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# Improving dopamine monitoring with NCAM and the effects of intranasal oxytocin on dopamine signaling in the rat brain

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# IMPROVING DOPAMINE MONITORING WITH NCAM AND THE EFFECTS OF INTRANASAL OXYTOCIN ON DOPAMINE SIGNALING IN THE RAT BRAIN

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By
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**Thesis for Master of Science**

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## I. ABSTRACT

Dopamine (DA) is a neurohormone highly involved in learning and memory. Oxytocin (OXT), another neurohormone, has also been implicated in learning and memory. Fast-scan cyclic voltammetry (FSCV) is a method used for the real-time examination of DA neurotransmission in the brain. Implanting FSCV electrodes is an invasive technique that likely results in an inflammatory response (*i.e.* gliosis) that can restrict FSCV recording of DA signals. Neural cell adhesion molecule (NCAM) may reduce gliosis and potentially improve the ability of FSCV electrodes to monitor DA signaling. *Chapter 1 Methods*: FSCV electrodes were coated with NCAM and implanted in the rat brain. Non-NCAM coated electrodes were implanted in the contralateral hemisphere. Electrode recoveries (stability of electrode signals) were monitored for 8 weeks post surgery. *Chapter 1 Results*: NCAM coated electrodes stabilized sooner than their control counterparts (n=8). *Chapter 2 Methods*: FSCV electrodes were implanted in the rat brain and DA signals were experimentally evoked (i.e. electrically stimulated). Once clear DA signals were evoked and recorded, rats were exposed to intranasal OXT (n=3) or saline (n=3) and OXT-induced changes DA signaling was monitored for 1 h. *Chapter 2 Results*: Intranasal OXT increased DA signaling (i.e. peak height of DA current), relative to controls. *Conclusions:* NCAM coating expedites the ability of FSCV electrodes to monitor DA signaling in the rat brain. In addition, intranasal OXT increases DA neurotransmission, elucidating a potential mechanism for a modulatory role of OXT in learning and memory.

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**B. List of Figures**

**Table of Contents**

# CHAPTER 1

## II. INTRODUCTION

### A. Dopamine

Dopamine (DA) is a neurotransmitter highly implicated in learning and memory, as well as motor function (Schultz et al., 1997; Schultz, 2007). Central nervous system (CNS) diseases, such as schizophrenia, Parkinson's disease, and addictive behavior have all been suggested to have a relationship to DA function, either through dysfunction (*e.g.* schizophrenia and Parkinson's disease) or normal function, but abnormal learning (*e.g.* addictive behavior) (Howes et al., 2017; Kassinen & Vahlberg, 2017; Cooper et al., 2017). In regards to addictive behavior, understanding the underlying mechanisms behind reward learning, seeking, and prediction is crucial (Wise, 2004; Hart et al., 2014; Wenzel et al., 2015). Signaling related to these aforementioned behaviors are required for an organism's survival and can also be "hijacked" by drugs of abuse resulting in continual drug seeking behavior (Hyman et al., 2006; Daberkow et al., 2013; Escobar et al., 2015).

One of the regions of the brain highly innervated with DA neurons is the striatum. This brain region is divided into the dorsal and ventral striatum, both in function and anatomy (Wise, 2004). Transmission of DA is heavily relied upon in this region as the major contributor of information related to behavior (Schultz, 2013). Functionally, the dorsal striatum has been suggested to be highly involved in controlling motor function, whether goal orientated or otherwise, and the ventral striatum has been indicated to be involved in learning and memory (reward related or otherwise) (Schultz et al., 1997; Wise, 2004). Research has suggested that the dorsal striatum does have a role in learning and memory, but this tends to be limited to learning related to

habituation (Hyman et al., 2006). Due to the role of DA in these two regions of the striatum, techniques centered on objectively examining DA neurotransmission are necessary to further our understanding of DA activity in these sub-regions.

**B. Monitoring Dopamine**

There are two main methods used for examining DA activity *in vivo*, microdialysis and voltammetry. While invasive, both methods allow for recording of DA activity, as well as other compounds in the case of microdialysis. Microdialysis works though collecting cerebrospinal fluid (CSF) over a period of time, usually several minutes, from a specific region in the brain. The collected samples are then analyzed using analytical chemistry methods, such as high pressure liquid chromatography. This allows for multiple, stable compounds that are present in the CNS to be studied (Jackowska & Krysinki, 2013). Some technical concerns with microdialysis are that there are compounds used in the CNS that break down rather quickly, as well as the relatively large size of the probes used and the length of time the technique requires to collect analytes, which limits the use of microdialysis. Probes typically employed in voltammetry, in contrast to microdialysis, are much smaller and the technique allows for collection of data over the time course of milliseconds (Robinson et al., 2003; Rodeberg et al., 2017).

Fast-scan cyclic voltammetry (FSCV), a particular voltammetric method, uses reduction-oxidation chemistry as a means to describe local DA concentrations. A voltage is applied to the tip of the electrode which cycles between the known reduction and oxidation potentials of DA and its oxidized form. This reduction and oxidation results in a current change around the electrode that is then recorded (Fig. 1) (Bath et al., 2000; Wu et al., 2001; Tsunoda, 2006;

Takmokov et al., 2010; Rodeberg et al., 2017). Due to the speed of acquisition, typically every 100 ms (ten records every second), FSCV recordings can be considered to occur in real-time. This allows for changes in DA signaling to be directly related a presented stimulus (Wightman et al., 1988; Bilder et al., 2004).

Thanks to current advancements in manufacturing techniques, FSCV electrodes can be constructed in a manner that allows for chronic placement and monitoring of DA neurotransmission long-term (*e.g.* weeks to months) (Clark et al., 2010). These silica housed carbon fiber electrodes can be implanted and recorded from for months at a time, conferring the ability to examine how DA signals change over continued re-exposure to a stimulus (Clark et al., 2010; Rodeberg et al., 2017). These particular FSCV electrodes have also been shown to be effective at recording DA signals in humans as well (Kishida et al., 2011). While these chronic FSCV electrodes experience a dramatic reduction in inflammation post implantation, there is still a period of signal loss immediately following implantation that may be due to short-term inflammation (Clark et al., 2010; Rodeberg et al., 2017).

**C. Neural Cell Adhesion Molecule**

Neural cell adhesion molecule (NCAM) is a protein expressed by both glial cells and neurons in the CNS. Functionally, NCAM has been implicated in neuroprotection, synapse formation and maintenance, as well as neuronal organization (Li et al., 2017; Westphal et al., 2017; Ko et al., 2018). Cell adhesion molecule L1 (L1CAM), another protein that is in the same superfamily as NCAM, has been suggested to reduce inflammation (*i.e.* gliosis) when adhered to an electrode

that is chronically implanted (Azemi et al., 2011). Therefore, potentially NCAM can confer similar benefits when adhered to chronic FSCV electrodes.

Adhering NCAM to a substrate, such as a neural implant, has been previously associated in increase in cell-substrate adhesion, *in vitro* (Wiertz, 1974). More recent work has implicated NCAM as a means to further increase biocompatibility of implants, *in vivo* (Zhong et al., 2001). Furthermore, glial cells were found to more readily anchor themselves to glass electrodes that had bioactive compounds adhered to them, such as NCAM (Kam et al., 2002; Polikov et al., 2005). Using NCAM as a means to anchor cells to an electrode reduced the micro-movements electrodes can experience after implantation, thereby reducing the amount of surrounding tissue damage (Polikov et al., 2005). Adhesion molecules in the same superfamily (*i.e.* L1CAM) reduce reactive glial cell response to invasive implants. This also reduces the inflammatory response around the implant (Azemi et al., 2011).

Some of the previous research in this area has been focused on adhering bioactive compounds, like NCAM, to glass electrodes (Wiertz, 1974). More recently, methods have shown success at adhering L1CAM to silica housed electrodes (Azemi et al., 2008). Using similar coating techniques, our lab has be able to develop a protocol that allows for adhering NCAM to silica housed chronic FSCV electrodes (Daberkow EWU FGRCW 2015-16). Interestingly, during *in vitro* (experimental "flow cell" DA/buffer perfusion system) testing, we found that our NCAM treated electrodes had a greater response to 1μM DA compared to flow cell testing prior to NCAM coating (Fig. 2) (Daberkow EWU FGRCW 2015-16). This further emphasizes the potential benefit electrodes may have after exposure to our NCAM coating procedure. We

therefore aimed to determine if NCAM coating is a viable means to increase biocompatibility *in vivo* through measurement of FSCV electrode signal loss/return (*i.e.* electrode recovery), as well as stability of the electrode, as monitored by a background current measurement. We hypothesize that *FSCV electrodes that were exposed to our NCAM coating procedure would have a faster electrode recovery and a background current that stabilizes sooner compared to non-treated controls.*

## III. METHODS

### A. NCAM Coating

After construction of chronic FSCV electrodes (Clark et al., 2010), the carbon fiber tip and ~4-8mm of the silica housing was washed in 100% ethanol for 6 h. A 30 min 8M $HNO_3$ wash promptly followed. The purpose of these two steps was to clean the carbon fiber and silica housing in preparation for the subsequent steps, which were performed in an anoxic environment (*i.e.* under inert argon gas). Electrodes were then soaked in dry toluene (98-100%) for 30 min, in order to remove any excess water. A 10 min soak in a 2% (3-mercaptopropyl) trimethoxysilane (MTS, 2%) solution followed, in order to modify the silica housing with a carbon chain containing a thiol (R-SH) group. Subsequently, the electrodes were soaked in 4-maleimidobutryic acid N-hydroxysuccinimide ester (GMBS) dissolved in dimethylformamide (2 mM) for 1 h covalently attached the maleimide group of GMBS to the thiol group of MTS. The electrodes were then treated with NCAM, 100µg/mL for 1 h in sterilized water at 4°C, followed with a phosphate buffered saline rinse at pH 7.4 to bind the lysine residue of NCAM to the

terminal end of GMBS. A 30 min Poly(ethylene glycol)-$NH_2$ bath followed in order to further stabilize the aforementioned chemical functionalization.

**B. Animal Care and Use and Electrode Implantation**

Male Sprague-Dawley *Rattus norvegicus* (400-500g) were used (n=8). All animal handling and care complied with *EWU Institutional Animal Care and Use Committee* (IACUC). Rats were initially anesthetized with 5% isoflurane in 100% oxygen in a sealed plexiglass box. Once animals were unconscious, animals were immobilized in a stereotaxic frame. Anesthesia was then maintained throughout surgery with 1.9-2.1% isoflurane in 100% oxygen as delivered through a nose cone attached to the stereotaxic frame. An unconscious state was repeatedly tested via a foot pinch, to confirm animals were maintained under the proper amount of anesthesia. Respiration rates were also used as a measurement to confirm isoflurane setting was set properly. While conscious and alert, rat respiration rates were approximately 85 breaths per minute. During initial anesthesia, respiration rates dropped to roughly 24 breaths per minute, and were maintained between 32 and 24 breaths per minute. During instances of increasing rates of respiration, indicating that proper anesthesia was not being maintained, the isoflurane setting was increased to a maximum of 5% isoflurane saturated in 100% oxygen. The isoflurane setting was reset to the 1.9-2.1% saturation once the respiration rate decreased to 32 breaths per minute, or below. If respiration rates dropped below 24 breaths per minute, the isoflurane setting was lowered to 0% saturation in 100% oxygen. Once respiration rates increased to 24 breaths per minute, or higher, the isoflurane setting was once again reset to 1.9-2.1% saturation.

With animals immobilized in the stereotaxic frame, skin and fascia were cleared and small holes (1-2 mm) were drilled in the skull for the placement of FSCV, stimulating and reference

electrodes, as well as for skull screws. FSCV electrodes were then implanted in the striatum (AP = +1.0; ML = +2.0; DV = -4.5 – 6.0) (Paxinos and Watson, 1986). Electrodes were implanted bilaterally; a NCAM-treated electrode in the striatum of one brain hemisphere and a non-NCAM treated electrode in the striatum of the other brain hemisphere, in the same rat. In order to evoke a DA signal, a twisted bipolar stimulating electrode (Plastics One, Roanoke, VA) with a ~1mm tip separation, was incrementally placed just above the medial forebrain bundle (AP = -4.6; ML= +1.4; DV = -7.0), a DA fiber tract that projects to the striatum. A chlorinated silver wire (Ag/AgCl) reference electrode was placed just below dura a short distance (approximately 1 to 2 mm) from FSCV electrodes. After electrodes were implanted, a head mount (Datamate M80, Harwin, Salem, NH) was used to allow for the connecting and disconnecting of electrodes for subsequent recordings. Electrodes and head mount were permanently fixed into place using skull screws and dental cement. The rats were monitored carefully after surgery and given a 7 d period in order to recuperate from surgery.

**C. Electrode recovery**

Beginning 7 d post-surgery, rats were anesthetized following the previously described procedure. Once fully anesthetized, rats were reconnected to the recording equipment and anesthesia was maintained (as described previously) while the pertinent data were collected (i.e. presence, or absence, or electrode signals and background current of electrode signals, if present). First, electrodes were monitored on an oscilloscope to determine if electrodes had recovered in a manner allowing for a background signal to be recorded. If the oscilloscope indicated a stable electrode signal within the recording range of the FSCV system, a measure of the background current was recorded and collected. Electrodes were considered recovered following three consecutive recording sessions of visibility on the oscilloscope ("*electrode recovery*", Fig. 3).

Due to electrodes having a wide range of baseline background currents, analyses were performed through examining percent change of each electrode. These comparisons were made by taking the day's background current recording, and normalizing the recordings to the background current of the electrode on the day it was implanted. Once the background signal plateaued, an electrode was determined to be stable ("*electrode stability*", Fig. 4-6). All electrodes, whether or not to be determined as stable, were left implanted and examined three times a week, for 8 wks (~56 d) post-surgery.

**D. Statistical analysis**

A *generalized additive mixed model* was used to assess statistical differences between control and NCAM-treated electrodes. This model incorporates both an additive model and random effects. The additive model allows one to fit non-linear relationships based on smooth splines, while the random effects allows one to incorporate non-independence due to repeated sampling of the same individuals over time (Zuur et al., 2007). The model we used had the percent change in background current as the response variable, electrode type as a fixed parametric effect, day post-surgery as a smooth non-parametric effect, and the individual rat as the random effect, with different relationships between day post-surgery and percent change in background current allowed for the different electrode types. The power of the model allows for ample prediction of how electrode background current changes based off of previous changes (*i.e.* background current changes on day 20 are related to background current changes on day 17). An ANOVA of the model provided all values. The model and ANOVA were conducted with the statistical model R (R Core Team, 2018).

## IV. RESULTS

Control electrodes recovered 27 d post surgery and NCAM electrodes recovered 21 d post surgery, which did not significantly differ (p=0.435, Fig. 3). The *generalized additive mixed model* analysis indicated a significant differences between both control electrodes and NCAM electrodes based on the day post-surgery (p=0.0213; Fig. 4). Control electrodes were considered stable at approximately day 12, as determined by a horizontal line test (*e.g.* examining whether or not a horizontal line can fit across a period that would not intersect the 95% confidence interval) (p=0.00233;Fig. 5). NCAM electrodes were seen to change less based on the day post-surgery (p=000017;Fig. 6). As determined by a horizontal line test, NCAM electrodes were considered stable by day 8 post surgery. Comparing percent change over the 8 wks of the experiment, NCAM electrodes experienced less change in recorded background current than control electrodes (p=0.0213, Fig. 7). On average, NCAM electrodes changed 8% less than control electrodes throughout the entire 8 wks (confidence interval: -1% to -16%).

## V. DISCUSSION

NCAM treatment of FSCV electrodes resulted in a smaller range in electrode recovery times (Fig. 3) and electrode stabilization time (Fig. 4), relative to control electrodes. Electrodes that reached stability sooner were more readily available for consistent recording functions at an earlier time point. This permits initiating an experiment involving the recording DA activity shortly after the animal has recuperated from FSCV electrode implantation surgery.

When characterizing the chronic DA electrode, Clark et al. (2010) indicated that there was little to no gliosis surrounding the electrode one, two, or four months after implantation; however, the short-term effects (1 – 8 wks) of invasively implanting a FSCV electrodes into brain has not been previously investigated empirically. Furthermore, Rodeberg et al. (2017) suggest the requirement of a one-month period wherein electrodes are left unused in order to allow for immune response (*i.e.* gliosis) to reduce to the point of regaining functionality of chronically placed FSCV electrodes. Demonstrated in this study, FSCV electrodes treated with NCAM stabilize in approximately 8 days, shorter than the one-month period previously suggested (Rodeberg et al., 2017). This may, in part, be due to a reduction in gliosis around the FSCV electrode (Polikov et al., 2005; Zhong et al., 2001). This shorter waiting period is where the data gained from the current study indicates there can be an improvement of electrode function. Considering the loss of electrode functionality during the one-month post-implantation period is likely related to gliosis, NCAM potentially reduces gliosis around the FSCV electrode and therefore reduces the time for electrode recovery and stabilization. NCAM, is a bioactive compound that is expressed on both neurons and glial cells and is a ligand and receptor for itself (Sytnyk et al., 2017). Neurons and glial cells more readily anchor themselves to electrodes coated with bioactive compounds like NCAM (Polikov et al., 2005). This can be thought of as a "like recognizes like" concept, where glial cells and neurons are interacting with the implanted probe as though it "belongs" in the tissue. This may be the reason behind why NCAM electrodes recover their signal faster than control electrodes; inflammation is no longer isolating the electrode, allowing it to come in contact with the surround tissue and extracellular fluid, and thereby permitting the electrode to more readily record a clear and consistent signal.

Thanks to advancements in construction techniques, the currently constructed chronic FSCV electrode (Clark et al., 2010) has been further modified and successfully used to record human DA signals *in vivo* (Kishida et al., 2011). While it has been reported that chronic FSCV electrodes can continue to function over long periods of time, there is still the aspect that early, post-surgical function is unreliable (Rodeberg et al., 2017). With the application of cell adhesion molecules, such as NCAM or L1CAM, there lies potential to increase the reliability of electrode function sooner after implantation. Previous work has indicated that L1CAM reduces inflammation around the electrophysiology electrodes, which may be the cause of the instability (Azemi et al., 2011). With application of NCAM to chronic FSCV electrodes, a closed circuit, self-regulating system intended for deep brain stimulation of neurodegenerative diseases, such as Parkinson's disease, would allow for earlier function of the system (Kuo et al., 2018). This could allow for a not only sooner maintenance of motor function, but better regulation of stimulated DA release which may reduce psychiatric disorders related to over-release of DA (Bouthour et al., 2019)

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## VII. FIGURES

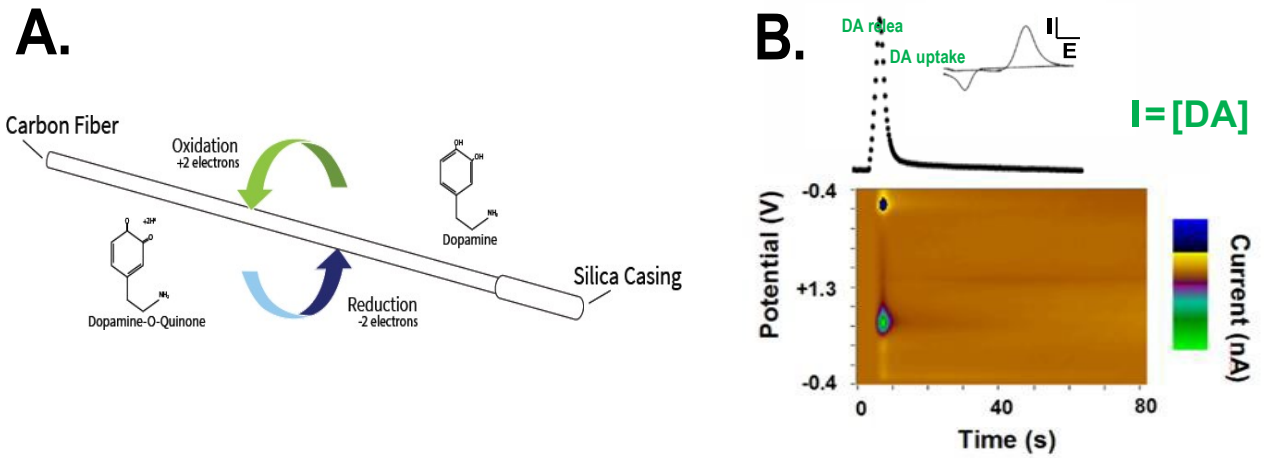


**Fig. 1. Fast Scan Cyclic Voltammetry (FSCV) at the carbon fiber microelectrode. (A)** Reduction/oxidation reaction at the carbon fiber of a microelectrode**(B)** A cyclic voltammogram (upper right) indicating oxidation (positive peak) and reduction (negative peak) of DA. Current is identified as DA (upper left). Three dimensional color plot (lower figure; x-axis= time; y-axis= applied potential; z-axis= measured current) of DA oxidation (green) and reduction (blue) typically used with FSCV. Peak voltages of DA for oxidation (+0.7V) and reduction (-0.3V) are shown. (Wu et al., 2001).

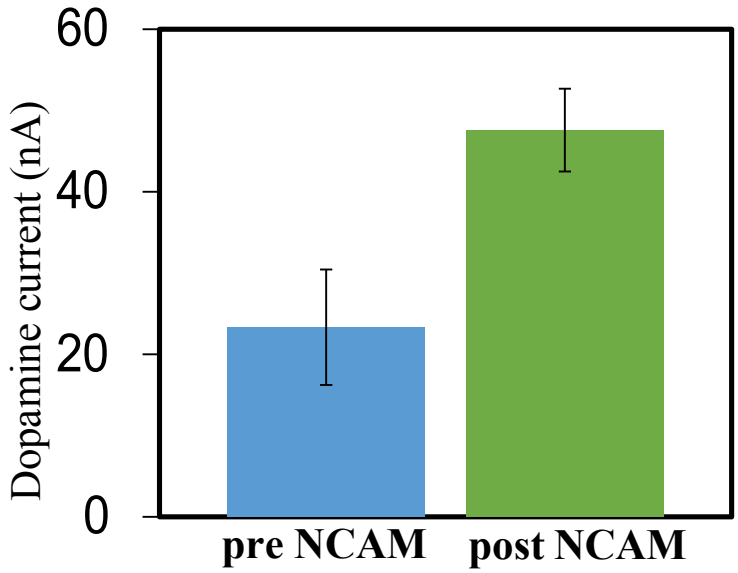


**Fig. 2. Pre- and Post- NCAM electrode *in vitro* response to 1 µM DA.** NCAM treatment significantly increased dopamine current *in vitro* (*p = 0.02, Student's t-test, n = 6).

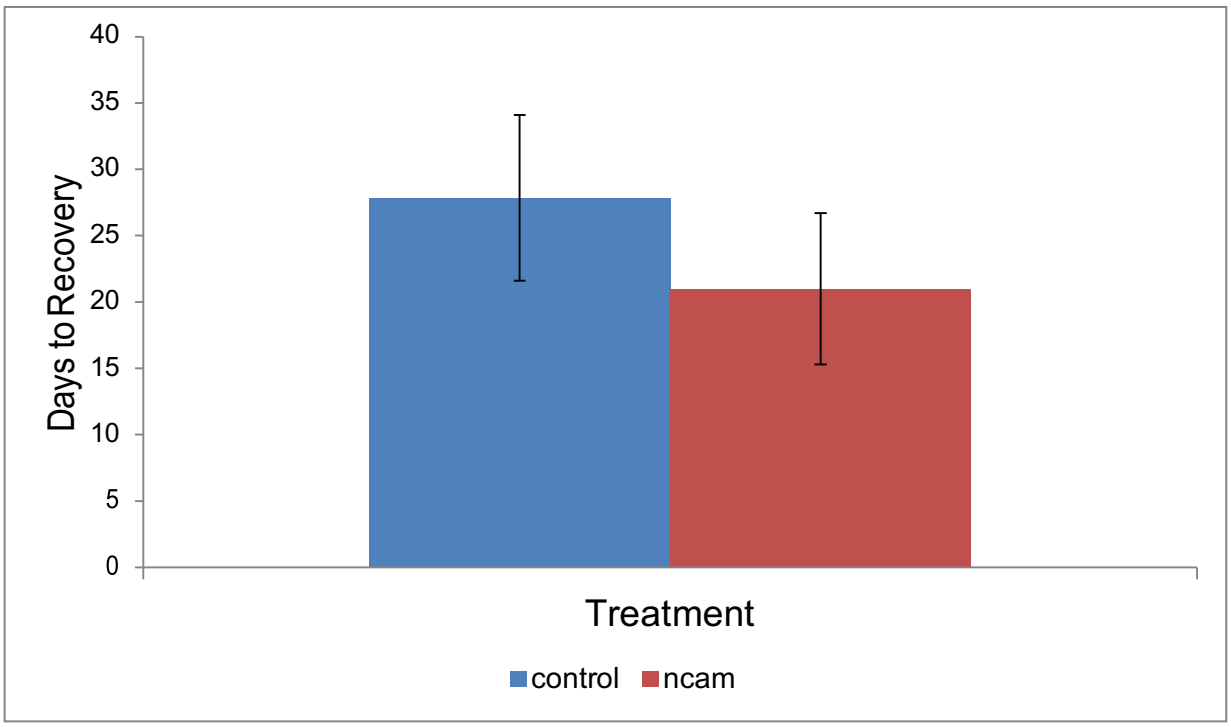


**Fig 3. Electrode recovery times for NCAM and control electrodes.** Control electrodes were considered recovered on day 27 and NCAM electrodes were considered recovered by day 21, on average. Differences were non-significant (p=0.435, *Student's t-test*, n=8 per group).

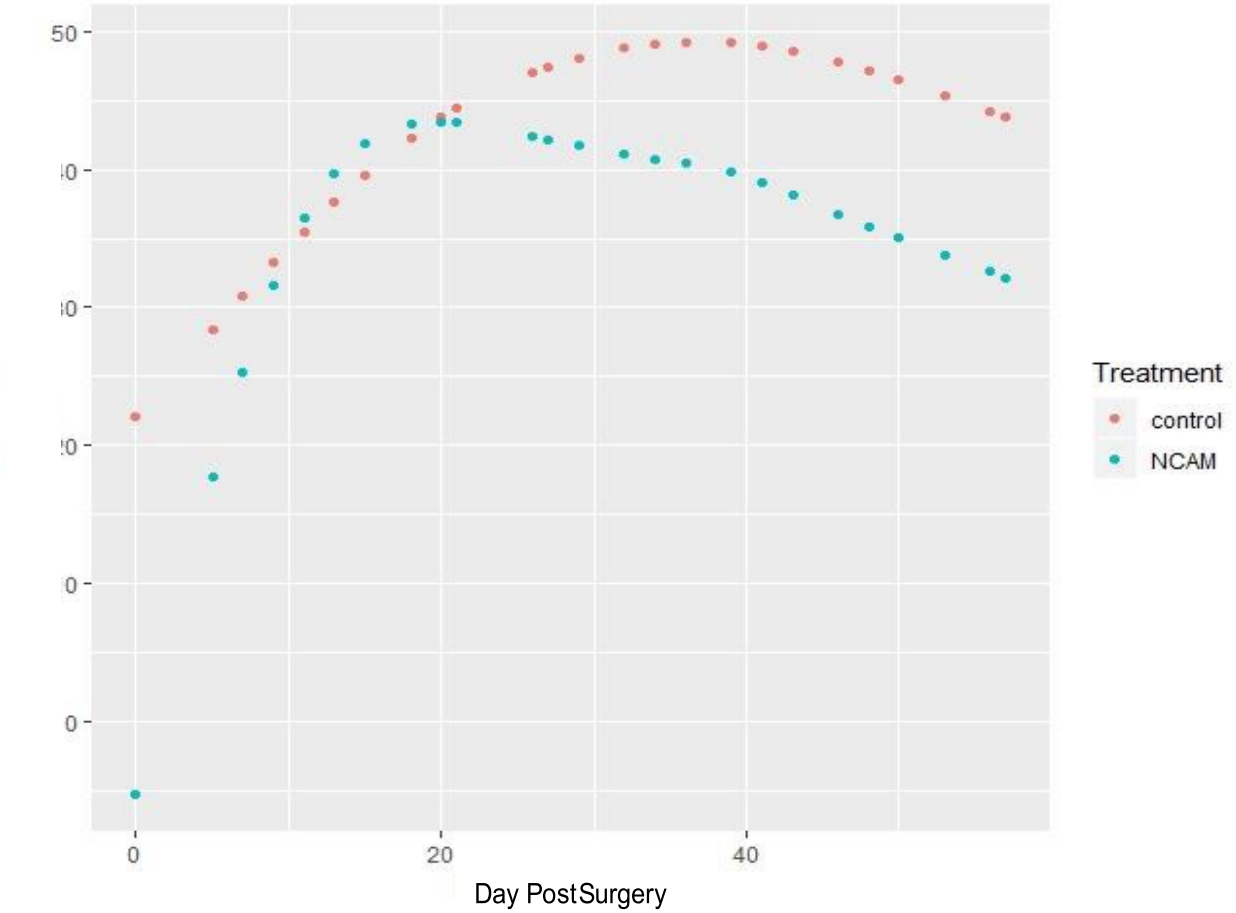


**Fig. 4. Time course of NCAM and control electrode percent change over 56 days a predicted by the model.** NCAM and control electrodes experienced significant differences in background current over 56 days (p=0.0213, *generalized additive mixed model,* n=8 per group).

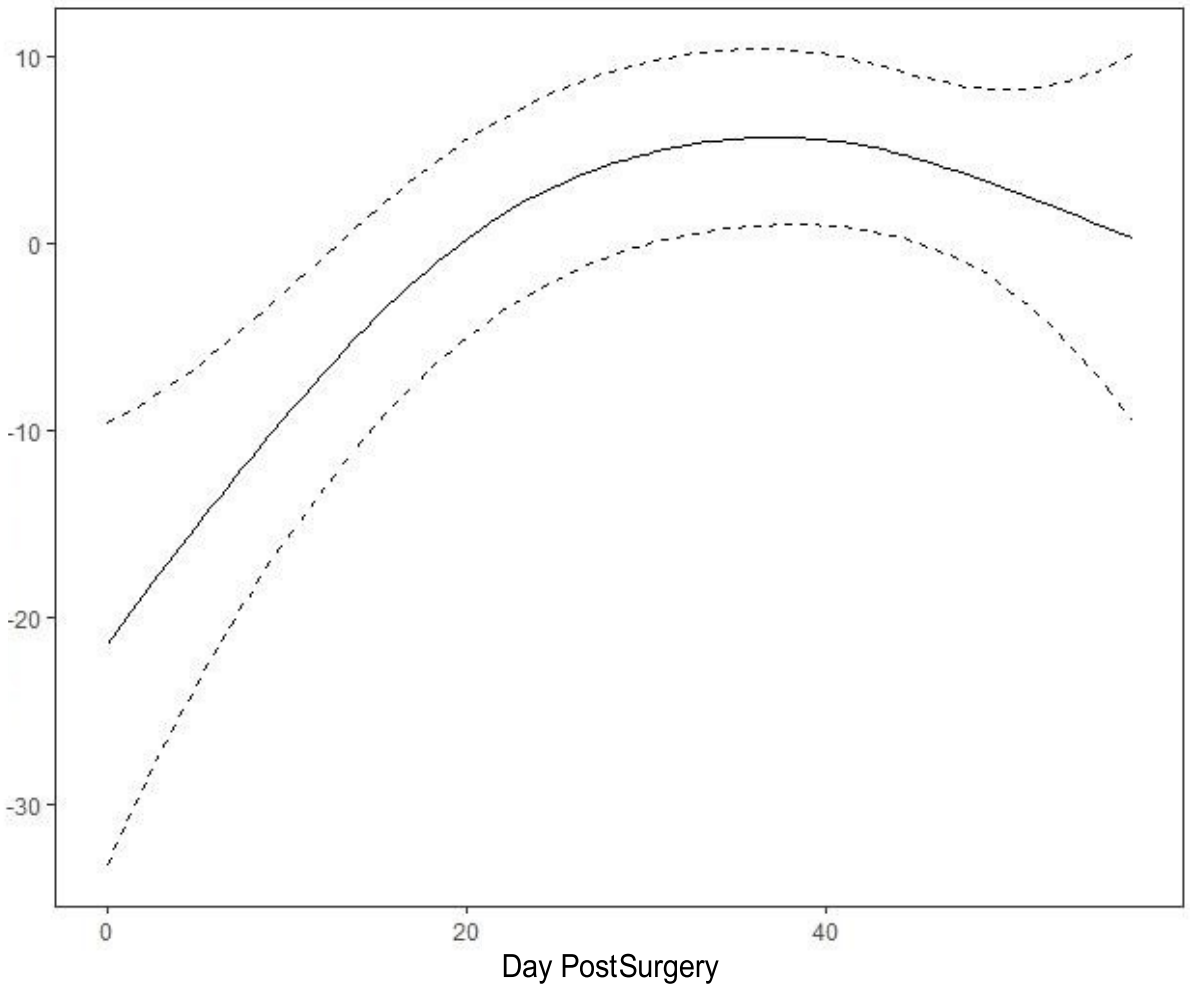


**Fig. 5. The effect of day post surgery on the percent change of control electrodes.** Over 56 days, control electrodes changed significantly (p=0.00233, *generalized additive mixed model,* n=8 per group). Control electrodes were no longer considered to be effected after day 12 post surgery . This time point is when control electrodes were considered stable (*horizontal line test*). Dotted lines indicate a 95% confidence interval.

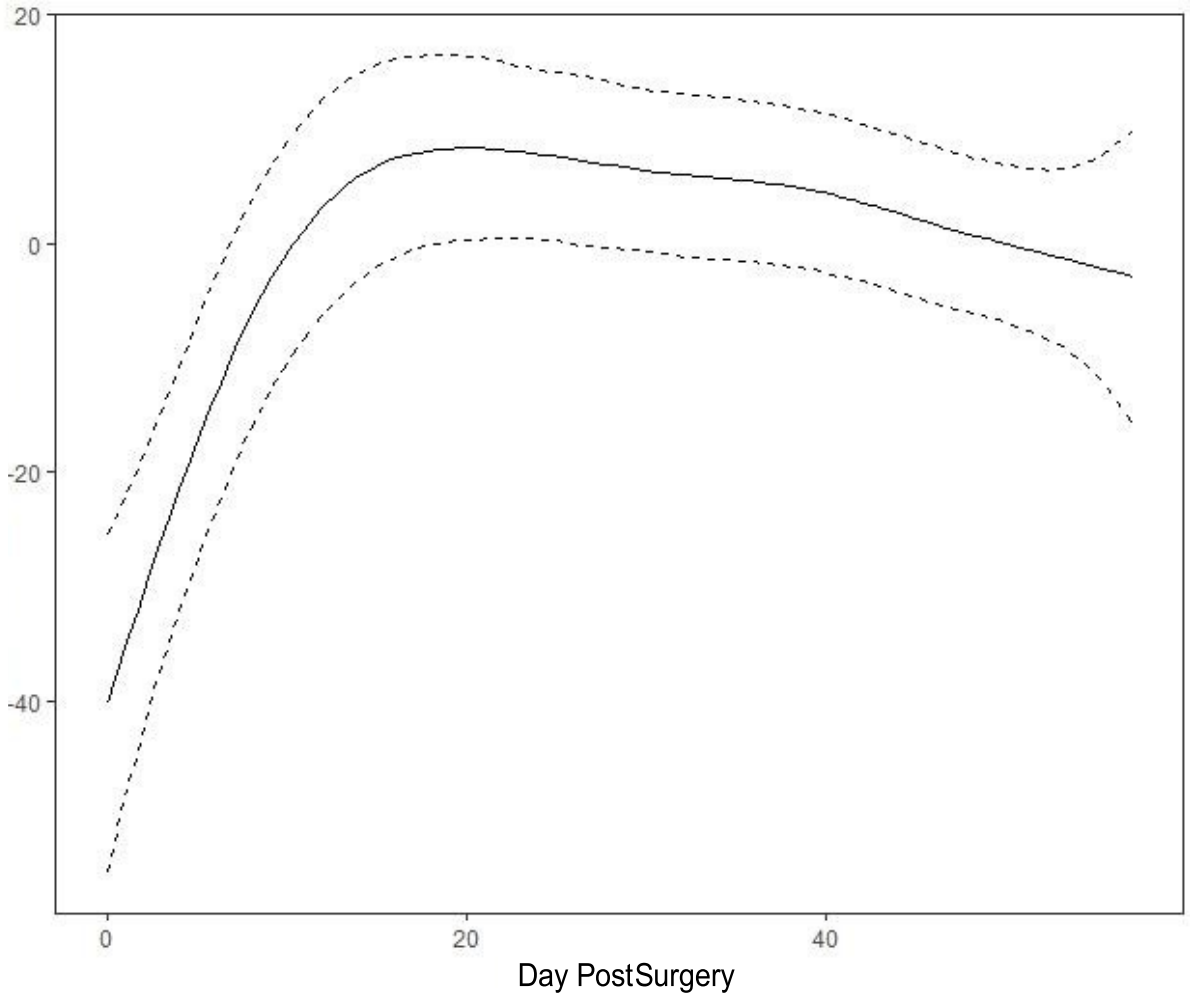


**Fig. 6. The effect of day post surgery on the percent change of NCAM electrodes.** Over 56 days, NCAM electrodes changed significantly (p=000017, *generalized additive mixed model,* n=8 per group). NCAM electrodes were no longer considered to be effected after day 8 post surgery . This time point is when control electrodes were considered stable (*horizontal line test*). Dotted lines indicate a 95% confidence interval.

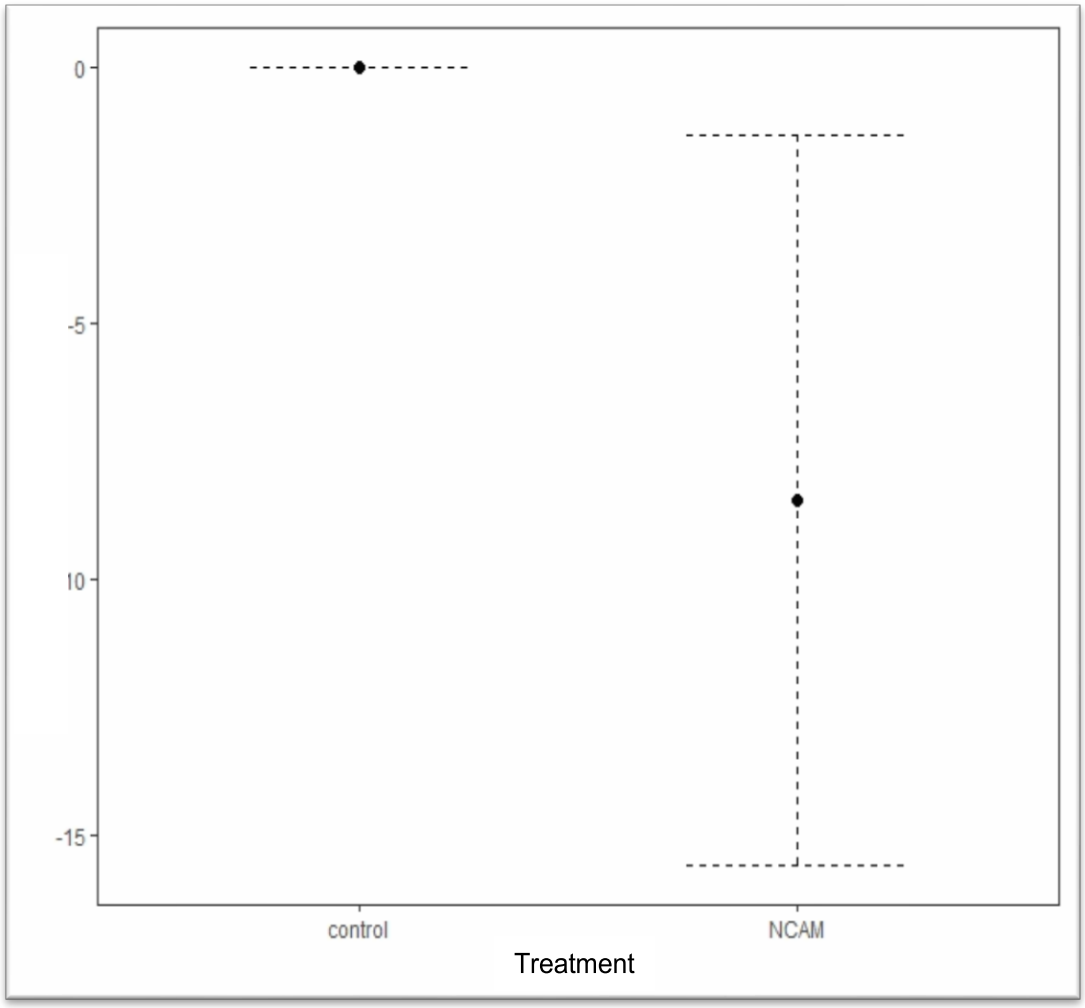


**Fig. 7. Amount of change NCAM electrodes experienced over the course of the experiment compared to control electrodes.** NCAM electrodes were normalized against control electrodes. As determined by the, the percent change NCAM electrodes experienced was 8% less than control electrodes (p=0.0213, *generalized additive mixed model*, n=8). The 95% confidence interval is indicated by the dotted line.

# CHAPTER 2

## II. INTRODUCTION

### A. Oxytocin

Oxytocin (OXT) is a long studied neurohormone involved in social behavior, childbirth, learning, memory, and emotion (reviewed in Jurek & Neumann, 2018). More recently, OXT has been suggested to have a role in fear conditioning (Renicker et al., 2018) and stimulus salience (*e.g.* how rewarding a stimulus is or is not) (Parr et al., 2018). The role of OXT in learning and memory is likely related to regions innervated with OXT neurons. As mentioned in the previous chapter, dopamine (DA) is a neurotransmitter highly implicated in learning and memory. Many OXT neurons have projections to regions that contain DA neurons, such as the VTA, which sends DA projections to regions involved in reward learning (Love, 2014; Wise, 2004). OXT also influences activity in the amygdala, a brain region highly implicated in fear (Frijling, et al., 2016; Love, 2014), further strengthening the role of OXT in aversive learning and memory. This interaction of OXT and DA suggests a role of OXT in learning and memory, possibly via mediation of DA neurotransmission.

Currently, OXT is in being used in clinical trials as a means to treat alcohol use disorder through re-learning of social perception and appetitive behaviors (*e.g.* behaviors aimed at obtaining alcohol) (Mitchell et al., 2016). OXT has also been used as a treatment for post-traumatic stress disorder (PTSD) (Acheson et al., 2013). Chronic exposure to this fear inducing stimuli results in over exaggeration of the fear/stress response, thereby resulting in PTSD (Lee et al., 2016). Furthermore, research has indicated that behaviors involving OXT can be context dependent; positive stimuli (*i.e.* altruism, pair-bonding, trust, etc.) elicits positive social response while a negative stimuli (*i.e.* aggression, fear, etc.) promote a negative social response (Love, 2014).

**B. Oxytocin and Stress**

Focusing on negative stimuli, previous work has indicated that endogenous OXT release can be a result of stressful context, as measured in plasma and saliva (Jurek & Neumann, 2018). This increase in endogenous OXT may be a means to reduce the potential fear responses that often result from a stressful context (Love, 2014). This implies that endogenous OXT release can act as a means to protect an animal from a fearful or anxious state during a fearful or stressful context. Increasing the concentration of OXT through exogenous administration could be beneficial and help an individual overcome anxiety or fear related to a stressful or a fearful stimulus. In rats, administration of OXT has been indicated as a means to not only reduce a fear or anxiolytic response to a previously fearful stimulus (Missig et al., 2010), but to also to enhance fear extinction to the previously associated stimuli (Triana-Del Rio et al., 2018). The anxiolytic effects of exogenous OXT have also been shown to be present in humans (Acheson et al., 2013; Fischer-Shofty et al., 2010). Furthermore, functional magnetic resonance imaging had shown that following OXT administration in individuals with PTSD, activity in the amgydala reduced in response to previously fear-associated stimuli (Frijling, 2017).

**C. Intranasal Oxytocin**

Exogenous delivery of OXT requires a means to bypass the blood-brain barrier. The blood-brain barrier inhibits most molecules from crossing from the blood into the brain, this includes peptides (*e.g.* OXT) (Ermisch et al., 1985). A means to bypass the blood-brain barrier is necessary to determine if administered OXT does in fact modify the stress response. Intranasal drug delivery is a relatively easy, non-invasive means of administration of readily absorbable compounds. Nasal physiology, which includes many capillary beds and nerve endings that are in direct connection to the brain, allows for rapid uptake of a compound into the brain (Talegaonkar

& Mishra, 2004; Wu et al., 2008). Further work has indicated that OXT administered intranasally does persist in the saliva (2-4 hours), in plasma (2 hours) (Huffmeijer et al., 2012; Weisman et al., 2012; Neumann et al., 2013; Bread et al., 2018) and increases OXT levels in the cerebrospinal fluid (Chang et al., 2012; Neumann et al., 2013; Striepens et al., 2013). Therefore, administering OXT intranasally is a noninvasive means to quickly get OXT into the brain.

**D. Oxytocin and Dopamine**

As previously mentioned, DA is a neurotransmitter in the brain involved in learning and memory (Schultz et al., 1997; Schultz, 2007). The role of DA in learning and memory is often examined through operant conditioning, typically through reward training, but DA activity also occurs with fear conditioning (Lee et al., 2016; Wise, 2004; Hart et al., 2014; Wenzel et al., 2015). Elucidating the role of both OXT and DA in learning and memory has become a topic of interest to many scientists, since both compounds are found to have some involvement in behavior related to reward or aversion learning. Furthermore, more recent work found that OXT receptors are found to be expressed on DA neurons located in the ventral tegmental area (VTA) in mice (Peris et al., 2017). The VTA is a region in the brain that sends projections to many other areas of the brain associated with learning and memory (Love, 2014; Schultz, 1997; Wise, 2004). Examining the impact of intranasal OXT on DA neurotransmission *in vivo* could elucidate potential mechanisms of action between OXT and the dopaminergic system in the brain.

As detailed in Chapter 1, Fast-scan cyclic voltammetry (FSCV) is a technique that can monitor and record current changes directly related DA activity (Bath et al., 2000; Tsunoda, 2006; Takmokov et al. 2010). These recordings occur in what is considered real-time, allowing for the examination of DA activity in response to a stimuli in freely moving animals (Rodeberg et al.,

2017). Given that OXT receptors are expressed on DA neurons (Peris et al., 2017), both OXT and DA have a role in learning and memory (Love, 2014; Schultz, 1997; Wise, 2004), and that intranasal OXT can bypasses the blood-brain barrier and effectively enter the brain, we hypothesize that *administering intranasal OXT will increase DA neurotransmission in the striatum.*

## III. METHODS

### A. Animal Care and Use and Electrode Implantation

Male Sprague-Dawley *Rattus norvegicus* (250-450g) were used (n=6). All animal handling and care complied with *EWU Institutional Animal Care and Use Committee* (IACUC). Animal care, both initially and during surgery, consisted of initially anesthetizing animals with 5% isoflurane in 100% oxygen in a sealed plexiglass box (as previously described). Once unconscious, animals were placed in a stereotaxic frame and anesthesia was delivered via a nose cone attached to the stereotaxic frame. The proper level of anesthesia was determined by a foot pinch and tracking respiration rates. Anesthesia was approximately set to 1.9-2.1% isoflurane in 100% oxygen throughout surgery, adjusting as necessary.

While under the appropriate amount of anesthesia and immobilized in the stereotaxic frame, skin and fascia on the head were cleared and small holes were drilled in the skull for placement of FSCV electrodes, stimulating electrodes, and reference electrodes. All holes drilled for FSCV and stimulating electrodes were drilled bilaterally, to allow for the opportunity to evoke and record DA signals on both sides of the brain, if needed. Coordinates for FSCV electrodes were gained via Paxinos and Watson, 1986. FSCV electrodes were placed in the dorsal striatum, a

region highly innervated with DA neurons (AP=+1.0; ML=+2.0; DV=-4.5). A twisted bipolar stimulating electrode (Plastics One, Roanoke, VA) which had ~1 mm separation at the tip, was incrementally placed above the medial forebrain bundle, a region that sends DA neuron projects to the dorsal striatum (AP=-4.6; ML=+1.4; DV=-7.0). A chlorinated silver (Ag/AgCl) reference electrode was placed just below the dura, a short distance (approximately 2-4 mm) from FSCV electrodes. For evoking DA release, a constant current of biphasic pulses (60 Hz, 60 pulses, 300 µA, Daberkow et al., 2013) were applied (NL 800, Neurolog, Medical Systems, Great Neck, NY) to the stimulating electrode. With electrodes initially placed, both FSCV and stimulating electrodes were incrementally lowered and electrical stimuli were applied until a clear and robust DA signal was evoked. In the instance of an unsuccessful evoked DA release on the initial side of electrode placement, electrodes were incrementally placed on the opposite side (with new FSCV and stimulating electrodes) and the DA optimization procedure was repeated until a clear and robust DA signal was evoked (as described in Chapter 1).

**B. Oxytocin administration, recording, and termination**

Once three clear DA signals were recorded, rats were intranasally administered 0.6 – 0.8 µg/kg of OXT (VetOne®) dissolved in saline (NaCl 0.9%), or an equivalent dose of saline. Due to the need of the nose cone to continually administer anesthesia, OXT (or saline) was administrated by carefully micropipetting the oxytocin solution (or saline) into the anesthesia line that fed into the nose cone. The point of administration was approximately 5cm from the rat's nose. Before administration, three DA signal were stimulated and evoked at 5 min intervals. Following administration, evoked DA signal recordings were collected every 5 min for 60 min. One hour post administration, rats were deeply anesthetized and lesions were made around the FSCV electrode by applying a strong current to the electrode (to create an electrical lesioned area to

identify the electrode placement post-surgery). Then, animals were sacrificed and brain tissue was collected, preserved, cryoprotected, and frozen at -20°C first, then -80°C for long term storage. Brains were then sectioned (30 μm) with a Lecia Cryostat in order to determine exact electrode placement.

**C. Statistical Analysis**

A linear mixed model was used to effectively model differences between OXT/saline treated animals at the different time points of the experiment. The mixed model has percent change in background current as the response variable, treatment and time as fixed effects and individuals as the random effect. Pairwise analyses were also conducted in order to further determine differences between time points, as well as between treatment types. Fixed effects for the mixed model included time and treatment type (OXT or saline). All statistical analyses were conducted with the statistical software R using the package lme4 (R Core Team, 2018).

## IV. RESULTS

The mixed model indicated significant differences between OXT and controls at two specific time points, 20 min ($p=0.012$) and 40 min ($p=0.0243$). A pairwise analysis indicated that other time points did not achieve significant differences, with a significance level below 0.05, but many were trending towards being significantly different ($p<0.10$) (time: 15 min, 25 min, 35 min, and 50 min). Confidence intervals (95%) indicated much overlap between both groups (Fig. 7), even with the two time points that did reach significant differences. These data are presented again without error bars for ease of viewing the percent change of OXT or saline treated animals over the recording period (Fig. 8).

## V. DISCUSSION

These data suggest that intranasal OXT increases DA neurotransmission in the dorsal striatum. While only two time points indicated significant differences between OXT and controls, there was a trend toward significance with other time points. This lack of significance during these time points may be related to the small sample size (n=3, rats per group). An increase in sample size could result in obtaining significant differences between OXT and controls at those time points where DA signals were close to reaching significant differences. Currently, these data support the hypothesis that intranasal OXT increases DA signaling, *in vivo*.

As discussed previously, DA is involved in learning and memory, both reward learning and aversion learning (Schultz et al., 1997; Wise, 2004; Schultz, 2007; Haack et al., 2014; Miranda et al., 2017). Also, as previously mentioned, OXT receptors are found in brain regions highly innervated with DA neurons, such as the VTA and many regions of the basal ganglia (*i.e.* caudate, putamen, nucleus accumbens, globus pallidus) (Love, 2014; Jurek & Neumann, 2018). The increase in DA release in the striatum of the animals used in this study can be considered to be related to the activation of OXT receptors in this region, facilitating DA release. Due to rat basal ganglia structure and function being similar to humans, it is likely that there will be similar effects in humans when administered intranasal OXT.

The dorsal striatum is involved in both DA neurotransmission and some aspects of reward seeking and aversion (Schultz, 2007). Since OXT and DA neurons and receptors are typically found in close proximity (Love, 2014; Jurek & Neumann, 2018), the OXT induced increase in DA signaling suggested in this study, could be seen in other brain regions containing DA

neurons and projections. When more closely examining OXT and DA neurocircuitry, we can see a better picture of how OXT interacts with DA neurons, influencing DA release in regions involved with encoding fearful stimuli. The hypothalamus sends OXT projections to the VTA, which then sends DA projections to the amygdala. Simultaneously, the hypothalamus sends OXT projections to the amygdala (Love, 2014). Due to the role of the amygdala in fear conditioning, both DA and OXT activity in this region likely have some interaction with fearful stimulus encoding (Triana-Del Rio et al., 2018). Also, understanding that OXT closely interact with DA D2 receptors (Jurek & Neumann, 2018), which are typically an inhibitory subset of DA receptor type (Neves et al., 2002), OXT could reduce fearful response to aversive stimuli. OXT activates DA neurons in the VTA (Love, 2014), which then sends DA projections to the amygdala which activate D2 receptors (Greba et al., 2001). The activation of D2 receptors is likely enhanced by the nearby activation of OXT receptors, reducing activity in the amygdala (Jurek &Neumann, 2018). This is suggested to be true in fMRI studies done on human individuals that experienced a traumatic event and administered intranasal OXT (Frijling et al., 2016). OXT reduces the fear/stress response to a previously associated fearful/stressful stimulus, due to a reduction in amygdala activity.

In conclusion, the results of this study support the hypothesis that intranasal OXT increases DA activity in the striatum. Furthermore, this OXT-induced increase in DA neurotransmission could potentially modulate the neurocircuitry related to fear and anxiety.

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## VII. FIGURES

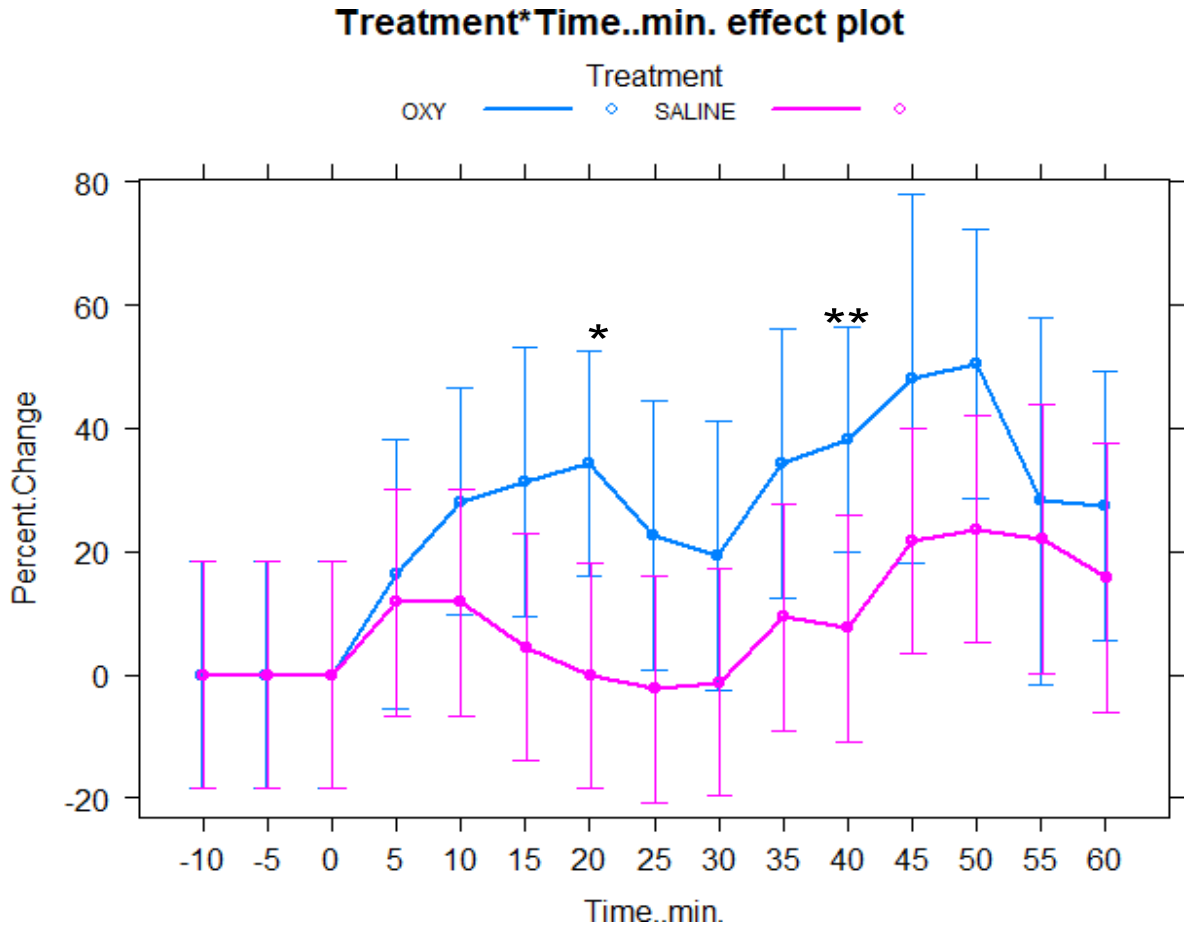


**Fig 8. Time course of percent change in DA signals post OXT/saline administration.** Error bars represent a 95% confidence interval. There is a significant difference in percent change between OXT and saline at time 20 min (*p=0.0122) and 40 min (**p=0.0243, *linear mixed model*).

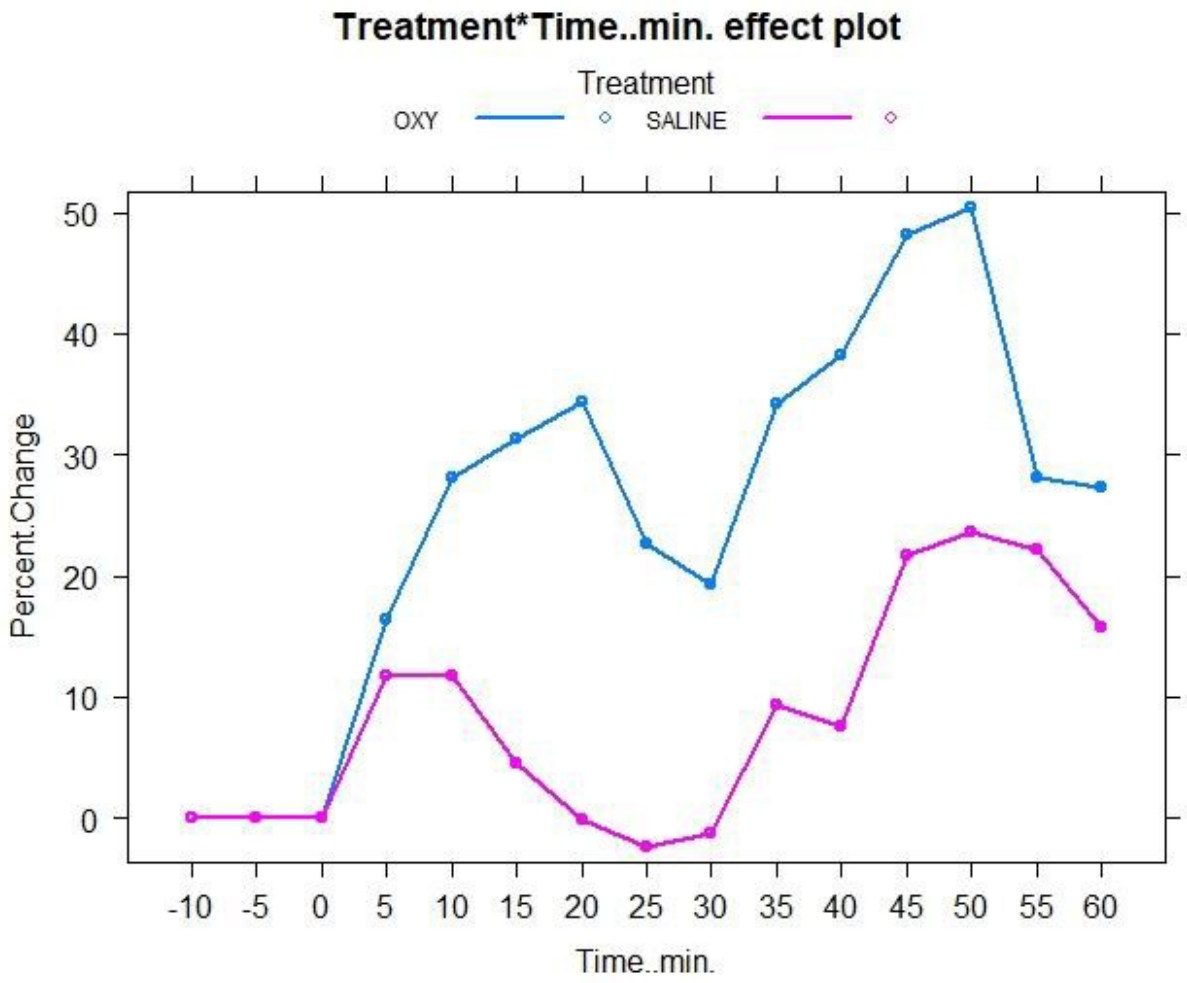


**Fig. 9. Time course of percent change of DA signals post OXT/saline administration presented without error bars.** Figure generated to allow for easier viewing of the time course data.

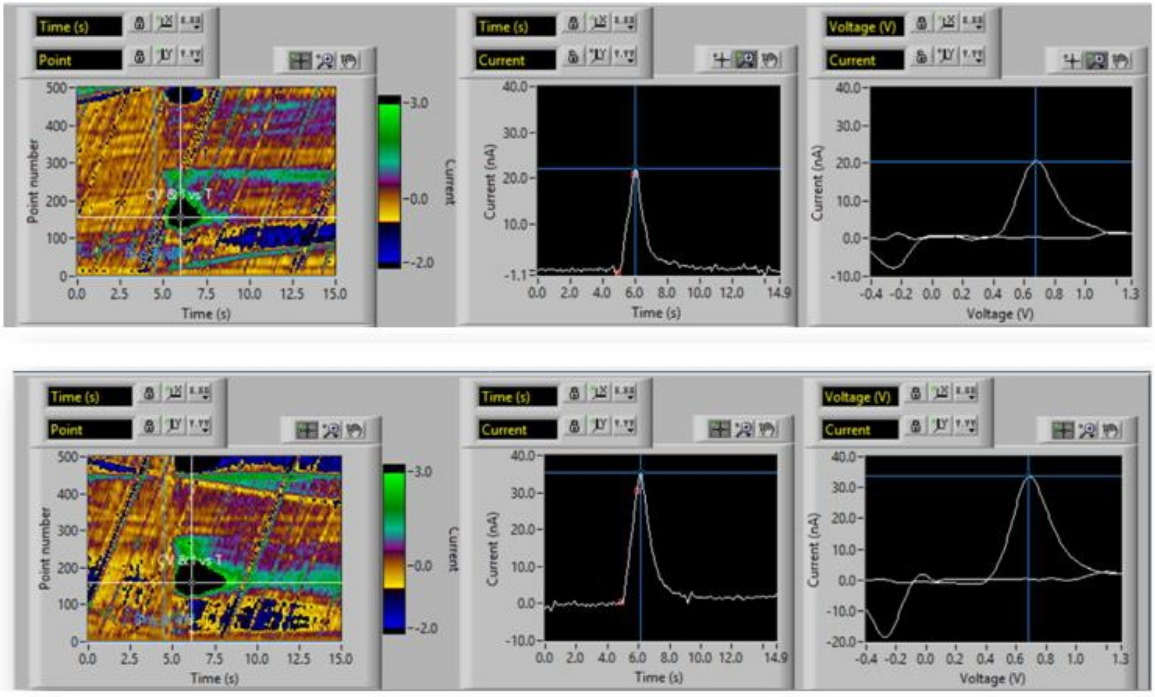


**Fig. 10. Example FSCV recording of DA response to intranasal OXT.** Top panel is electrically-evoked DA signal pre-OXT treatment and bottom panel is 20 min post-OXT treatment. Both images were taken from the same rat.

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Graduation: December 2015

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- 2 years research experience examining dopamine in freely moving animals
- B.A. Behavioral Neuroscience, Minor Chemistry
- 5 years research experience in basic research on neural substrates of addiction

- 3 time presenter at Society for Neuroscience Annual Meeting (2013, 2014, 2018)
- Member of Society for Neuroscience

**RESEARCH SKILLS**

- Fast Scan Cyclic Voltammetry (FSCV)
    - FSCV Electrode Construction
    - FSCV Electrode Implantation

- Addiction Studies
    - Transcardial Perfusions

  Cranial Surgeries-Cannulae insertion

- Experimental Analysis of Behavior
    - Western Blot & Electrophoresis
    - Bicinchoninic Acid (BCA) Assay
    - Brain Extractions and Tissue Slicing
    - Animal Handling
    - Rat Brain Histology

**PROFESSIONAL DEVELOPMENT**

**RESEARCH EXPERIENCES**

- Master of Science in Biology Student/Research Assistant, Eastern Washington University, August 2017-Current

- Research focus: FSCV and electrode biocompatibility

Advisor: Dr. David Daberkow

  - Conducted studies that recorded dopaminergic response to external stimuli, increasing biocompatibility of chronically implanted FSCV electrodes, and immune response to neuronal implants.

- Research Assistant, Western Washington University, January 2012-June 2017

Research focus: basic neural substrates of addiction

Principle Investigator: Dr. Jeff Grimm

  - Conducted studies that examined the relationship between craving and behavior with changes in protein (c-fos and DARPP32) expression in the mesocortical and striatial cortical dopaminergic pathways.

- Animal Handling including: interperitoneal/subcutaneous injections, cranial surgeries, drug microinjections into specific brain regions, running rat behavior studies using operant conditioning chambers and open field training.

- Proficient in: rapid decapitation, transcardial perfusion, brain extraction, brain slicing, Western blot, $CO_2$ euthanasia, anesthesia (isoflurane and ketamine)

- Lab schedule management and leadership (training of new lab members in proper use of materials)

**TEACHING EXPERIENCE**

- Eastern Washington University
    - Anatomy and Physiology Teaching Assistant (TA) ( September 2017-Current) - ~20 students per lab section
        - Human cadaver dissections
        - Lab practical and quiz preparation and grading
        - Directed students on lab exercises
        - Proctored lecture exams (400+ students)
        - Graded lecture exams (400+ students)
    - Neurobiology TA (April 2018 – June 2018) – 12 students
        - Human cadaver dissection and brain extraction
        - Lab practical and quiz preparation and grading
- Western Washington University
    - Methods in Behavioral Neuroscience TA (April 2015 – June 2015) – 10 students
        - Operant conditioning chambers using sucrose reinforcement
        - Trained students on animal handling
    - Behavioral Neuroscience (September 2014 – December 2014) - ~100 students
        - Graded classwork and exams

**PUBLICATIONS:**

- Grimm, J., Glueck, E., Ginder, D., Hyde, J., North, K., and Jiganti, K. (2018) Sucrose abstinence and environmental enrichment effects on mesocorticolimbic DARPP32 in rats. Scientific Reports, 8.

- Glueck, E., Ginder, D., Hyde, J., North, K., and Grimm, J. (2017). Effects of dopamine D1 and D2 receptor agonists on environmental enrichment attenuated sucrose cue reactivity in rats. Psychopharmacology, 5, 815-825.

- Grimm, J., Barnes, J., Koerber, J., Glueck, E., Ginder, D., Hyde, J., and Eaton, L. (2016). Effects of acute or chronic environmental enrichment on regional Fos protein expression following sucrose cue-reactivity testing in rats. Brain Structure and Function, 221, 2817-2830.

**PRESENTATIONS AND PUBLIC APPEARANCES**

- Improving the sensitivity and effectiveness of the Fast-Scan Cyclic Voltammetry dopamine electrode, poster, EWU Research and Creative Works Symposium, Eastern Washington University, June 2017

- Abstinence and environmental enrichment related changes in Fos expression in rats responding for a sucrose-paired cue, Poster# UU2, Society of Neuroscience Conference, Washington D.C., November 2014

- Examination of a role for corticosterone in the anti-craving effect of environmental enrichment in rats, Poster# JJJ27, Society of Neuroscience Conference, San Diego, November 2013

- Systemic dopamine D1 receptor agonism reverses environmental enrichment attenuated sucrose cue-reactivity, poster, Western Washington University PsycFest, Spring 2013

- Examination of a role for corticosterone in the anti-craving effect of environmental enrichment in rats, poster, Western Washington University PsychFest, Spring 2012

**COMMUNITY OUTREACH**

- Volunteered as part of Neuroscience Research Driven Students (NeRDS) Club visiting elementary schools and teaching neuroscience content to students
    - Carl Cozier Elementary (2013)
        - 2nd grade and 3rd grade

- Volunteer outreach to local elementary classrooms teaching students basic information on brain structures and purposes
    - Northwest Christian Schools (2017)
        - Kindergarten, 1st grade, and 4th grade
    - Saint Paul's Elementary (2014)
        - 1st grade