle-modified electrode with an electrodeposition time of ~ 1 min. The disappearance of the cylindrical carbon fiber ridges suggests the presence of gold nanoparticles. EDS/EDX is an analytical technique used in conjunction with SEM for elemental analysis and chemical characterization purposes. Its characterization capabilities are largely due to the concept of each element having a distinct atomic structure, which then allows for distinct peaks on its electromagnetic spectrum.[68] The presence and relative abundance of gold was further verified using EDS/EDX measurements as shown in Figure 2d. The applied electrical field from cyclic voltammetry produced a thin uniform coat of gold nanoparticles on the carbon-fiber surface and was able to reduce Au^{3+} in solution to a solid Au^{0} at a high count (~100 counts) as portrayed by the EDS/EDX spectrum. However, carbon is still the most abundant chemical (~250 counts) due to presence of the carbon fiber core.

Sensitivity Comparison (Bare- and AuNP-CFMEs)

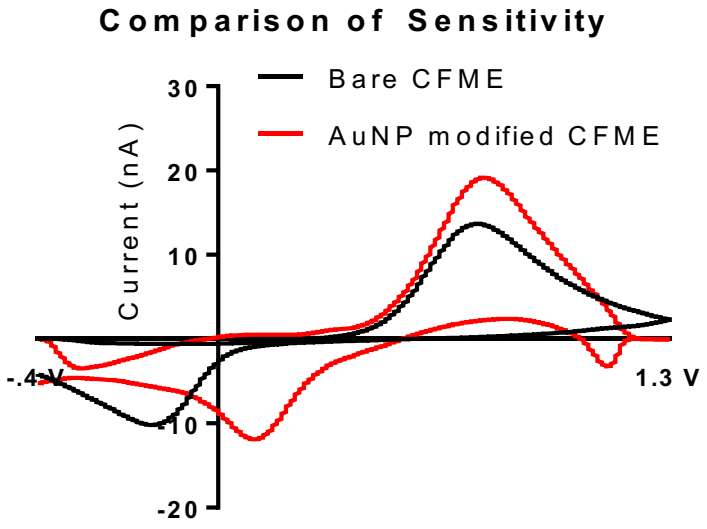

Figure 3(a): Superimposed Cyclic Voltammograms of the Bare- and AuNP-Modified Carbon Fiber Microelectrodes while Testing with 1µM Dopamine in the Flow Cell.[5]

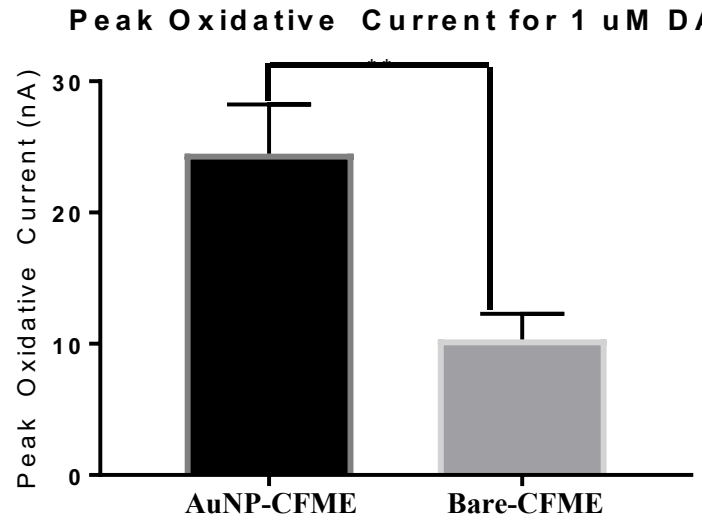

Figure 3(b): Bar Graph Depicting the Difference in Peak Oxidative Currents of the Bare- and AuNP-Modified Carbon Fiber Microelectrodes. Significance was Measured with an Unpaired t-Test, P=0.004.[5]

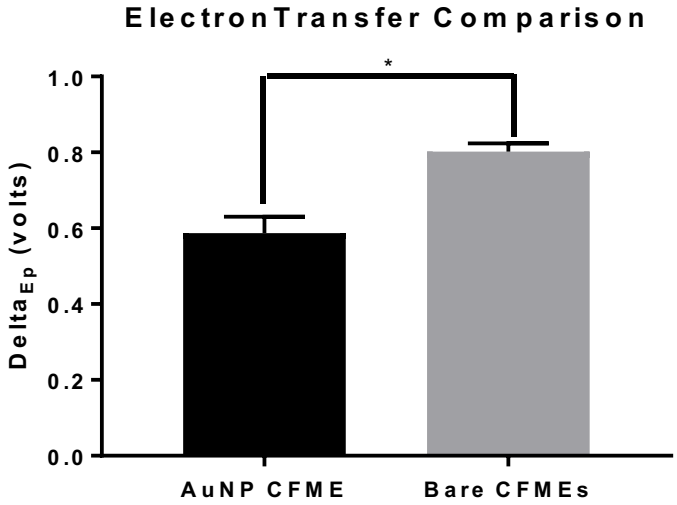

Figure 3(c): Bar Graph Depicting the Difference in Peak-to-Peak Separation of the Bare- and AuNP-Modified Carbon Fiber Microelectrodes. Both Types of Electrodes were Tested with1μM Dopamine in a Pine Custom Made Flow Cell. Significance was Measured with an Unpaired t-Test, P=0.0016.[5]

The superimposed cyclic voltammograms (Figure 3a) of both the bare- and AuNP-modified carbon-fiber microelectrodes are used to compare sensitivity and electron-transfer kinetics. The AuNP-modified carbon-fiber microelectrodes possess considerably higher peak oxidative currents (Figure 3b), indicating an increase in electroactive sites where more dopamine

adsorption can occur. Also seen on the cyclic voltammograms is a smaller peak-to-peak separation (ΔE_p) with the modified electrodes suggesting higher conductivity and faster electron-transfer kinetics of the AuNP-modified CFME substrate (Figure 3c), which could potentially be useful when measuring rapid neurochemical changes and phasic firing of dopaminergic neurons in the mesolimbic pathway of the brain. In addition to that, the small peak recorded following the switching potential could potentially be a result of water oxidation.

Stability Experiment (Bare- and AuNP-CFMEs)

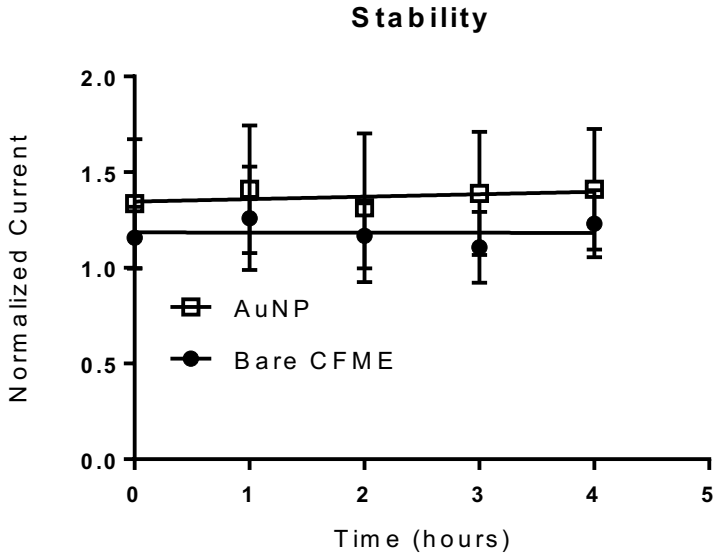

Figure 4: Stability Comparison of the Bare- and Gold Nanoparticle-Modified Carbon Fiber Microelectrodes while Testing with 1μM Dopamine.[5]

Figure 4 compares the stability of the bare- and AuNP-CFMEs. Both the unmodified- and modified-electrodes were placed in the flow cell for four hours scanning at a waveform of -0.4V to +1.3V at a 400V/s scan rate. Measurements were taken at every hour for at least four hours for the detection of 1μM dopamine. A uniform electrode response for over a four-hour period with respect to the normalized current (nA) of the lowest recorded peak oxidative current for the detection of 1 μM dopamine (the typical time period for *in vivo* experiments) is observed with

both types of electrodes. This is significant as it denotes the potential applicability of the AuNP-CFMEs to conduct *in vivo* neurochemical measurements.

Scan Rate Experiment (Bare- and AuNP-CFMEs)

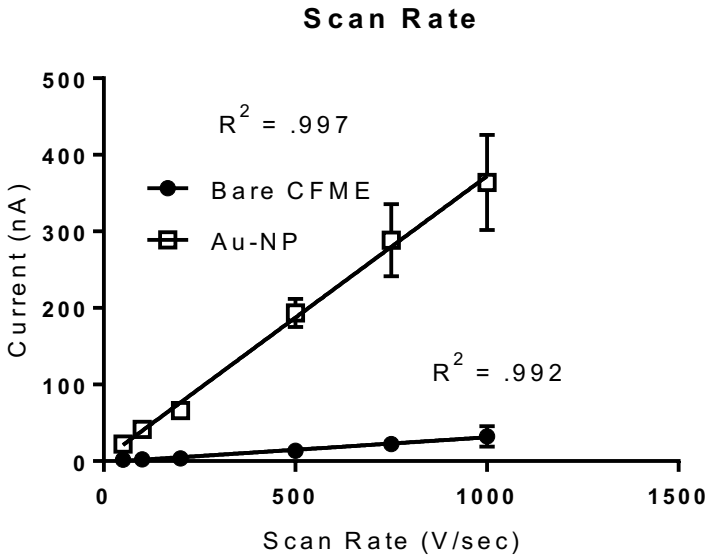

Figure 5: Scan Rate Comparison of the Bare- and Gold Nanoparticle-Modified Carbon Fiber Microelectrodes while Testing with 1μM Dopamine.[5]

The scan rate dictates the speed (in volts per second) at which the potential is applied and varied. Both unmodified- and AuNP-modified electrodes were tested with 1μM dopamine at varying scan rates from 50V/s to 1,000V/s and a waveform of -0.4V to +1.3V. It should also be noted that10Hz is the wave application frequency at which the electrode is held at the negative holding potential. The size of the diffusion layer decreases as a result of faster scan rates, which causes an increase in oxidative currents. A linear relationship is observed between both the bare- and AuNP-modified electrodes with regards to scan rate and the observed oxidative currents (Figure 5), indicative of dopamine adsorption control to both the bare- and AuNP modified-electrode surfaces.

Concentration Experiment (Bare- and AuNP-CFMEs)

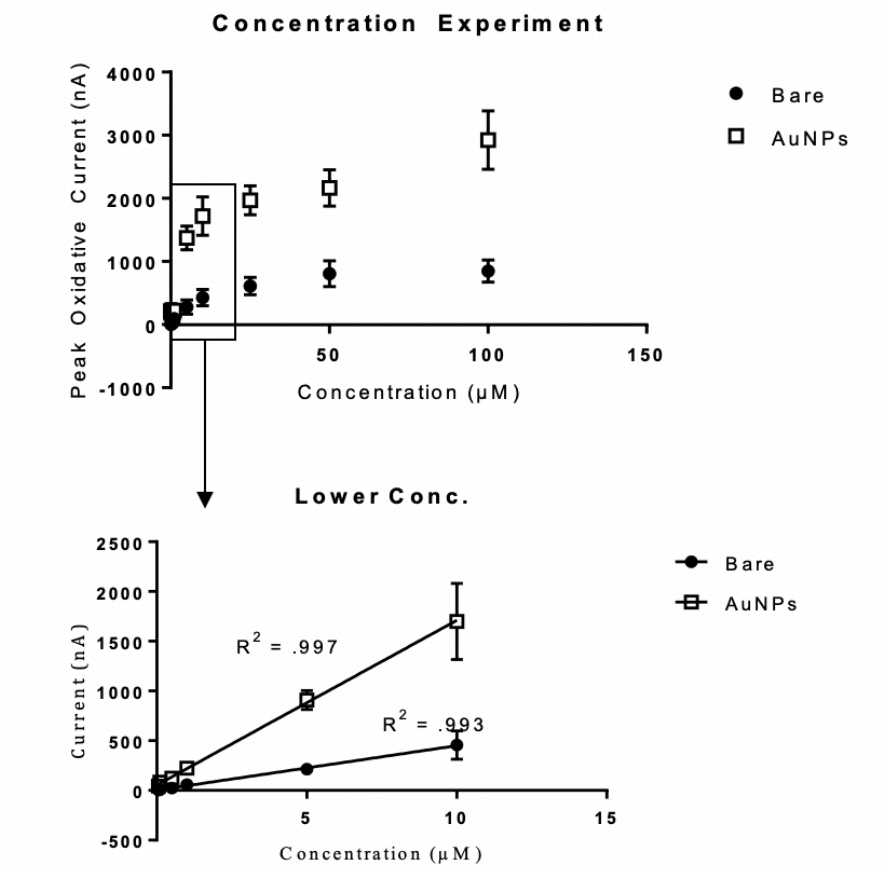

Figure 6: Concentration Comparison of the Bare- and Gold Nanoparticle-Modified Electrodes while Testing with Varying Dopamine Concentrations of 50nM to 100µM.[5]

Both types of electrodes were also exposed to dopamine concentrations varying from 50nM to 100µM and at a waveform of -0.4 V to +1.3 V with a 400V/s scan rate. A linear rise in peak oxidative currents of dopamine is observed from 50nM to 10µM dopamine, indicative of dopamine adsorption control to both types of electrode surfaces. However, an asymptotic curve is seen with concentrations above 10µM with the unmodified electrodes suggesting that dopamine is now saturated at the electrode surface with more diffusion control taking place at higher concentrations. On the other hand, the peak oxidative current continues to gradually increase with the AuNP-CFMEs at higher dopamine concentrations, indicative of kinetic control

to the electrode surface instead. It is also worth noting that physiologically relevant dopamine concentrations in the brain lie within the 50nM to 10μM range but differ between brain regions.[5]

CHAPTER 5

CONCLUSIONS

Over the last several decades, the field of *in vivo* electrochemistry has progressed tremendously and undergone a great deal of standardization. Electrochemistry is now frequently used for high-speed, spatially resolved *in vivo* neurochemical measurements. Though current assays are versatile and robust, there is still a need for innovations in sensor design and the development of analytical methods to create new possibilities for monitoring neurotransmitter dynamics. The distinctive physical and chemical characteristics of nanomaterials have shown much promise in sensor fabrication due to the high effective surface area, mass transport, catalytic properties, and cost-effectiveness. By combining these AuNPs characteristics and electrochemical technology, more novel and sensitive electrochemical sensing devices can be fabricated, thus furthering the field of nanoelectrochemistry.

Carbon fiber-microelectrodes (CFMEs) remain the standard biosensors for neurochemical monitoring due to their biocompatibility, size and optimal electrochemical properties. This includes a higher overpotential for water oxidation, in addition to the negatively-charged electrode which will allow for dopamine adsorption to the electrode surface. Furthermore, these electrodes are capable of making rapid neurochemical measurements that facilitate a more comprehensive understanding of dopamine signaling mechanisms. However, unmodified-CFMEs have been known to have several limitations. Because carbon fibers are largely comprised of basal plane carbon, unmodified electrodes have relatively low surface areas that leads to poor sensitivities for *in vivo* measurements. Additionally, surface fouling may occur with certain neurochemicals that may potentially obstruct further neurotransmitter adsorption. Recent studies have proven that the most widespread method of enhancing electrode

functionality is by coating the fiber surface with conductive substrates that enhance neurochemical detection.

A novel technique to fabricate AuNP-CFMEs to enhance neurotransmitter detection (i.e. dopamine) is demonstrated in this work. The development of AuNP-CFMEs allows for the fabrication of novel electrochemical sensors for measuring rapid changes in dopamine concentration and other neurochemicals at lower limits of detection. This technique is an efficient, quick, cost-effective and facile approach to improve electrode functionality for neurochemical detection. This study utilized a 0.5mM chloroauric acid ($HAuCl_4$) solution and an electrodeposition time of 64 seconds (~1 minute) to deposit a thin and uniform gold layer onto the fiber surface. The AuNP-CFMEs were shown to possess considerably higher electroactive surface areas and faster electron transfer kinetics than that of unmodified-electrodes. Increased sensitivities and lower limits of detection were observed with the AuNP-modified electrodes. Additionally, the modified-electrodes demonstrated uniform stability for dopamine detection for over four hours during *in vitro* testing, showing promise in its applicability for *in vivo* experiments. A linear relationship is observed between dopamine peak oxidative current with respect to both scan rate and concentration, denoting dopamine adsorption control to the modified-electrode surface. This study has a myriad of biomedical applications for enhanced *in vivo* dopamine detection to analyze the neurochemical effects of drug abuse, depression, and other behavioral conditions such as Parkinson's disease.

CHAPTER 6

FUTURE WORKS

Future works of this study include examining the detection of other neurochemicals such as norepinephrine, serotonin, histamine and others, as well as *ex vivo* neurochemical measurements in rodent and zebrafish models, and tuning deposition parameters to influence size, shape and distribution of the AuNPs.

Zebrafish models have now become ideal for neuronal function studies due to several factors.[69, 70] First, the similarities between the central nervous systems (CNS) of humans and zebrafish are greater than that of invertebrates.[71] It is also easier to genetically manipulate the CNS of zebrafish than that of rodents.[71] In addition to that, the use of intact brains leaves neuronal pathways intact and reduces tissue damage, allowing for remote pathway stimulation and prevention of interfering neurotransmitter release.[72, 73] Monitoring the release and uptake of electroactive neurotransmitters using electrochemistry has lately become of interest. In particular, monitoring the potassium-stimulated release dynamics of dopamine in intact whole zebrafish brains using unmodified electrodes is one of the research topics of Zestos *et al.* thus far. A depletion in dopamine concentration was observed with prolonged sugar exposure (i.e. glucose and mannitol).[74] Changes in dopamine concentration between male and female zebrafish models were also observed.[74] The next step of this project includes the use of the AuNP-CFMEs to monitor potassium-stimulated dopamine release to test its potential applicability to conduct *ex vivo* neurochemical measurements.

Moreover, optimization of electrodeposition parameters to study size, shape, catalytic properties, and distribution of the AuNPs will also be carried out. Manipulation of the deposition potential at a constant chloroauric acid concentration can alter the interplay between particle

growth rate and mass transport rate.[75, 76, 77] Additionally, the thickness of the AuNP distribution deposited on the electrode surface can be exploited by varying deposition time.[78] Studies have shown that the overall performance of AuNPs as enhancing platforms for electrocatalysis and electrochemical sensor is significantly influenced by the size and shape of the nanoparticles, in addition to the nature of their supporting materials.[79, 80]

REFERENCES

1. Li, J., Xie, H., and Chen, L. (2011) A Sensitive Hydrazine Electrochemical Sensor Based on Electrodeposition of Gold Nanoparticles on Choline Film Modified Glassy Carbon Electrode. *Sensors and Actuators B: Chemical153*, 239–245.
2. Huffman, M. L., and Venton, B. J. (2009) Carbon-Fiber Microelectrodes for In-Vivo Applications. *The Analyst134*, 18–24.
3. Williams, G., and Millar, J. (1990) Concentration-Dependent Actions of Stimulated Dopamine Release on Neuronal Activity in Rat Striatum. *Neuroscience39*, 1–16.
4. Michael, AC.; Wightman, RM. Laboratory Techniques in Electroanalytical Chemistry. Marcel Dekker; New York: 1996. p. 367-402. Chapter 12
5. Mohanaraj, S., Wonnenberg, P., Cohen, B., Zhao, H., Hartings M. R., Zou, S., Fox, D., and Zestos, A. G. (2019) Gold Nanoparticle Modified Carbon-Fiber Microelectrodes for Enhanced Neurochemical Detection. *Journal of Visualized Experiments*., in-press.
6. Gerhardt, G. A., Oke, A. F., Nagy, G., Moghaddam, B., and Adams, R. N. (1984) Nafion-Coated Electrodes with High Selectivity for CNS Electrochemistry. *Brain Research290*, 390–395.
7. Nagy, G., Gerhardt, G., Oke, A., Rice, M., Adams, R., Szentirmay, M., and Martin, C. (1985) Ion Exchange and Transport of Neurotransmitters in Nation Films on Conventional and Microelectrode Surfaces. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry188*, 85–94.
8. Robinson, D. L., Hermans, A., Seipel, A. T., and Wightman, R. M. (2008) Monitoring Rapid Chemical Communication in the Brain. *Chemical Reviews 108*, 2554–2584.

9. Yeh, Y.-C., Creran, B., and Rotello, V. M. (2012) Gold Nanoparticles: Preparation, Properties, and Applications in Bionanotechnology. *Nanoscale 4*, 1871–1880.
10. Sardar, R., and Shumaker-Parry, J. S. (2011) Spectroscopic and Microscopic Investigation of Gold Nanoparticle Formation: Ligand and Temperature Effects on Rate and Particle Size. *Journal of the American Chemical Society 133*, 8179–8190.
11. Hu, M., Chen, J., Li, Z.-Y., Au, L., Hartland, G. V., Li, X., Marquez, M., and Xia, Y. (2006) Gold Nanostructures: Engineering Their Plasmonic Properties for Biomedical Applications. *Chemical Society Reviews 35*, 1084.
12. Murphy, C. J., Gole, A. M., Stone, J. W., Sisco, P. N., Alkilany, A. M., Goldsmith, E. C., and Baxter, S. C. (2008) Gold Nanoparticles in Biology: Beyond Toxicity to Cellular Imaging. *Accounts of Chemical Research 41*, 1721–1730.
13. Jain, P. K., Lee, K. S., El-Sayed, I. H., and El-Sayed, M. A. (2006) Calculated Absorption and Scattering Properties of Gold Nanoparticles of Different Size, Shape, and Composition: Applications in Biological Imaging and Biomedicine. *The Journal of Physical Chemistry B110*, 7238–7248.
14. Miller, M. M., and Lazarides, A. A. (2005) Sensitivity of Metal Nanoparticle Surface Plasmon Resonance to the Dielectric Environment. *The Journal of Physical Chemistry B109*, 21556–21565.
15. Srivastava, S., Frankamp, B. L., and Rotello, V. M. (2005) Controlled Plasmon Resonance of Gold Nanoparticles Self-Assembled with PAMAM Dendrimers. *Chemistry of Materials 17*, 487–490.

16. Kelly, K. L., Coronado, E., Zhao, L. L., and Schatz, G. C. (2003) The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape, and Dielectric Environment. *ChemInform 34*.

17. Link, S., and El-Sayed, M. A. (2000) Shape and Size Dependence of Radiative, Non-Radiative and Photothermal Properties of Gold Nanocrystals. *International Reviews in Physical Chemistry 19*, 409–453.

18. Huo, Q., Colon, J., Cordero, A., Bogdanovic, J., Baker, C. H., Goodison, S., and Pensky, M. Y. (2011) A Facile Nanoparticle Immunoassay for Cancer Biomarker Discovery. *Journal of Nanobiotechnology 9*, 20.

19. (a) Fang S-B, Tseng WY, Lee H-C, Tsai C-K, Huang J-T, Hou S-Y. J.Microbial. Meth. 2009; 77:225–228 (b) Laderman EI, Whitworth E, Dumaual E, Jones M, Hudak A, Hogrefe W, Carney J, Groen J. Clin. Vaccine Immunol. 2008; 15:159–163. [PubMed: 18003814]

20. (a) Nam JM, Park SJ, Mirkin CA. J. Am. Chem. Soc. 2002; 124:3820–3821. [PubMed: 11942805] (b) Nam JM, Thaxton CS, Mirkin CA. Science. 2003; 301:1884–1886. [PubMed: 14512622] (c) Nam JM, Stoeva SI, Mirkin CA. J. Am. Chem. Soc. 2004; 126:5932–5933. [PubMed: 15137735] (d) Georganopoulou DG, Chang L, Nam JM, Thaxton CS, Mufson EJ, Klein WL, Mirkin CA. Proc. Natl. Acad. Sci. U.S.A. 2005; 102:2273–2276. [PubMed: 15695586] (e) Thaxton CS, Hill HD, Georganopoulou DG, Stoeva SI, Mirkin CA. Anal. Chem. 2005; 77:8174–8178. [PubMed: 16351173] (f) Hill HD, Mirkin CA. Nat. Protocols. 2007; 1:324–336 (g) Nam J-M, Jang K-J, Groves JT. Nat. Protocols. 2007; 2:1438–1444.

21. Thaxton CS, Elghanian R, Thomas AD, Stoeva SI, Lee J-S, Smith ND, J.Schaeffer A, Klocker H, Horninger W, Bartsch G, Mirkin CA. Proc. Natl. Acad. Sci.U.S.A. 2009; 106:18437–18442. [PubMed: 19841273]

22. (a) Bunz UHF, Rotello VM. Angew. Chem. Int. Edit. 2010; 49:3268–3279.(b) Miranda OR, Creran B, Rotello VM. Curr. Opin. Chem. Bio. 2010; 14:728–736. [PubMed: 20801707] (c) De M, Rana S, Akpinar H, Miranda OR, Arvizo RR, Bunz UHF, Rotello VM. Nat. Chem. 2009; 1:461–465. [PubMed: 20161380]
23. Phillips RL, Miranda OR, You C-C, Rotello VM, Bunz UHF. Angew. Chem. Int. Edit. 2008; 47:2590–2594.
24. Bajaj A, Rana S, Miranda OR, Yawe JC, Jerry DJ, Bunz UHF, M.Rotello V. Chem. Sci. 2010; 1:134–138.
25. Miranda OR, Chen H-T, You C-C, Mortenson DE, Yang X-C, Bunzand VM, Rotello UHF. J. Am. Chem. Soc. 2010; 132:5285–5289. [PubMed: 20329726]
26. (a) Arvizo RR, Miranda OR, Thompson MA, Pabelick CM, Bhattacharya R, Robertson JD, Rotello VM, Prakash YS, Mukherjee P. Nano Lett. 2010; 10:2543–2548. [PubMed: 20533851] (b) Cho EC, Zhang Q, Xia Y. Nat. Nanotech. 2011; 6:385–391.(c) Verma A, Stellacci F. Small. 2010; 6:12–21. [PubMed: 19844908]
27. (a) Verma A, Uzun O, Hu Y, Hu Y, Han H-S, Watson N, Chen S, Irvine andF, Stellacci DJ. Nat. Mater. 2008; 7:588–595. [PubMed: 18500347] (b) Chou LYT, Ming K, Chan WCW. Chem. Soc. Rev. 2011; 40:233–245. [PubMed: 20886124]
28. Rosi NL, Giljohann DA, Thaxton CS, Lytton-Jean AKR, Han MS, Mirkin CA. Science. 2006; 312:1027–1030. [PubMed: 16709779]
29. Giljohann DA, Seferos DS, Prigodich AE, Patel PC, Mirkin CA. J. Am.Chem. Soc. 2009; 131:2072–2073. [PubMed: 19170493]
30. Ghosh PS, Kim C-K, Han G, Forbes NS, Rotello VM. ACS Nano. 2008; 2:2213–2218. [PubMed: 19206385]

31. Venton BJ, Seipel AT, Phillips PE, Wetsel WC, Gitler D, Greengard P, Augustine GJ, Wightman RM. J.Neurosci 2006;26:3206–3209. [PubMed: 16554471]
32. Wagner AK, Sokoloski JE, Ren D, Chen X, Khan AS, Zafonte RD, Michael AC, Dixon CE. J.Neurochem 2005;95:457–465. [PubMed: 16190869]
33. Stuber GD, Roitman MF, Phillips PEM, Carelli RM, Wightman RM. Neuropsychopharmacology 2004;30:853–863. [PubMed: 15549053]
34. Wightman RM, Heien MLAV, Wassum KM, Sombers LA, Aragona BJ, Khan AS, Ariansen JL, Cheer JF, Phillips PEM, Carelli RM. European Journal of Neuroscience 2007;26:2046–2054. [PubMed: 17868375]
35. Cheer JF, Wassum KM, Heien ML, Phillips PE, Wightman RM. J.Neurosci 2004;24:4393–4400. [PubMed: 15128853]
36. Venton BJ, Wightman RM. Synapse 2007;61:37–39. [PubMed: 17068772]
37. Heien MLAV, Phillips PEM, Stuber GD, Seipel AT, Wightman RM. Analyst 2003;128:1413–1419. [PubMed: 14737224]
38. Phillips PEM, Stuber GD, Heien ML, Wightman RM, Carelli RM. Nature 2003;422:614–618. [PubMed: 12687000]
39. Stuber GD, Wightman RM, Carelli RM. Neuron 2005;46:661–669. [PubMed: 15944133]
40. Day JJ, Roitman MF, Wightman RM, Carelli RM. Nature Neuroscience 2007;10:1020–1028.
41. Justice, JB., editor. Voltammetry in the Neurosciences. Clifton, NJ: Humana; 1987.
42. Kawagoe KT, Garris PA, Wiedemann DJ, Wightman RM. Regulation of transient dopamine concentration gradients in the microenvironment surrounding nerve terminals in the rat striatum. Neuroscience. 1992; 51:55–64. [PubMed: 1465186]

43. Robinson DL, Hermans A, Seipel AT, Wightman RM. Monitoring rapid chemical communication in the brain. Chem Rev. 2008; 108:2554–84. [PubMed: 18576692]

44. Bucher ES, Wightman RM. Electrochemical Analysis of Neurotransmitters. *Annu Rev Anal Chem (Palo Alto Calif)*. 2015;8:239–261. doi:10.1146/annurev-anchem-071114-040426

45. Park J, Takmakov P, Wightman RM. In vivo comparison of norepinephrine and dopamine release in rat brain by simultaneous measurements with fast-scan cyclic voltammetry. J Neurochem. 2011; 119:932–44. [PubMed: 21933188]

46. Venton BJ, Michael DJ, Wightman RM. Correlation of local changes in extracellular oxygen and pH that accompany dopaminergic terminal activity in the rat caudate-putamen. J Neurochem. 2003; 84:373–81. [PubMed: 12558999]

47. Bunin MA, Prioleau C, Mailman RB, Wightman RM. Release and uptake rates of 5-hydroxytryptamine in the dorsal raphe and substantia nigra reticulata of the rat brain. J Neurochem. 1998; 70:1077–87. [PubMed: 9489728]

48. Triarhou, L. C. (1970, January 1) Dopamine and Parkinson's Disease. *Madame Curie Bioscience Database [Internet]*. U.S National Library of Medicine.

49. (2018, February 21) What Role Does Dopamine Play in Addiction? Inspire Malibu.

50. Owesson-White CA, Cheer JF, Beyene M, Carelli RM, Wightman RM. Dynamic changes in accumbens dopamine correlate with learning during intracranial self-stimulation. PNAS. 2008; 105:11957–62. [PubMed: 18689678]

51. Carelli RM. Nucleus accumbens cell firing and rapid dopamine signaling during goal-directed behaviors in rats. Neuropharmacology. 2004; 47(Suppl 1):180–89. [PubMed: 15464136]

52. Addy NA, Daberkow DP, Ford JN, Garris PA, Wightman RM. Sensitization of rapid dopamine signaling in the nucleus accumbens core and shell after repeated cocaine in rats. J Neurophysiol. 2010; 104:922–31. [PubMed: 20554845]

53. Owesson-White CA, Ariansen J, Stuber GD, Cleaveland NA, Cheer JF, et al. Neural encoding of cocaine-seeking behavior is coincident with phasic dopamine release in the accumbens core and shell. Eur J Neurosci. 2009; 30:1117–27. [PubMed: 19735286]

54. Ehrich JM, Phillips PE, Chavkin C. Kappa opioid receptor activation potentiates the cocaine-induced increase in evoked dopamine release recorded in vivo in the mouse nucleus accumbens. Neuropsychopharmacology. 2014; 39:3036–48. [PubMed: 24971603]

55. Raju, D., Mendoza, A., Wonnenberg, P., Mohanaraj, S., Sarbanes, M., Truong, C., and Zestos, A. G. (2019) Polymer Modified Carbon Fiber-Microelectrodes and Waveform Modifications Enhance Neurotransmitter Metabolite Detection. *Analytical Methods11*, 1620–1630.

56. R. B. Keithley, P. Takmakov, E. S. Bucher, A. M. Belle, C. A. Owesson-White, J. Park and R. M. Wightman, Anal. Chem., 2011, 83, 3563–3571.

57. P. Takmakov, M. K. Zachek, R. B. Keithley, P. L. Walsh, C. Donley, G. S. McCarty and R. M. Wightman, Anal. Chem., 2010, 82, 2020–2028.

58. B. P. Jackson and R. M. Wightman, Brain Res., 1995, 674, 163–166.

59. M. L. Pajski and B. J. Venton, ACS Chem. Neurosci., 2010, 1, 775–787.

60. A. E. Ross and B. J. Venton, Anal. Chem., 2014, 86, 7486–7493.

61. M. E. Denno, E. Privman, R. P. Borman, D. C. Wolin and B. J. Venton, ACS Chem. Neurosci., 2016, 7, 407–414.

62. S. E. Cooper and B. J. Venton, Anal. Bioanal. Chem., 2009, 394, 329–336.

63. S. Demuru and H. Deligianni, J. Electrochem. Soc., 2017, 164, G129–G138.

64. R. F. Vreeland, C. W. Atcherley, W. S. Russell, J. Y. Xie, D. Lu, N. D. Laude, F. Porreca and M. L. Heien, Anal. Chem., 2015, 87, 2600–2607.

65. M. J. Peairs, A. E. Ross and B. J. Venton, Anal. Methods, 2011, 3, 2379–2386.

66. C. B. Jacobs, T. L. Vickrey and B. J. Venton, Analyst, 2011, 136, 3557–3565.

67. L. Yang, D. Liu, J. Huang and T. You, Sens. Actuators, B, 2014, 193, 166–172.

68. (2018, December 19) Energy-dispersive X-ray spectroscopy. *Wikipedia*. Wikimedia Foundation.

69. Friedrich RW, Jacobson GA, Zhu P. Circuit Neuroscience in Zebrafish. Curr Biol. 2010; 20:R371– R381. [PubMed: 21749961]

70. Fetcho JR, Liu KS. Zebrafish as a Model System for Studying Neuronal Circuits and Behaviora. Annals of the New York Academy of Sciences. 1998; 860:333–345. [PubMed: 9928323]

71. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. Developmental dynamics: an official publication of the American Association of Anatomists. 1995; 203:253–310. [PubMed: 8589427]

72. Ullrich C, Daschil N, Humpel C. Organotypic vibrosections: novel whole sagittal brain cultures. J Neurosci Methods. 2011; 201:131–141. [PubMed: 21835204]

73. Ferris MJ, Calipari ES, Yorgason JT, Jones SR. Examining the complex regulation and drug-induced plasticity of dopamine release and uptake using voltammetry in brain slices. ACS chemical neuroscience. 2013; 4:693–703. 1948–7193. [PubMed: 23581570]

74. Zestos, A. G. (2019) Zebrafish FSCV Data. American University Washington, DC. PowerPoint Presentation Slides

75. Hau, N.Y.; Chang, Y.H.; Huang, Y.T.;Wei, T.C.; Feng, S.P. Direct electroplated metallization on indium tin oxide plastic substrate. Langmuir 2013, 30, 132–139.

76. Hau, N.Y.; Chang, Y.H.; Feng, S.P. Kinetics study of silver electrocrystallization on (3-mercaptopropyl) trimethoxysilane-grafted indium tin oxide plastic substrate. Electrochim. Acta 2015, 158, 121–128.

77. Feng, H.P.; Paudel, T.; Yu, B.; Chen, S.; Ren, Z.F.; Chen, G. Nanoparticle-Enabled Selective Electrodeposition. Adv. Mater. 2011, 23, 2454–2459.

78. Zhao, G., and Liu, G. (2018) Electrochemical Deposition of Gold Nanoparticles on Reduced Graphene Oxide by Fast Scan Cyclic Voltammetry for the Sensitive Determination of As(III). *Nanomaterials9*, 41.

79. C. Welch and R. Compton, Anal. Bioanal. Chem., 384 (2006) 601

80. M. S. El-Deab, Electrochim. Acta, 54 (2009) 3720