2015

Long-term characterization of the chronic dopamine microelectrode and effect of electrical conditioning

Eric P. Marr
Eastern Washington University

Follow this and additional works at: https://dc.ewu.edu/theses

Part of the Biology Commons

Recommended Citation
https://dc.ewu.edu/theses/299

This Thesis is brought to you for free and open access by the Student Research and Creative Works at EWU Digital Commons. It has been accepted for inclusion in EWU Masters Thesis Collection by an authorized administrator of EWU Digital Commons. For more information, please contact jotto@ewu.edu.
LONG-TERM CHARACTERIZATION OF THE CHRONIC DOPAMINE MICROELECTRODE AND EFFECT OF ELECTRICAL CONDITIONING

A Thesis
Presented to
Eastern Washington University
Cheney, Washington

In Partial Fulfillment of the Requirements for the Master of Science in Biology

By
Eric P. Marr
Summer 2015
Thesis of Eric P. Marr is approved by:

____________________ __________________________  Date ___________
David Daberkow, Ph.D., Assistant Professor, Graduate Study Committee Chair

____________________ __________________________  Date ___________
Kristen Keefe, Ph.D., Professor, Graduate Study Committee

____________________ __________________________  Date ___________
Krisztian Magori, Ph.D., Assistant Professor, Graduate Study Committee

____________________ __________________________  Date ___________
Andrew Oster, Ph.D., Assistant Professor, Graduate Study Committee
Thesis for Master of Science

In presenting this thesis in partial fulfillment of the requirements for a master’s degree at Eastern Washington University, I agree that JFK Library shall make copies freely available for inspection. I further agree that copying of this project in whole or in part is allowable only for scholarly purposes. It is understood, however, that any copying or publication of this thesis for commercial purposes, or for financial gain, shall not be allowed without my written permission.

Signature________________________

Date_____________________________
I. Abstract

Background: Dopamine (DA) is a neurotransmitter involved in movement, reward learning and addiction. Fast-scan cyclic voltammetry (FSCV) has long been an indispensable tool for monitoring real-time DA signaling. Development of polyimide fused silica-encased FSCV microelectrodes have made the technique more suitable for chronic DA monitoring (months) in vivo.

Methods: In this study, electrically stimulated DA signals were evoked weekly in an effort to characterize the recovery time and stability of DA signals recorded long-term with silica-encased chronic DA microelectrodes. Additionally, electrical conditioning (etching), previously shown to improve microelectrode sensitivity in vitro, was performed to investigate the long-term impact on DA monitoring in vivo. Changes in sensitivity were assessed by kinetic analysis of recorded DA signals resulting in parameters describing DA release ([DA]p; the concentration of DA release per stimulus pulse) and uptake (Vmax; maximal rate of DA uptake).

Results: Data from this study demonstrate that the peak amplitude of evoked DA signals (DAmax) significantly decreases after surgery, recovers in about 4.5 weeks, and then stabilizes and remains consistent long-term (> 6 weeks). The same trend holds for kinetic parameters describing DA release and uptake. Additional data also demonstrate that electrical conditioning increases the magnitude and quality of DA signals recorded long-term in vivo.

Conclusions: Once recovered, electrically evoked DA signals recorded at the silica-encased chronic DA microelectrode, and resulting kinetic parameters describing DA release and uptake, are stable long-term (months) and can be enhanced with electrical conditioning.
Acknowledgements

I would to thank my graduate advisor Dr. David Daberkow for his insight, guidance, and support throughout all aspects of this project.

I would also like to thank my graduate committee; Dr. Kristen Keefe, Dr. Krisztian Magori, and Dr. Andrew Oster for investing their time for the betterment of my project.

I would like to thank my wife for her continued love, encouragement, and support throughout my education. I would also like to thank my parents for providing a great example of pursuing further education at any point in your life.

Lastly, I would like to thank the undergraduate research assistants in the Daberkow lab; Stephanie Davis, Peter Rinne, Britt Yazel, Peter Martsin, and Taylor Caires for their hard work and dedication to our lab and this project.
Table of Contents

I. Abstract iv
Acknowledgements v
List of Figures vi

II. Introduction 1
   A. Dopamine 1
   B. Microdialysis 1
   C. Fast-Scan Cyclic Voltammetry 2
   D. Kinetic Analysis 4
   E. Electrical Conditioning 4

III. Methods 5
   A. Animals 5
   B. Microelectrode placement and in vivo monitoring 5
   C. Kinetic analysis 7
   D. Electrical conditioning 8
   E. Statistical analysis 8

IV. Results 9

V. Discussion 11

VII. Reference List 14

VIII. Figures 18

IX. Vita 32
List of figures

Figure 1 – Fast-scan cyclic voltammetry at the carbon fiber microelectrode
Figure 2 – Histogram of electrode recovery times
Figure 3 – Box plot of electrode recovery times
Figure 4 – Representative electrically evoked dopamine signals
Figure 5 – Total evoked dopamine from implantation to 12 weeks after surgery
Figure 6 – Total evoked dopamine 3 to 12 weeks after surgery
Figure 7 – Dopamine release from implantation to 12 weeks after surgery
Figure 8 – Dopamine release 3 to 12 weeks after surgery
Figure 9 – Dopamine uptake from implantation to 12 weeks after surgery
Figure 10 – Dopamine uptake 3 to 12 weeks after surgery
Figure 11 – Representative electrically evoked dopamine signals before and after electrical conditioning
Figure 12 – Percent change in dopamine current after electrical conditioning
Figure 13 – Evoked dopamine current prior to electrical conditioning compared to that after electrical conditioning
Figure 14 – Dopamine cyclic voltammograms before and after electrical conditioning as compared to a standard dopamine cyclic voltammogram
II. Introduction

A. Dopamine
Dopamine (DA) is a neurotransmitter implicated in motor function (Delaville et al., 2015; Emborg et al., 1998; Freed and Yamamoto, 1985; Haber, 2014) and reward-motivated behaviors (Haber, 2014; Schultz, 1997, 2002, 2007; Yawata et al., 2012). DA activity is also implicated in learning and memory (Packard and Knowlton 2002; Puig et al., 2014; Yawata et al., 2012). DA is highly implicated in reward-dependent learning (Cox et al., 2015; Schultz, 1997, 2002; Wise, 2006), and drugs of abuse have been shown to increase DA levels in the brain (Di Chiara and Imperato, 1988; Cheer et al., 2005, 2007; Covey et al., 2013; Daberkow et al., 2013). Dysfunction of the DA system, through depletion of DA neurons, underlies the symptoms of Parkinson’s disease (Albin et al., 1989; Benazzouz et al., 2014). Because DA is involved in these important brain functions and dysfunctions, long-term monitoring of DA activity in the brain is crucial to investigate and elucidate these processes further.

B. Microdialysis
Upon activation, DA is exocytotically expelled (dopamine release) into the synapse and then taken back up into dopamine neurons, and adjacent cells (dopamine uptake). Monitoring DA neurotransmission (release and uptake) in vivo is challenging. One method of DA detection is microdialysis and subsequent chemical detection using high performance liquid chromatography (HPLC) or gas chromatography mass spectrometry (GC-MS). HPLC and GC-MS have a high degree of sensitivity and specificity of detected substances, making them widely used methods for neurotransmitter detection. However, use of microdialysis to obtain extracellular perfusate containing neurotransmitters has spatial and temporal limitations. The microdialysis probe implanted in the brain is approximately 200 – 300 μm in diameter which can cause significant tissue damage to the areas of implantation (Nesbitt et al., 2013). In addition, DA activity related to behavior
happens on short time scales (milliseconds to seconds); therefore, capturing DA in the dialysate in relation to a behavioral event can be a challenge (Nesbitt et al., 2015; Zhang et al., 2012). To effectively investigate how DA neurotransmission is related to behavior, it is important to most accurately measure the exact time of DA release and uptake in relation to behavioral events in real-time.

C. Fast-scan cyclic voltammetry

DA is included in a class of neurotransmitters known as the catecholamines. Catecholamines contain a dihydroxyphenol group with an amine group. These functional groups play an important role in their detection through electrochemical methods, as catecholamines are easily oxidized to their quinone form (Tsunoda, 2006). Fast-scan cyclic voltammetry (FSCV) is an electrochemical method to detect electroactive chemicals such as DA (Ewing et al., 1983). It has become a vital technique in the field of DA research due to the small size of the FSCV sensor (resulting in very little tissue damage in the brain) and ability to monitor DA on a millisecond time scale (i.e., “real-time”).

FSCV utilizes a micron-sized carbon fiber (sensor of the microelectrode) to monitor DA (Fig. 1A). The electrochemical measurements are made every 100 ms, affording the opportunity to monitor DA activity in real-time with sub-second temporal resolution at the carbon fiber microelectrode (CFM) (Wightman et al., 1988). During FSCV, detection of DA is done by applying an electrical potential through the CFM and quantification of DA is done through the measurement of current change at the CFM in response to the applied potential. The potential applied at the CFM is cycled between a low voltage (-0.4 V) to high voltage (+1.3 V) and back to low voltage (-0.4 V) in a triangular waveform. During DA monitoring, these scans are repeated every 100 ms to give a cycle rate of 10 Hz (normal cycling). If DA is present at the CFM, DA is oxidized during the anodic sweep of the electrical potential to form dopamine-o-quinone, which is then reduced back to DA in the cathodic sweep of the potential (Fig. 1A). DA is identified by the characteristic oxidation peak (approx. +0.7 V; green in Fig. 1B colorplot) and reduction peak (approx. -0.3 V, blue
in Fig. 2B colorplot). Graphing the measured oxidative and reductive current against voltage provides a visual depiction known as a cyclic voltammogram (CV; upper right of Fig. 2B) that is specific to DA. FSCV also allows the quantification of concentration in release events. The flow of electrons to and from DA as it is oxidized and reduced are measured as current at the CFM and are directly proportional to the number of DA molecules undergoing electrolysis. This current can subsequently be converted to DA concentration by comparison to measurements of known concentrations in vitro (Kristensen et al., 1986; Sinkala et al., 2012). Therefore, FSCV at the CFM allows for monitoring DA with high temporal resolution, as well as high chemical specificity.

There are two main methods of fabricating DA microelectrodes for FSCV. While both types utilize a single strand of carbon fiber as their DA sensor, two different materials are used to house (encase) the carbon fiber sensor. More commonly, glass-housed electrodes have been used in FSCV. This glass-housed FSCV microelectrode has been used with success in vivo for detection of DA primarily in acute recordings (several hours). The microdrive apparatus used to permanently affix the glass electrodes is relatively large and is not conducive for long-term DA monitoring in freely moving behavioral experiments. In addition, glass housings cannot be utilized in humans; therefore, an alternative housing material has been employed. A new type of silica-encased electrode has been developed which more readily allows for chronic (weeks to months) implantation and recording of DA in vivo (Clark et al., 2010). This CFM is smaller than then typical glass-housed CFM and requires no microdrive apparatus for chronic implantation, making it physically easier to affix and successfully use in long-term behavioral experiments with freely moving animals. Importantly, a modified version of this relatively new silica-housed chronic electrode has been approved and is being further developed for the therapeutic use in humans with DA dysfunction, such as Parkinson’s disease (Kishida et al., 2011).
D. Kinetic Analysis

Employing computer analysis and modeling based on Michaelis-Menten enzyme kinetics, parameters describing DA release and uptake can be determined from individual DA signals (Wu et al., 2001, 2002). The ability to determine these changes in DA neurotransmission can be beneficial in elucidating possible mechanisms related to behavior or in response to drugs (Covey et al., 2013; Daberkow et al., 2013). In the present study, they provide additional measures to quantify the stability of DA signals over time. Total evoked DA (DA$_{\text{max}}$) is a balance between DA release, and DA uptake by the dopamine transporter (DAT) (Wu et al., 2001). During the stimulation of DA neurons, the DA signal is a combination of both release and uptake. Upon extinction of stimulation, the signal is composed solely of DA uptake while the DAT clears DA from the synaptic cleft, returning extracellular DA to basal levels. Kinetic parameters defining changes in DA release and uptake determined from recorded DA signals can be useful in more specifically describing specific DA activity in the brain, as well as further describing changes in implanted microelectrodes used long-term in vivo.

E. Electrical Conditioning

Electrical conditioning (etching) creates a fresh surface on the carbon fiber of the DA microelectrodes by sloughing off the outer layer of the carbon fiber sensor (Keithley et. al., 2011; Takmakov et al., 2010). Etching increases surface roughness of the carbon fiber, increasing the available surface area for electrons from the oxidation and reduction of DA to be detected. Etching is done by increasing the voltammetric scan frequency parameters from the normal 10 Hz cycle rate (DA monitoring) to a 60 Hz cycle rate (Etching) for a period of time (e.g., 30 min). After etching, the cycle rate is returned to the original/normal 10Hz cycle rate to allow the electrode to stabilize for 15 min prior to DA monitoring. With this procedure, there is no increase in current or voltage applied to the electrode, only an increase in the frequency that the potential sweeps from its low voltage (-0.4 V) to its high voltage (+1.3 V) and back (-0.4 V). This etching procedure only removes a minuscule
amount of the carbon from the surface of the microelectrode (Takmakov et al., 2010); therefore, the etching procedure should not cause any significant damage to brain tissue. Etching has been shown to increase electrode sensitivity to DA in vitro (Takmakov et al., 2010). Etching has only been used sparingly in vivo, prior to implantation surgery in acute experiments with glass-housed DA microelectrodes (Keithley et al., 2011; Sombers et al., 2009), as well as in one published experiment with silica-housed DA microelectrodes (Oleson et al., 2014). However, in these experiments, etching was only utilized prior to DA electrode implantation surgery, and therefore, results relating to any increase in DA sensitivity in vivo were not reported. The effects of etching surgically implanted DA microelectrodes after recovery from implantation surgery, as well as the effect of successive (weekly) etching events in vivo, has not been previously described.

III. Methods

A. Animals
Male, Sprague Dawley rats (250g-300g) were housed individually in a temperature-controlled room, under a natural light/dark cycle (light cycle regulated by sunrise and sunset through windows in the animal housing area) and allowed access to food and water ad libitum. All animal care and experimental procedures conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Use and Care Committee at Eastern Washington University.

B. Microelectrode placement and in vivo monitoring
Rats (n=13) were implanted with stimulating, reference and chronic silica-encased DA microelectrodes. Implantation surgeries followed procedures similar to what is described in Daberkow et al. (2013) with the modification of utilizing silica-encased chronic electrodes (Clark et al., 2010) instead of a glass-housed acute electrode. Maximal DA
release and kinetic parameters describing DA release and uptake were tracked weekly over 12 weeks following implantation surgery, these measures were then compared to determine and quantify changes in the DA signals recorded long-term with the chronic DA FSCV microelectrode.

On the day of surgery, rats were first deeply anesthetized. Initially anesthesia was induced with 4% isoflurane in 100% oxygen while the animal was housed in a sealed plexiglass box. After being rendered unconscious, rats were transferred to a stereotaxic frame (Stoelting Co. Wood Dale, IL, USA). A warm (37°C) heating pad was placed under the rat, and a nose cone was used to administer anesthesia throughout the electrode implantation surgery. During surgery, 1.9%-2.1% isoflurane in 100% oxygen was delivered through the nose cone. Percentage of isoflurane vaporized was monitored and maintained with a precision nebulizer. The appropriate depth of anesthesia was established through monitoring respiration rate and the absence of responses to a firm paw pinch. Depth of anesthesia was carefully assessed throughout the DA recording sessions.

Once rats were immobilized in the stereotaxic frame. Skin and fascia were cleared, and small holes drilled in the skull for placement of electrodes. Stereotaxic coordinates were obtained from the atlas of Paxinos and Watson (1986). Using stereotaxic micromanipulator arms, a DA recording electrode was positioned in the dorsal striatum (+1.0 AP, +2.0 ML, -5.8 DV), a brain region highly innervated with DA neurons and strongly implicated in learning (Salamone and Correa, 2012). Then, a chlorinated silver wire (Ag/AgCl) reference electrode was lowered into the contralateral cortex just below the dura. Finally, a twisted bipolar stimulating electrode (Plastics One, Roanoke, VA) with ~1-mm tip separation was incrementally lowered and positioned just above the medial forebrain bundle (-4.6 AP, +1.4 ML, -7.5 DV). Constant current, biphasic stimulus pulses (60 Hz, 60 pulses, 300 µA; Oleson et al., 2013) were applied (NL 800, Neurolog, Medical Systems, Great Neck, NY), and the stimulating electrode was incrementally lowered until a robust DA signal was evoked. Once DA signals were monitored, a small (6mm x 4mm) headstage (Datamate
M80, Harwin, Salem, NH) was connected to the electrodes and affixed to the rat’s skull with dental cement. The headstage allowed for simple reconnection to recording equipment during subsequent DA monitoring sessions. Once the dental cement dried (25-30 min), the rat was removed from the stereotaxic frame and allowed to regain consciousness under delivery of 100% oxygen.

One week after implantation surgery, electrode recovery testing began. Chronic DA microelectrodes require a period of recovery time before clear DA signals can be detected (Clark et al., 2010; Personal discussion at the Society for Neuroscience annual meeting 2013 and 2014). Once a week, after surgery, rats were reconnected to recording equipment with a simple headstage clip (Datamate M80-667, Harwin, Salem, NH). Small stimulations (60 Hz, 24 pulses, 125 µA; Daberkow et al., 2013) were applied in attempt to evoke identifiable DA signals (NL 800, Neurolog, Medical Systems, Great Neck, NY). This procedure was repeated weekly until such DA signals were detected. Once clearly identifiable DA signals were observed, indicating recovery from surgery, rats began anesthetized stimulations for larger electrically-evoked DA signals conducive to kinetic analysis. For DA recording during these larger stimulations, rats were deeply anesthetized as described above, and reconnected to recording equipment with the headstage clip. Constant current, biphasic stimulus pulses (60 Hz, 60 pulses, 300 µA; Oleson et al., 2013) were applied, and DA signals were monitored. After three electrically-evoked DA signals were recorded, rats were disconnected from recording equipment, given pure oxygen, then returned to their home cage and allowed to regain consciousness.

C. Kinetic Analysis

DA signals were analyzed using a kinetic model describing evoked DA levels as a balance between DA uptake and release mechanisms (Wu et al., 2001) using the equation \( \frac{d[DA]}{dt} = [DA]_p * f - \frac{V_{max}}{K_m/\langle DA \rangle + 1} \). Based on curve fitting of DA signals to this equation utilizing computer modeling software, parameters describing exocytotic DA
release ([DA]₀; DA release per stimulus pulse) and DA uptake (V_{max}; maximal velocity of DA uptake) were determined (Wu et al., 2001, 2002).

**D. Electrical Conditioning**

Once electrodes were surgically implanted and clear DA signals re-appeared after recovery from surgery, electrical conditioning (etching) treatments began. A series of three electrically evoked DA signals were recorded prior to etching as described above (pre-etch signals). Etching was then done for a period of 30 min at an increased frequency of 60 Hz voltammetric cycling rate (Takmakov et al., 2010). The cycling rate was then returned to the normal 10 Hz cycle rate for a period of 15 min after etching to allow the electrode to stabilize. A series of three electrically evoked DA signals were recorded after etching (post-etch signals). Rats were then disconnected from recording equipment and returned to their home cage to regain consciousness. Peak height of DA signals ([DA]_{max}) and cyclic voltammograms, from pre-etch and post-etch were compared to determine the impact of etching on microelectrode sensitivity and ability to accurately detect DA (Fig. 11-14).

**E. Statistical analysis**

Changes in [DA]_{max}, [DA]₀, and V_{max} were statistically analyzed with a one-way ANOVA, using weeks post-surgery as the independent variable, followed by sequential Tukey’s *post-hoc* tests. Etching data was also analyzed with a one-way ANOVA, using weekly etching treatment event as the independent variable. Cyclic voltammogram (CV) data were analyzed using a Spearman’s rank correlation test followed by a Williams’s test to determine significance of the fit of the data compared to the standard CV. All statistical analyses were performed utilizing R 3.2.0 software program for windows (The R Foundation for Statistical Computing). Significance level was set at p<0.05.
IV. Results

The range of electrode recovery time was 3 to 7 weeks (Fig. 2, n=13). The average recovery time was 4.5 weeks post-surgery with a median of 4 weeks (Fig. 3).

Once recovered from surgery, electrically evoked DA stimulation was preformed to characterize the changes in recovered DA signals. Initially, there was a significant reduction in the total evoked DA from the day of implantation surgery to subsequent recovered DA signals (Fig. 5, [DA]_{max}, p=0.024). However, once DA signals recovered there were no significant differences in total evoked DA values between weekly recorded DA signals (Fig. 6, [DA]_{max}, p=0.482).

A similar trend was found for kinetic parameters describing DA release ([DA]_p). There was a significant decrease between the DA release at the time of surgery compared to values from signals recorded in subsequent weeks following post-surgery signal recovery (Fig. 7, [DA]_p, p<0.001). However, there were no differences found in DA release parameters following recovery out to twelve weeks post-surgery (Fig. 8, [DA]_p, p=0.173).

The kinetic parameters describing uptake similarly showed a significant decrease between the recovered DA uptake values when compared to the uptake values recorded at the time of surgery (Fig. 9, V_{max}, p<0.001). Importantly, DA uptake values remained stable after signal recovery with no significant changes over subsequent weeks (Fig. 10, V_{max}, p=0.115).

Total evoked DA currents were also recorded and analyzed prior to, and after, etching. The mean DA current prior to etching was 11.37 nA (± 1.34 nA, standard error), and the mean DA current after etching was 17.08 nA (± 2.20nA, standard error), demonstrating a significant increase in DA current after etching for 30 minutes at 60 Hz (Fig. 13, p=0.0321). Figure 12 shows the percent increase in DA current by etch number through six etching
treatments. The range of DA current percent change was -20.21% to +176.79%. The mean percentage increase averaged across all six etchings was 52.23% (± 9.40%, standard error). These data demonstrate a significant increase from pre-etch signals to post-etch signals (Fig. 13, p=0.0321). When the number of etching events was added as an independent variable in statistical analysis, there was no significant difference between the etchings (Fig. 12, p=0.529).

While most animals had an increase in DA current after etching, there were two animals that displayed a decrease in DA current after etching treatment. However, even though the DA current was reduced in these animals, the cyclic voltammograms were “cleaner”. A cleaner voltammogram is defined as a voltammogram shape that is more similar to a standard DA voltammogram obtained during flow cell injection of a known concentration of DA (1 µM; Kristensen et. al., 1986, Sinkala et. al., 2012). A series of DA signals of various sizes (nA) were obtained during implantation surgery. A surgery DA signal of similar size (nA) as the pre-etch and post-etch DA signals was defined as the standard voltammogram. This standard signal was plotted together with the pre-etched and post-etched voltammograms (Fig. 14). To determine if there was a significant improvement in the voltammogram after etching, the pre-etched and post-etched voltammograms were compared to the standard voltammogram using a Spearman rank correlation test to obtain the correlation value. The correlation parameter for the pre-etch voltammogram compared to the standard was 0.66, and the post-etch voltammogram compared to standard correlation parameter was 0.74. A Williams’s test was then used to determine that these correlation values were significantly different from each other (p<0.001). This analysis verified that the post-etch voltammogram was more similar to a standard voltammogram than the pre-etched.
V. Discussion

The aim of the present study was three fold. The first objective was to determine the amount of time required for viable DA signals to return after implantation of the chronic DA microelectrode. The results of this study demonstrate that electrically evoked DA signals require approximately 4.5 weeks to recover after implantation surgery (Fig. 2-3).

Secondly, this study aimed to determine if recovered DA signals, as well as kinetic parameters describing DA release ([DA]_p) and uptake (V_max), remain stable long-term. The results demonstrate that there is an initial reduction in total evoked DA ([DA]_{max}), [DA]_p, and V_max until DA signal recovery (Fig. 5, 7, 9). However, once DA signals recover from implantation surgery, these values remain consistent long-term (Fig. 6, 8, 10). These data suggest that once recovered, the DA signals recorded at the chronic DA microelectrode remain stable long-term.

While these data suggest long-term stability of the CFM, the data do not explain the significant reduction in total evoked DA, as well as the decrease in the kinetic parameters describing DA release and uptake after implantation surgery. A possible explanation for these effects is the likely disturbance of tissue surrounding implanted electrodes during and after placement. It has been shown previously that similar electrodes implanted in the brain can lead to encapsulation of the electrodes by microglial cells and astrocytes (Duff and O’Neill 1994; Markwardt et al., 2013; Seymore and Kipke 2007; Kozai et al., 2012). However, it has also been previously demonstrated that small probes such as the CFM do not cause significant levels of encapsulation by microglia and astrocytes around the carbon fiber sensor (Seymore and Kipke 2007; Clarke et al., 2010). Therefore, it is more likely that encapsulation is occurring around either the reference electrode and/or surrounding the stimulating electrode as these electrodes are significantly larger in size. In either case, encapsulation could likely explain the reduction of signals recorded during surgery until electrode signal recovery. Encapsulation of the stimulating electrode could create
additional cell layers which the electrical stimulating current would have to travel through prior to reaching the medial forebrain bundle (Kozai et al., 2012). This could induce a reduction in realized current at the medial forebrain bundle, and thus cause a reduction in the magnitude of stimulation resulting in an overall smaller evoked DA release event. Encapsulation of the reference electrode, causing an additional cell layer, could reduce the effective applied waveform current which could potentially reduce the ability to oxidize and reduce the DA surrounding the CFM. Further research is needed to determine the precise location(s) and extent of encapsulation(s) after chronic implantation of all electrodes. Additionally, it would be useful to investigate potential procedures to minimize electrode encapsulation (Azemi et. al., 2008, 2011).

The first two aims of this study support the continued use and further development of the CFM for use in long-term studies. While there are still some unanswered questions regarding the mechanisms causing reduction in post-implantation signals, the fact that the DA signals recover and remain stable for several weeks demonstrates that these chronic DA microsensors are beneficial for long-term in vivo studies where use of glass electrodes would be more difficult or prohibited (i.e., in humans; Kishida 2011).

Finally, the third objective of this study was to investigate the effect of electrical conditioning (etching) in vivo after electrode implantation. The results suggest that electrical etching provides a significant increase in the DA current measured at the CFM (Fig. 13). While most animals had an increase in DA current after etching, there were two animals that displayed a decrease in DA current after etching treatment. Analysis of voltammograms verified that the post-etch voltammogram is more similar to a standard voltammogram than the pre-etched. These data validate that electrical conditioning significantly improves the DA signature signal (cyclic voltammogram, CV) measured with the chronic DA microelectrode long-term (Fig. 14). A cleaner cyclic voltammogram allows
for greater microelectrode reliability and certainty that the current detected at the CFM is DA.

It is important to note that there is a limit to the amount of etching that can be done on an electrode. In 2010, Takmakov et al. showed that carbon fiber in an electrode will completely disappear after etching for a period of 30 hrs. In addition, the data from the present study suggest a trend in which the first, and last two etchings displayed the greatest increase in DA current, while etchings 3 and 4 remain below the average percent increase (Fig. 12). It may be beneficial to limit the etching in an experiment to the initial recording sessions in order to preserve the amount of carbon fiber available.

Overall, this study demonstrates the viability of the CFM for use in long-term in vivo studies. In addition, these results support the use of successive etchings in vivo as a treatment to increase the magnitude and quality of long-term recorded DA signals in experiments utilizing FSCV.
VI. References


Covey DP, Juliano SA, Garris PA. Amphetamine elicits opposing actions on readily releasable and reserve pools for dopamine. 2013;8:e60763.


Wise RA. Role of Brain Dopamine in Food Reward and Reinforcement. 2006;361:1149-58.


Fig. 1. Fast Scan Cyclic Voltammetry (FSCV) at the carbon fiber microelectrode. A, Diagrammatic carbon fiber microelectrode sensor (radius = 7 μm) shown in dark blue encased in silica tubing (light blue). Oxidation of dopamine to dopamine-o-quinone and reduction of dopamine-o-quinone to dopamine. B, Upper right, dopamine cyclic voltammogram showing dopamine oxidation (positive peak) and reduction (negative peak) identifying measured current as dopamine. Lower figure, FSCV color plot with dopamine oxidation (green) and reduction (blue) showing peak voltages of for dopamine oxidation (+0.7 V) and dopamine reduction (-0.3 V).
Fig. 2. Histogram of electrode recovery times. The range of recovery times was 3 to 7 weeks for the electrode signal to recover.
Fig. 3. Box plot of electrode recovery times. The gray box represents 50 percent of the data, whiskers include the inter-quartile range representing 75 percent of the data, and the bold solid line is the median. The mean microelectrode recovery time was 4.538 weeks after implantation surgery. Symbols represent outlier (°) and mean (Δ).
Fig. 4. Representative electrically evoked dopamine signals. A, Evoked dopamine signal recorded during implantation surgery. Upper right, dopamine voltammogram showing dopamine oxidation (positive peak) and reduction (negative peak) identifying measured current as dopamine. Middle, dopamine current plotted against time showing total evoked dopamine signal. Lower figure, FSCV color plot with dopamine oxidation (green) and reduction (blue) showing peak voltages for dopamine oxidation (+0.7 V) and dopamine reduction (-0.3 V). B, Evoked dopamine signal recorded 5 weeks after surgery. Upper right, dopamine voltammogram showing dopamine oxidation (positive peak) and reduction (negative peak) identifying measured current as dopamine. Middle, dopamine current plotted against time showing total evoked dopamine signal. Lower figure, FSCV color plot with dopamine oxidation (green) and reduction (blue) showing peak voltages for dopamine oxidation (+0.7 V) and dopamine reduction (-0.3 V).
Fig. 5. Box plot showing total evoked dopamine from implantation to 12 weeks after surgery. There was a significant reduction in total evoked dopamine from implantation surgery values to the recovered signal values, while weeks 3 to 12 had no significant differences. Symbols represent mean (A). * p < 0.05, significantly different than weeks 3 through 12 determined by one-way ANOVA and Tukey’s post hoc analysis (n=7).
Fig. 6. Box plot of total evoked dopamine after electrode recovery, 3 to 12 weeks after surgery. Symbols represent mean (Δ). Once recovered, total evoked dopamine remained stable with no significant differences (one-way ANOVA, p=0.482).
Fig. 7. Box plot of dopamine release from implantation to 12 weeks after surgery. Dopamine release ([DA]_p) has a significant reduction from implantation surgery values to the recovered signal values, while weeks 3 to 12 had no significant differences. Symbols mean (Δ). * p < 0.01, significantly different than week 3 through 12 determined by one-way ANOVA and Tukey’s post hoc analysis (n=7).
Fig. 8. Box plot of dopamine release ([DA]p) after electrode recovery to 12 weeks after surgery. Symbols represent mean (Δ). [DA]p remains stable after electrode recovery with no significant differences between weeks (one-way ANOVA, p=0.173).
Fig. 9. Box plot of dopamine uptake from implantation to 12 weeks after surgery. There was a significant reduction in dopamine uptake ($V_{\text{max}}$) from implantation surgery values to the recovered signal values, while weeks 3 to 12 had no significant differences. Symbols represent outlier (◦) and mean (Δ). * $p < 0.01$, significantly different than week 3 through 12 determined by one-way ANOVA and Tukey’s post hoc analysis (n=7).
Fig. 10. Box plot of dopamine uptake ($V_{\text{max}}$) after electrode recovery to 12 weeks after surgery. Symbols represent outlier (◦) and mean (Δ). $V_{\text{max}}$ remains stable after electrode recovery with no significant differences between weeks (one-way ANOVA, p=0.115).
Fig. 11. Representative electrically evoked dopamine signals before and after electrical conditioning (etching). A, Evoked dopamine signal recorded prior to etching. Upper right, dopamine voltammogram showing dopamine oxidation (positive peak) and reduction (negative peak) identifying measured current as dopamine. Middle, dopamine current plotted against time showing total evoked dopamine signal. Lower figure, FSCV color plot with dopamine oxidation (green) and reduction (blue) showing peak voltages for dopamine oxidation (+0.7 V) and dopamine reduction (-0.3 V). B, Evoked dopamine signal recorded after etching at 60 Hz for 30 min. Upper right, dopamine voltammogram showing dopamine oxidation (positive peak) and reduction (negative peak) identifying measured current as dopamine. Middle, dopamine current plotted against time showing total evoked dopamine signal. Lower figure, FSCV color plot with dopamine oxidation (green) and reduction (blue) showing peak voltages for dopamine oxidation (+0.7 V) and dopamine reduction (-0.3 V).
Effect of Electrical Conditioning

Fig. 12. Box plot showing the percent change in dopamine current after electrical conditioning (etching) for 30 min at 60 Hz. Percent dopamine increased an average of 52.23% over 6 etching treatments. The percent change in dopamine current had a range from -20.21% to +176.79%. Symbols represent outlier (°) and mean (Δ).
Fig. 13. Box plot showing evoked dopamine current (nA) prior to electrical conditioning (etching) compared to that after etching for 30 min at 60 Hz. Electrically evoked dopamine current increased significantly after electrical conditioning. Symbols represent outlier (○) and mean (Δ). * p < 0.05, significantly different than post-etch determined by one-way ANOVA (n=6).
Fig. 14. Dopamine is identified by the characteristic oxidation peak (approx. +0.7 V) and reduction peak (approx. -0.3 V). Graphing the measured oxidative and reductive current against voltage provides a visual depiction, known as a cyclic voltammogram (CV), specific to dopamine. Pre-etch and post-etch dopamine CVs were compared to a standard dopamine CV. The post-etch dopamine CV was more similar to a standard dopamine CV verified through a Spearman’s rank correlation test with subsequent Williams’s test (p<0.0001).
Vita

Author: Eric Marr

Place of Birth: Loveland, Colorado

EDUCATION

Eastern Washington University, Cheney, WA, B.S., Biology, Cum laude, August 2013

Spokane Community College, Spokane, WA, A.A., Biology, 2010

AWARDS

Swartz-Narrance Biotechnology Graduate Student Fellowship. Awarded November 2014.


Eastern Washington University Biology Graduate Student Travel Grant. Awarded April 2014.


PROFESSIONAL SOCIETIES

Society for Neuroscience
INVITED PODIUM PRESENTATIONS

2013 TEDxSpokane, Eric Marr. “Brain Rush: Dopamine’s Effects on Learning and Memory”, Nov. 2013

POSTER PRESENTATIONS


TEACHING

Eastern Washington University, Teaching Assistant. Neurobiology (Biol 496), Spring 2015.

Eastern Washington University, Teaching Assistant. Human Anatomy and Physiology for Biology Majors (Biol 333), Winter 2015.

Eastern Washington University, Teaching Assistant. Human Anatomy and Physiology for Biology Majors (Biol 332), Fall 2014.

Eastern Washington University, Teaching Assistant. Neurobiology (Biol 496), Spring 2014.
Eastern Washington University, Teaching Assistant Biological Investigation (Biol 270), Spring 2014.

Eastern Washington University, Teaching Assistant. Human Anatomy and Physiology for Biology Majors (Biol 333), Winter 2014.

Eastern Washington University, Teaching Assistant. Human Anatomy and Physiology for Biology Majors (Biol 332), Fall 2013.

Eastern Washington University, Teaching Assistant. Neurobiology (Biol 496), Spring 2013.

RESEARCH EXPERIENCE

Carbon fiber microelectrode fabrication, flow cell chemical analysis, fast-scan cyclic voltammetry, surgical electrode implantation surgery, anesthesia using vaporized isoflurane, spectrophotometer, gas chromatography/mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance.