EVALUATION OF DOPAMINE SIGNALING IN THE RAT VENTROMEDIAL STRIATUM FOR VARYING REWARD PROXIMITY

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By

Muhammad Haziq

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TROMEDIAL STRIATUM FOR VARYING REWARD PROXIMITY

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We certify that we have read this thesis and that in our opinion it is fully adequate,
in scope and in quality, as a thesis for the degree of Master of Science.

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

EVALUATION OF DOPAMINE SIGNALING IN THE RAT VENTROMEDIAL STRIATUM FOR VARYING REWARD PROXIMITY

Muhammad Haziq

M.S. in BIOMEDICAL ENGINEERING AND BIOINFORMATICS

Advisor: Assist. Prof. Dr. Mehmet Kocatürk

August, 2020

Dopaminergic neurons play an important role in reward-mediated learning and motor control. In this work, we studied the transient changes in dopamine concentration in the ventromedial striatum (VMS) or nucleus accumbens (NAcc) during movement of a robotic actuator which is visible and brings reward to the rat through different trajectories. The goal of our work was to identify the relationship between the spatial proximity of the reward and the dopamine concentration in the VMS. In order to monitor the changes in dopamine concentration with subsecond temporal resolution, we chronically implanted two Wistar rats with carbonfiber microelectrodes in the ventromedial striatum (VMS) unilaterally and performed recordings using the fast-scan cyclic voltammetry (FSCV) system we developed. Presentation of unexpected reward and instrumental conditioning was used for engaging the rats with the behavioral tasks. Offline analysis was performed on the acquired data using principle component regression (PCR). During presentation of unexpected reward, a phasic increase in dopamine concentration was measured as 20.96 ± 7.0 nM for Rat-1 and $25.93.46 \pm 8.1$ nM for Rat-2 (n = 15 trials). During instrumental conditioning in which rats performed a nose poke through a hole for delivery of reward, the phasic increase in dopamine concentration was 29.1 ± 9.1 nM for Rat-1 and 50.72 ± 17.3 nM for Rat-2 (n= 15 trials). In experiments with reward carrying robotic actuator movements, an increase in dopamine concentration was observed as robotic actuator approaches toward the reward area. Fluctuations in dopamine concentration were observed during varying trajectories of the robotic actuator. Based on our voltammetric recordings with two rats, the phasic increase in dopamine concentration was significantly larger in instrumental conditioning in a comparison with presentation of unexpected reward (p<0.0005). With different trajectories of the robotic actuator, increase in dopamine concentration occurred only when the actuator, in

other words reward cue, reaches the reward area (121.01 ± 13.9 nM for Rat-1 and 61.98 ± 20.01 nM for Rat-2; n= 15 trials). Our results based on the recordings from two rats indicate that the spatial proximity of the reward cue can be identified by monitoring the fluctuations of dopamine concentration in the ventromedial striatum.

ÖZET

SIÇAN VENTROMEDİAL STRİATUMUNDA DEĞİŞEN ÖDÜL YAKINLIĞI İÇİN DOPAMİN İŞARETİNİN DEĞERLENDİRİLMESİ

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Dopaminerjik nöronlar, ödül aracılı öğrenme ve motor kontrolde önemli bir rol oynar. Bu çalışmada, sıçana görünür ve farklı gezinişlerle ödül getiren robotik bir eyleyicinin hareketi sırasında ventromedial striatum veya akkumbens çekirdeğindeki dopamin derişimindeki geçici değişiklikleri inceledik. Çalışmamızın amacı ödülün uzamsal yakınlığı ile ventromedial striatumdaki dopamin konsantrasyonu arasındaki ilişkiyi belirlemekti. Dopamin konsantrasyonundaki değişiklikleri bir saniyeden daha az zamansal çözünürlükle izlemek için iki Wistar sıçanın ventromedial striatumuna tek taraflı ve kronik olarak karbonfiber mikroelektrot implante ettik ve geliştirdiğimiz hızlı tarama döngüsel voltametri sistemini kullanarak kayıtlar gerçekleştirdik. Sıçanların davranışsal görevlere ilgisini çekmek için beklenmedik zamanlarda verilen ödüller ve araçsal koşullardırma yöntemi kullanıldı. Temel bileşen regresyonu kullanılarak elde edilen veriler üzerinde çevrimdışı analiz gerçekleştirildi. Beklenmedik ödülün verilmesi sırasında dopamin konsantrasyonunda anlık bir artış Sıçan-1 için 20.96 ± 7.0 nM ve Sıçan-2 için $25.93.46 \pm 8.1$ nM olarak ölçüldü (n = 15 deneme). Sıçanların ödül almak için bir delikten burnunu uzattığı aletsel koşullandırma denemeleri sırasında dopamin konsantrasyonundaki ani artış Sıçan-1 için 29.1 ± 9.1 nM ve Sıçan-2 için 50.72 ± 17.3 nM olarak gerçekleşti (n = 15 deneme). Ödül taşıyan robotik eyleyici hareketleriyle yapılan deneylerde robotik aktüatör ödül alanına yaklaştıkça dopamin derişimi artışı gözlemlendi. Robotik aktüatörün değişen yörüngeleri sırasında dopamin derişiminde dalgalanmalar gözlemlendi. İki sıçanla yaptığımız voltametrik kayıtlarımıza göre dopamin konsantrasyonundaki ani artış beklenmedik ödülün sunumuna kıyasla araçsal koşullandırma sırasında daha yüksekti (p<0.0005). Robotik aktüatörün farklı yörüngeleriyle ödülün verildiği

denemelerde dopamin konsantrasyonundaki artış sadece eyleyici, diğer bir deyişle ödül belirteci, ödül alanına ulaştığında meydana geldi (Sıçan-1 için 121.01 ± 13.9 nM ve Sıçan-2 için 61.98 ± 20.01 nM; n = 15 denemeler). İki sıçandan alınan kayıtlara dayanan sonuçlarımız ödül belirtecinin uzamsal yakınlığının ventromedial striatumdaki dopamin konsantrasyonundaki dalgalanmalar izlenerek belirlenebileceğini göstermektedir.

Anahtar sözcükler: Dopamin, Voltametri, Akümbens Çekirdeği, Ventromedial Striatum, Öğrenme.

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Chapter 1

Introduction

Brain is a vital organ which is responsible for controlling most of the body functions through the network of nervous system. It interprets information from the outside world and transforms all this raw information received through its sensory inputs into meaningful information and can store that information in memory for future use. Intelligence, creativity, emotion, and memory are a few of many things governed by the brain. The brain is comprised of two types of cells:. Gila cells are responsible for providing nourishment, protection, and structural support to neurons. Nerve cells are responsible for conveying information in terms of electrical and chemical signals.

How do neurons communicate with other neurons? This process occurs through synapses which is between the source neuron and target neuron. presynaptic neuron receives inputs from other neurons through dendrites, information comes from other neurons and transferred to neuron body which determines the importance of information and accordingly transfer the information to neighbouring neuron through synaptic transmission. Synaptic transmission can be of two types, i.e. electrical or chemical. Chemical synaptic transmission occurs commonly through vesicles located at axon of the presynaptic neuron excrete some chemicals known as neurotransmitters into synapse where they are bound to the receiving sites of other neurons, which stimulate the neighbour neuron to pass on

the message as shown in Figure 1.1.

Regular functionalities of the body such as controlling the brain activity, breathing and heart rate etc. are performed by the interaction of billions of neurotransmitter molecules. Furthermore these neurotransmitters play a vital role in physiological functions such as fear, mood, pleasure, joy etc. Well-known neurotransmitters in the brain are glutamate, gamma-amino butyric acid (GABA), acetylcholine, norepinephrine, serotonin and dopamine.
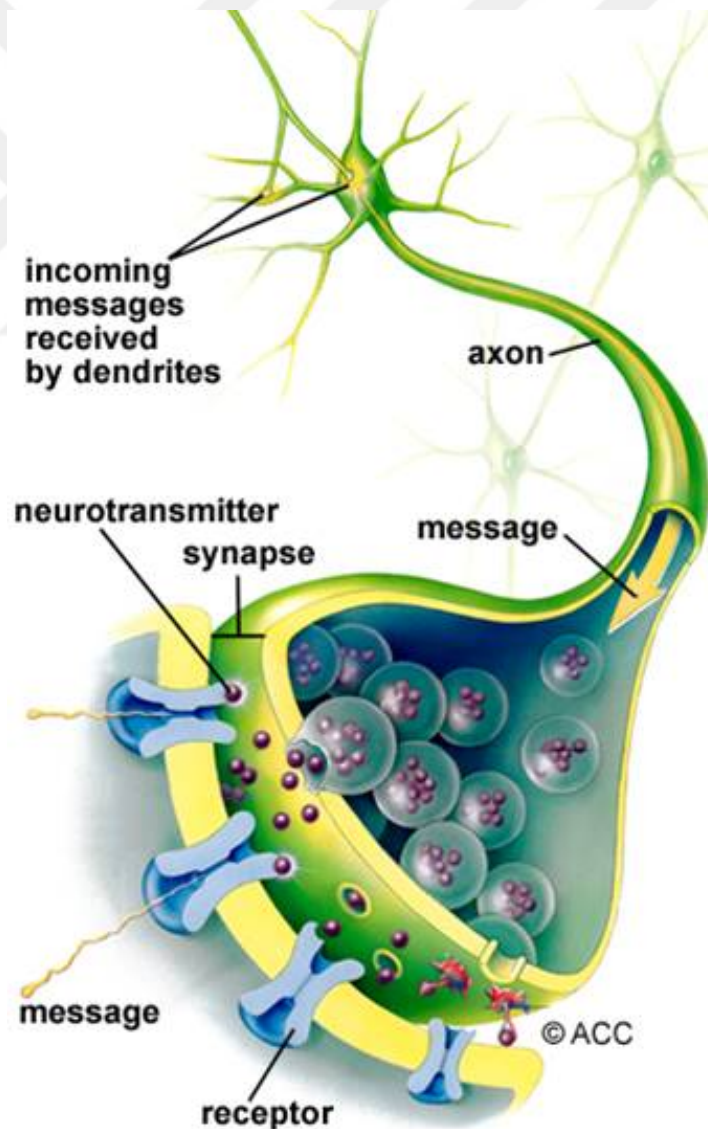
Figure 1.1: Nerve cell consist of a cell body, dendrites and axons [1].

Neurotransmitters act as messengers during synaptic transmission process. This process is essential for human health. Imbalance in concentration of these neurotransmitters result in various mental disorders or abnormal behavior. In order to diagnose such mental illness or to study function of particular region of the brain concentration of various neurotransmitters are monitored. For this purpose there are various invasive and non-invasive techniques used to monitor these neurotransmitters which include micro dialysis, positron emission topography (PET), functional magnetic resonance imaging (FMRI) and voltammetry.

Dopamine is one of the important neurotransmitters which is secreted by basal ganglia. It has been a subject of interest for neuroscientist for last several decades due to its vast impact on various critical mechanism of the brain such as movement, memory, reward, learning and motivation etc. The main focus of this thesis is to observe phasic changes in dopamine concentration in the striatal region of the brain of a behaving rat.

1.1   Dopamine and Dopaminergic Pathway

Dopamine is an important neurotransmitter which belongs to the catecholamine family, i.e. it transfers electrical signal chemically between the neurons. Despite being smaller in number as compared to other neurotransmitters (only 1%) , it plays a vital role and impact on human cognitive performance and emotional drive [4] [5]. Dopamine modulates various neural circuits of reward process, motor movement and cognition. More over it plays an important role in cardiovascular [6] and immune systems. Dopamine causes desire, curiosity and motivates thinking. It creates reward seeking loops and organizes repeat pleasurable behavior.

Since dopamine is incapable of penetrating blood brain barrier, it is created locally at Adrenal Medulla from its precursor molecules L-phenylalanine, L-tyrosine, and L-DOPA [7]. It controls various physiological functions within the brain via regulation through its receptors D1, D2, D3, D4 and D5. These receptors are divided into two groups namely D1 type and D2 type receptors.

D1 type receptor (D1 and D5) are mostly postsynaptic receptors while D2 type receptor (D2, D3 and D4) are both pre and postsynaptic receptors [8]. These receptors behave as excitatory or inhibitory on predefined neuronal pathways. Dysfunction of theses dopaminergic pathways leads to psychiatric disorders [6].

The dopaminergic system is comprised of four pathways, namely nigrostrialtal, tuberoinfundibular, mesolimbic and mescortical as shown in the Figure 1.2. The function of each pathway is described in following passage.
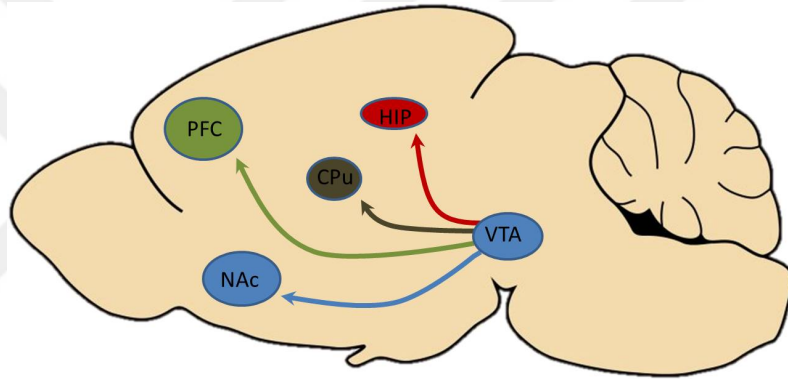
Figure 1.2: Dopaminergic pathways of rat brain. modified from [2].

• Mesolimbic Pathway: This pathway comprises of the projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). This pathway is associated with reward and pleasure behavior [9]. Stimulation of this pathway motivates the subject to perform day to day activities. However access of dopamine in this region can lead to addiction or state of euphoria [10].

• Mesocortical Pathway: Similar to mesolimbic pathway the projection of this pathway emerges from the ventral tegmental area (VTA) to prefrontal cortex structures. The prefrontal cortex (PFC) is associated with working memory, motivation and emotion. Excess amount of dopamine in this pathway can lead to mental disorders like schizopherenia [11]. On the other hand lack of dopamine can cause attention deficit hyperactivity disorder (ADHD).

• Nigro-striatal Pathway: Dopamine formed due to synthesis in substantia nigra is delivered to dorsal striatum. This pathway is comprised of 80% of the brain's dopamine and is mainly associated [12] with motor planning. Reduced number of dopamine neurons or damage to this pathway results in movement disorders

such as Parkinson disease [13].

• Tuberoinfundibular Pathway: This pathway is associated with the regulation of prolactin [14] and is originated from arcuate and areventricular neuclei of hypothalamus and projected to infundibular region of hypothalamus, specifically median eminence.

1.2 Techniques for the Detection of Neurotransmitters

There are two commonly used chemical assay for the monitoring the activities of neurotransmitters.

1) Microdialysis

2) Fast Scan Cyclic Voltammetry

Microdialysis is a minimally invasive technique that is commonly used for the continuous monitoring of neurotransmitters and other molecules in extracellular environment. As compared to push pull or cortical cup perfusion, it displaces smaller area of tissue and it provides a physical barrier to tissue during perfusion process. Despite of the advancements in this technique in terms of electrode size and coupling with other analytical methods, microdialysis still possess some limitation such as low temporal and spatial resolution (minutes to hour) [15] [16], disruption of the surrounding tissues. On the other hand, fast scan cyclic voltammetry (FSCV) which is advanced version of cyclic voltammetry used to study chemical changes on subsecond time scale. This technique is preferably used with carbon fiber microelectrodes [17] due to its various advantages such as biocompatibility, wider voltage range as compared to other electrode materials, Due to its small size, application at high scan rate becomes possible. In this technique, voltage applied to electrode is linearly increased from a holding potential with scan rate of 400V/s and 10 Hz frequency, after reaching to stimulating potential it comes back to resting potential. For detection of dopamine,

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the applied voltage range is -0.4V to 1.3V. since the applied waveform is bipolar in nature, during the forward cycle of the wave dopamine oxidized to become dopamine o-quinone after losing two electrons On the reverse cycle, this oxidized dopamine is then reduced to form dopamine again as shown in Figure 1.3.
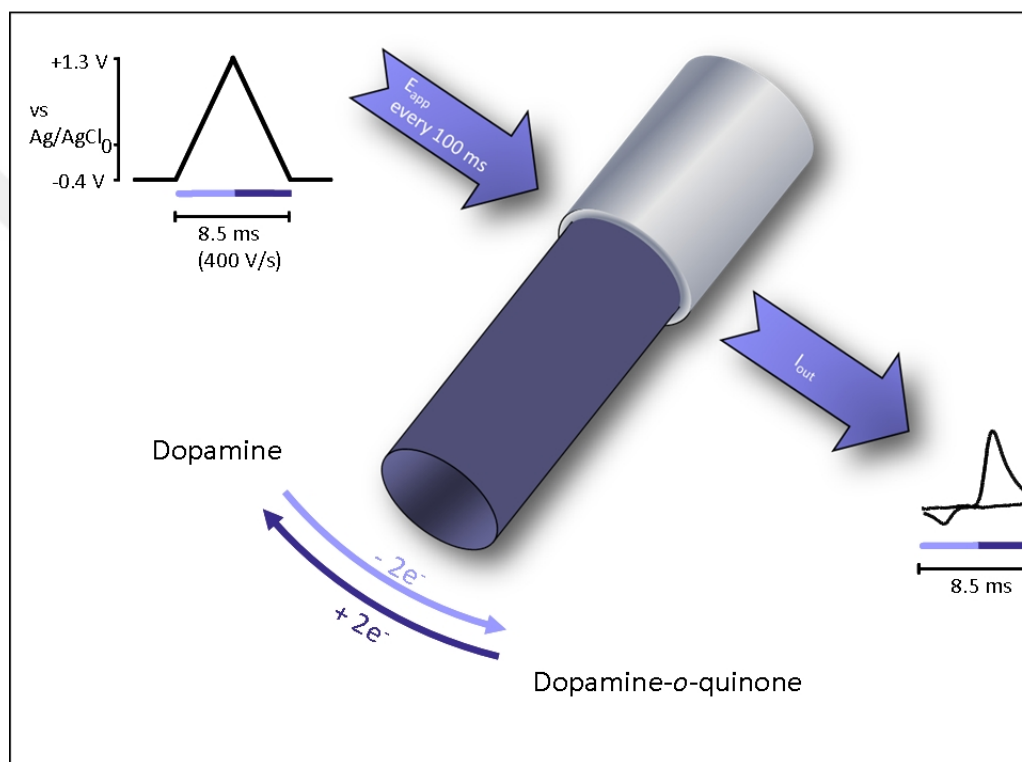
Figure 1.3: Principle of Fast Scan Cyclic Voltammetry [3].

1.3 Literature Review

To understand the relationship between dopamine and reward, various approaches have been used to understand dopamine release in the brain in absence or presence of reward. In earlier studies, rodents were trained to perform self-administered rewarding tasks (lever pressing), which results in electrically stimulation of dopaminergic pathway [18] [19]. Later on another approach was used to correlate dopamine and reward in which dopamine receptors were made dysfunctional by introducing neurotoxin before behavioral experiments were performed in which food pellet was given to rodent as reward. It was seen that the blockage

of dopamine receptor led the animal to not being motivated to eat food, hence suggesting that dopamine also plays a role in motivation (in addition to reward) [20].

Various studies also suggest that dopamine plays a role in predicting reward stimuli. An experiment was performed by Hikosaka et.al [21] based on three condition stimuli experiment in which they propose a particular group of dopamine neuron in substantia nigra and ventral tegmental area which represents motivational values. The variation in its activity represents prediction error associated to reward which also helps in learning while the subject approaches reward [22]. In another study, inactivation of NMDA and inotropic glutamate receptors in dopamine neurons of midbrain structures provide evidence that dopamine is necessary for predicting reward and aversive event [21]-[23] as those animals were not even motivated to eat food.

Most experiments related to studying dopamine's role and functionality were performed either through Pavlovian conditioning, where animal is trained to conditioned stimulus [18], or lever press [24]. In these methods, tasks are in peripersonal space and therefore, animal can reach them easily due to which changes in dopamine concentration are phasic. Limitation of these methods is that role of dopamine behind motivation of performing longer goal oriented tasks which results in distant reward are still unclear. Therefore, to monitor the activity of dopamine during a goal oriented and reward proximity tasks, a new approach was used by [25] in which rodents were trained to perform a task in T maze [26] where animal runs and turns to either left side or right side depending on the auditory cue to have reward. Fast scan cyclic voltammetry is used to monitor these dopamine phasic changes which were gradually increasing as animal approaches the target i.e. where reward was presented.

1.4 Scope of the Thesis

This work analyzes the role of striatal dopaminergic neurons in behavioral and learning activities, specifically their role in pleasure and reward experiences, motivation and motor movements. Considering the studies performed by A.M. Graybiel et.al. [20], the aim of the thesis is to investigate physiological concentration of dopamine while animal pursues the reward. For this purpose rat animals were chronically implanted with carbonfiber microelectrode in nucleus accumbens. After recovery, animals were trained to perform a set of reward associated tasks; unexpected reward delivery, reward delivery through motor movements (nose poke) and reward delivery through robotic actuator movements (with unidirectional and bi-directional movements). During the behavioral experiments voltammetric recordings were performed through an in-housed designed fast scan cyclic voltammetry system (FSCV). The acquired data is then analyzed offline.

An issue that arises while analyzing voltammograms is the inability to see dopamine properly under the influence of pH change. Therefore a data analysis technique utilizing principal component regression (PCR) is applied to the data for the identification of the neurotransmitter of our interest, i.e. dopamine.

1.5 Outline of Thesis

Chapter 01 provides an introduction, literature review related to dopamine and dopaminergic pathway and its role in goal oriented task. Furthermore it gives an overview of in vivo measurement techniques used for detection of neurotransmitters.

Chapter 02 provides description of method followed during experiments including hardware, software, microsensor developments, surgical procedures and the paradigms used during behavioral experiments.

Chapter 03 contains results which are observed during different behavioral

paradigm including unexpected, two different configuration of reward proximity based experiments.

Chapter 04 concludes the thesis. It includes discussions and future work in order to improve the current system and increase signal quality

Chapter 2

Methods

2.1 Fast Scan Cyclic Voltammetry System

Fast scan cyclic voltammetry (FSCV) is an electrochemical technique which was developed by Julian Miller in early 1980's. In FSCV the classic cyclic voltammetry technique has been accelerated by several orders to provide a subsecond temporal resolution (typically 100 ms). For detection of dopamine a bipolar voltammetric waveform with voltage range of (-0.4V to 1.3V) is applied at 10 Hz with a scan rate of 400V/s. These parameters were set for the detection of dopamine due to reason discussed in following passage [27] which is then followed by the description of FSCV system which we developed in our lab.

In order for better adsorption of dopamine on the electrode surface, negative potential is used as holding potential. It is seen that as reduction peak occurs at -0.2 V therefore it is better to use -0.4 to see peak properly. Another reason to use -0.4v as holding potential is since 90% of the time electrode is kept at holding potential. From studies it is seen that oxygen is reduced at below voltage level -0.6V and producing by products which causes oxidative stress to cell around the electrode [27]. For switching potential, early studies show that 1V is enough for the oxidation of dopamine but in later studies it is seen that scanning performed

with large potential increases current potential which is due to the activation of CFME surface. However, if we increase potential too much it will lead to etching, hence decreasing the diameter of CFME [28]. Keeping this in mind and results obtained from various studies it is suggested to use 1.3V as switching potential. Lastly we know that FSCV waveform is almost 10ms long so FSCV system can run on 100 Hz but instead of that it runs at 10 Hz. At 100 Hz less adsorbtion of dopamine is done due to which we see 75% less current but with 10 Hz, since electrode stands mostly at its holding potential due to which maximum adsorbtion of dopamine take place at electrode surface[27].

Following section will describe both hardware and software parts of FSCV system which is designed and developed in our lab. Block diagram of this system is represented in Figure 2.1.
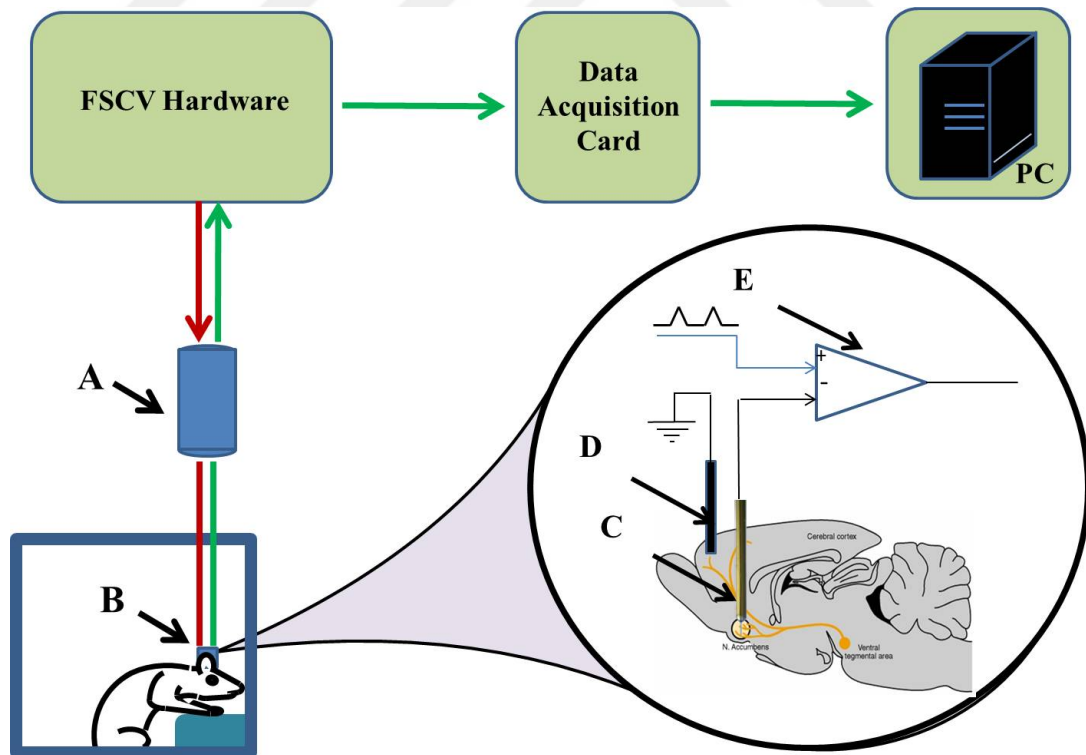
Figure 2.1: Generic overview of FSCV system .

2.1.1 Hardware

To design the hardware section of FSCV, we developed multiple hardware components and then linked them together for final results. These hardware components are as follows:

a. Waveform generation board

b. Headstage

c. Signal amplifier

d. Data acquisition

2.1.1.1 Wave form generation board:

The wave form generation board is responsible for generating triangular voltammetric waveform. It consists of following two subsections as shown in figure 2.2.

• Microcontroller section

• Analog Section

Microcontroller section: To generate the pulse waveform of the duration of 8.5 ms, we used PIC 16F877A microcontroller along with phase lock loop. The need of phase lock loop is to align the generated pulse with the mains lines so noise interference from mains line can be eliminated

Analog section: The analog section is consisting of a series combination of Op-Amps which are divided into three stages as shown in figure 2.2 (a).

• At first stage a comparator is responsible of converting unipolar pulses coming from microcontroller into bipolar pulses.

• At second stage an integrator is used which is responsible of integrating bipolar pulses to generate a triangular wave form. The slope of the triangle is adjusted by adjusting the input resistance.

• At third stage a summing amplifier is used in a scaling configuration which is

responsible for adjusting gain and offset of the generated triangular wave form to
our desired voltage range which is -0.4V to 1.3 V.
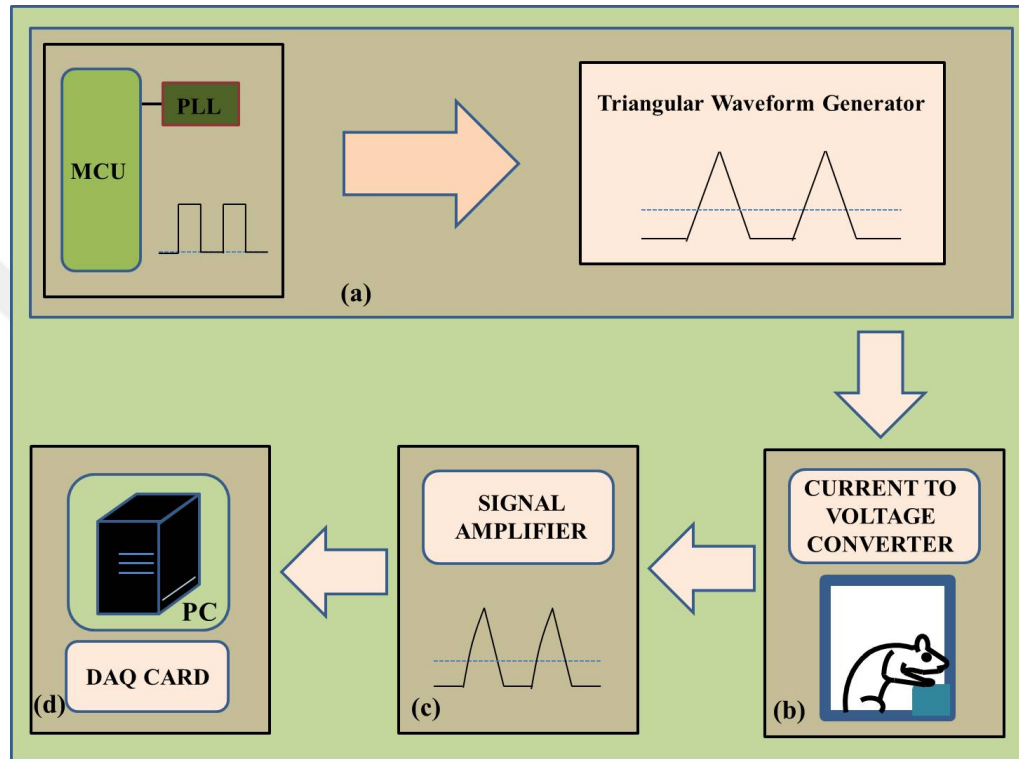
Figure 2.2: Block diagram of FSCV Hardware components.

2.1.2   Headstage

At headstage, we implement a current to voltage converter which is responsible
for converting current produced due to the oxidation and reduction of dopamine
into appropriate voltages. A TL071 IC is used and powered from separate power
supply in order to remove the noises generated by slip ring of commutator.

2.1.3   Signal Amplifier

In order to utilize the full voltage range of DAQ card the voltammetry voltage signal coming from headstage is first fed to an adjustable signal amplifier and then passed through a low pass filter in order to remove high frequency noises.

2.1.4   Data Acquisition

A 12 bit DAQ device (National Instruments, NI PCI6040E) is used to perform analog to digital conversion for data storage. For data acquisition 4.25 milliseconds pulse waveform is used to trigger the DAQ card. The output of the amplifier board is then connected to DAQ board through an analog input channels (AI0) to acquire the data. One digital port is also used as output in order to trigger the MATLAB program which is responsible for controlling the experimental setup. The computer used for this application has a Core i5 (Intel Corporation) processor (dual core) with the processing speed of 3.20 GHz and 12 GB RAM.

2.2   Software

The voltammetric experiments are recorded through two programs which are running simultaneously on the PC as shown in the figure 2.3. Theses program are as follows:
- Voltammetric recorder
- Timestamp recorder
- Data Analysis
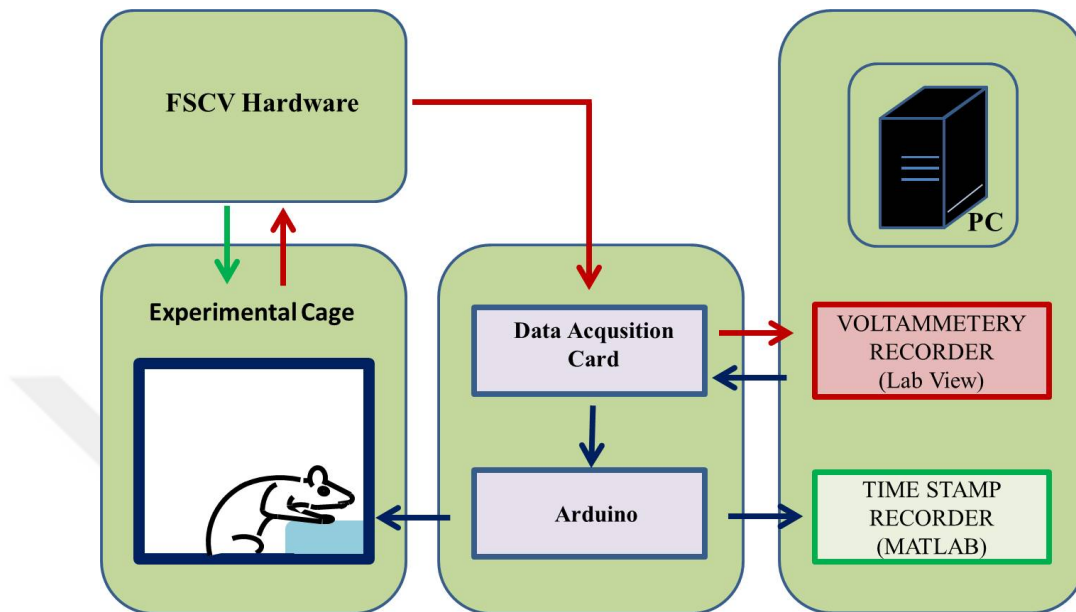
Figure 2.3: Block diagram representing software components of FSCV system .

2.2.1 Voltammetry Recorder

The voltammetry recorder is LABVIEW (National Instrument software) based program. The primary function of this program is to procure 3000 data samples with a rate of 200 KHz in each voltammetric cycle. Furthermore this program behaves as Master program which is also responsible for initiating the time stamp recorder program by generating a DC signal through DAQ card.

2.2.2 Timestamp Recorder

The Timestamp Recorder is a MATLAB-based program which is running continuously but initiates recording timestamp as it receives a high DC signal from voltammetry recorder through DAQ card, hence behaving as a slave program. The purpose of the program is to record and store each timestamp with its label e.g. the initiation and termination time of the voltammetry experiment, nose poke or button press (if the reward is delivered by experimenter) and reward presenting time.

2.2.3 Data Analysis

Data which is recorded through above discussed FSCV system is further analyzed in two steps, which are as follows:

- Offline analysis.
- PCR analysis.

2.2.3.1 Offline Analysis

The data analysis is done by another MATLAB-based in-house designed program. The program uses CSV format file generated by voltammetry recorder and its timestamp file. The program consists of various sections as shown in the block diagram figure 2.4. Since voltammetry experiments are performed with continuous five minutes fragments, therefore each voltammetry recorder file and timestamp have multiple trials data. In order to analyze this data, further subsections consisting of 40 seconds were extracted from the data with reference to nose poke time in such a fashion that nose poke lies at 10 seconds interval as shown in Figure 2.5.
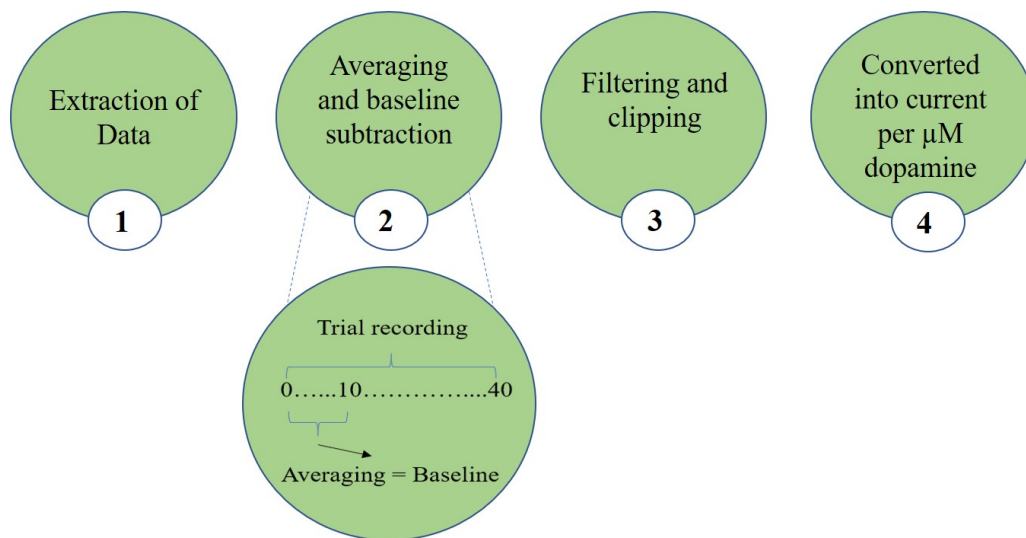
Figure 2.4: Block diagram of offline Analysis program.
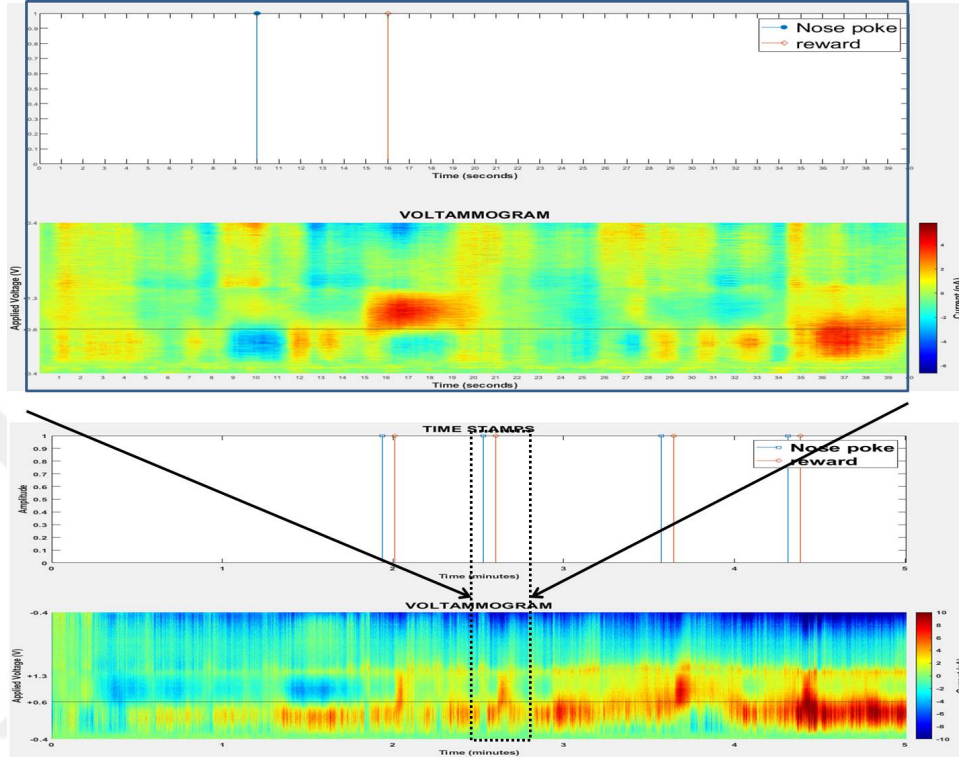
Figure 2.5: Extraction of one trial from multiple experimental trial recording.

From those 40 second subsections of the data, the first 10 second data is taken and averaging is performed. The average data is then taken as baseline and subtracted from the whole 40 second data. After subtraction of baseline, filtering is performed and samples were clipped to 1700 .These clipped sample voltages were then converted into appropriate currents per μM dopamine by using the following equation.

$$I_{out} = \frac{V_{out}}{R_{cf}} Ri(\frac{1}{R_f} + \frac{1}{R_f 1}\frac{1}{R_f 2} + ... + \frac{1}{R_f n}) \qquad (2.1)$$

Where V_{out} is the output of amplifier, R_{cf} is the feedback resistor of current to voltage converter, R_i is the input resistor of amplifier and R_f and R_{fn} are the feedback resistors of the amplifier. The above equation can be modified to the following equation

$$I_{out} = \frac{V_{out}*(n+1)}{10^6 *10} \qquad (2.2)$$

Where n is the number of resistor connected as the feedback resistors of amplifier

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2.2.3.2   PCR Analysis

To predict the dopamine concentration without pH effect for entire forty-second trial, we performed PCR analysis. For this purpose, training sample set A_{train} and training concentration set C_{train} are used to find new regression coefficients, which then used to predict new concentration for the whole trial by using following equation [29].

$$C = PA \tag{2.3}$$

Where P is the matrix containing regression coefficient and can be calculated from the following equation.

$$I_{out} = CA^T[AA^T]^{-1} \tag{2.4}$$

But since data is in high dimensions and there is a chance of having high degree of multi co-linearity between the measurements, therefore the term $[AA^T]^{-1}$ cannot be calculated. So to calculate P, we applied different approach that is Singular Value Decomposition (SVD) (by using SVD command in MATLAB). This approach reduced the dimensionality of the training dataset by decomposing the matrix into three matrices as seen by following equation.

$$A = U \sum V^T \tag{2.5}$$

Where U and V are the unitary matrix containing the left singular vectors and right singular vetcors of the matrix $[AA^T]$ respectively and $\sum$ is a matrix containing singular values in diagonal of matrix $[AA^T]$.

To reduce the dimensionality of the training data set we use the following equation.

$$T_{train} = W^T A_{train} \tag{2.6}$$

where W referred as loading matrix which is equal to U matrix and T_{train} is referred as scores calculated from training data. For simplicity we always truncate first m columns of W, which represent the principle components having maximum variance. The score calculated with these m column principle components are referred as truncated scores.

The regression equation is then reformulated into the following equation.

$$C = MT \tag{2.7}$$

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Where T is normal score matrix and M is calculated by following equation.

$$M = C_{train}T_{TS}^T[T_{TS}T_{TS}^T]^{-1} \qquad (2.8)$$

Where C_{train} is the training concentration matrix and T_{TS} is the truncated score matrix.

Now compare to equation.

$$P = C_{TS}T_{TS}^T[T_{TS}T_{TS}^T]^{-1}W_R^T = MW_R^T \qquad (2.9)$$

This is a fixed value matrix after SVD calculation and rank selection therefore $[T_{TS}T_{TS}^T]^{-1}$ can be calculated this time. Now this P will be used to predict the new concentration values.

$$C_{Predict} = P.Exp_{Data} \qquad (2.10)$$

2.3 Electrode Fabrication

This section will cover the the methods used for prepation of carbon fiber microelectrode (along its calibration) [30] and reference electrode [31].

2.3.1 Carbonfiber Micoelectrode

2.3.1.1 Fabrication

The process starts when a carbon fiber submerged in ethanol is pulled into polyimide coated fused silica capillary with an outer diameter of $90\mu m$ using a vacuum pump. To fix the carbon fiber one end of the capillary is sealed using two component Epotek 301 epoxy .The epoxy should be viscous so that a bulb shaped structure at the tip of capillary can be formed as shown in the figure (2.6) to prevent or minimize the tissue damage during the implantation of microelectrode. After curing, a metal wire of 1mm diameter is attached to the other end of capillary using epoxy to provide support. A silver adhesive is applied to provide an

electrical connection between carbon fiber and metal wire. After curing, a thin layer of dental acrylate is applied in order to secure the connection. Finally the protruding carbon fiber from the sealed end is trimmed to $150 - 200 \mu m$ using a sharp scalpel.
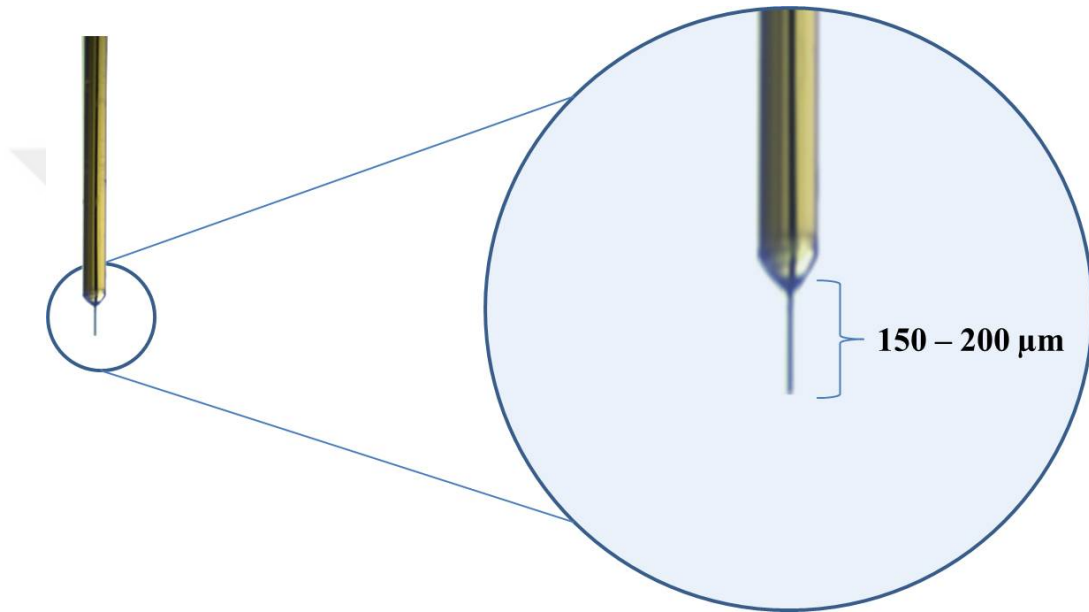
Figure 2.6: Carbonfiber Micro electrode sealed with biocompatible epoxy (Epotek 301)

2.3.1.2 Calibration:

For calibration process a Labview based program is developed locally and is operated by the experimenter. The program acquires 10s data for baseline signal and dopamine contained signal; the dopamine contained signal is then subtracted from the baseline signal in order to get dopamine concentration. The carbon fiber based micro sensors with a reference electrode are immersed in 0.9% isotonic saline solution till micro sensor becomes stabilized. After stabilization program acquires a base line signal, later a $1 \mu M$ of dopamine is added to the saline solution. New dopamine contained signals recording are then acquired and then subtracted from the pre acquired baseline and plotted on the graphs in terms of voltage values proportional to concentration of oxidized dopamine. The data acquisition for

dopamine contained signal continues till subtracted voltage is stabilized or stops increasing. This voltage value is then converted into current using equation 2.2. This current is known as the highest sensitivity of that electrode. Electrode with highest sensitivity and good physical shape are selected for the implantation purpose.

2.3.2 Reference Electrode

In this study, Silver/silver chloride is used as reference electrode. For the preparation of reference electrode, a silver wire of diameter 0.2 mm is coated with chloride ions via electrolysis. The silver wire is then connected to the anode terminal and stainless steel is connected on the cathodic terminal of a 5V Dc power supply with 500mA current limitation .Both wire are then submerged into Hydro-chloric acid solution (36%) and current is applied for 2 to 3 minutes till color of submerged silver wire becomes grey indicating the completion of electrolysis process.

2.4 Voltammetric Surgery

To perform voltammetry surgeries for our experiments, we used Wistar male rat animal. These animals along with all of the surgical procedures were first approved by and conducted in accordance with the regulations of the Istanbul Medipol University Ethics Committee on Animal Maintenance and Experimentation. The Wistar male rat weighing 450 g (6 months) was chronically implanted with a carbon fiber microelectrode in the ventromedial striatum (VMS) in left hemisphere and a reference electrode in the contralateral hemisphere. Implantation of the electrodes was performed after i.p. injection (1.5 cc/kg) of a mixture of ketamine and xylazine anesthesia (160 mg/kg and 16 mg/kg in saline, respectively). Maintenance of general anesthesia was performed with isoflurane.

An incision was created using scalpel in a plane parallel to sagittal structure to expose the cranium. Hydrogen peroxide was applied to remove the tissue residue

on the skull so that location of landmarks such as bregma and lamda become visible. The craniotomy was created and dura matter was peeled off carefully without damaging the brain, above the nucleus accumbens core (AP +1.5 mm and ML +2.1 mm from bregma) for micro sensors. The reference electrode (at position AP +1.5 mm and ML -2.1 mm from bregma) with four anchor screws at the convenient location was inserted and secured with dental acrylic such that the craniotomy location for implantation of the micro sensor remains exposed.

Then voltammetry amplifier was attached to micro sensor and using micro positioner (Narishige, MO-82), microsensor was lowered slowly (with a rate of $50 \mu m$ per minute) till it reaches to target position (6.4 mm Dorsal Ventral of dura mater for nucleus accumbens). Finally, dental acrylic is applied to cranium to secure the microsensor.

2.5 Experiments

This section consist the description of the various paradigms used to analyze the dopamine release during behavioral experiment. The experiments started with "unexpected reward delivery", which was then followed by another experiment in which reward delivery was associated with motor movements and then finally reward presentation was associated with unidirectional and bidirectional robotic actuator movements. Before the beginning of experimental training the weight of animal was reduced to 85% and it is kept constant throughout procedure days with diet restriction.

2.5.1 Rewards Presented Unexpectedly

In the first task, the rat is trained to anticipate the release of reward on hearing an audio cue, a click sound, produced by solenoid valve.

2.5.2 Rewards Presented in Response to Motor Movements

In this task animal is trained to perform a nose poke and reward is associated with conditional stimulus. For this experiment, animal does the nose poke after which a cue LED turns on for 2 sec and then reward is delivered.

2.5.3 Rewards Presented with Unidirectional Robotic Actuator Motion

In this task, when animal does the nose poke first a cue LED turns on for 2 seconds, after which robot arm starts moving from its initial position to the target position in 2 seconds. After reaching the target position, first it will wait for 2 seconds and then reward is delivered. After reward delivery, robot arm stays at target position for 5 seconds and then finally it starts moving back to its initial position.
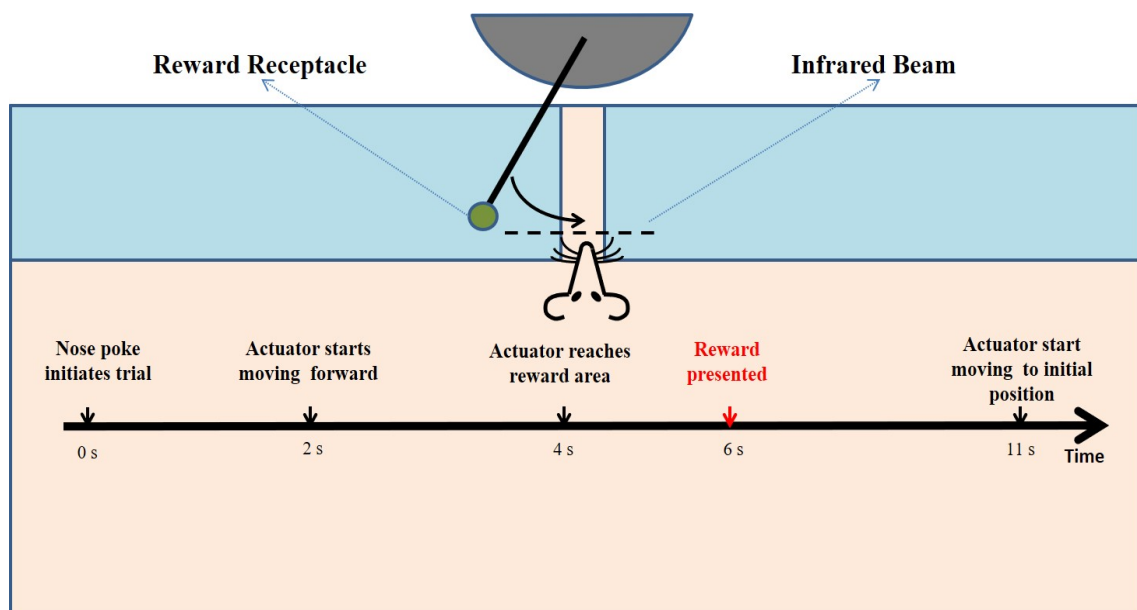
Figure 2.7: reward associated with uni-directional robotic actuator paradigm

2.5.4 Rewards Presented with Bidirectional Robotic Actuator Motion

2.5.4.1 Actuator Movement Type I

In Type I trajectory, the robotic actuator movement profile is similar to unidirectional robotic actuator movement. After recording trial was initiated through nose poke first reward predictive LED turned on which is then followed by the movement of robotic actuator (Figure 2.8 a).
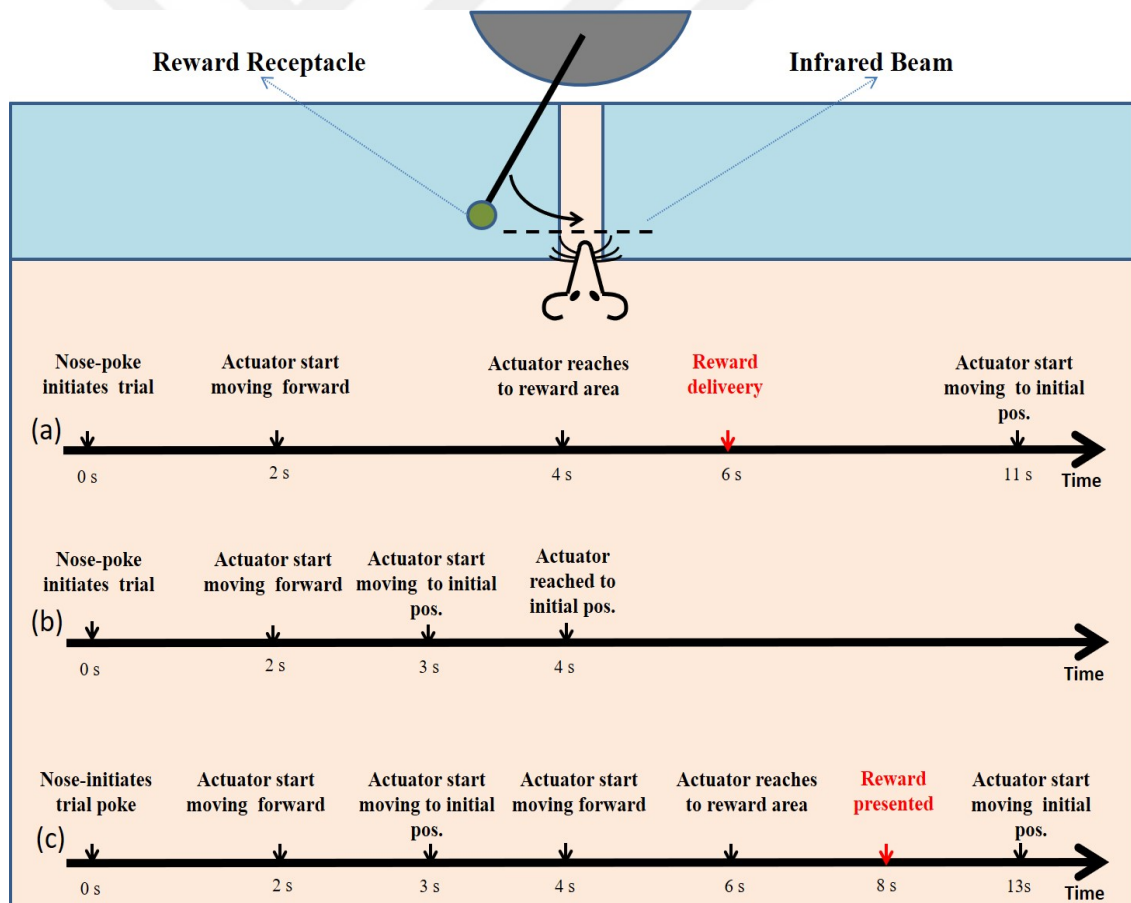
Figure 2.8: reward assosiated with bi-directional robotic actuator paradigm

2.5.4.2   Actuator Movement Type II

In type II trajectory when animal does the nose poke, first a cue LED will turn on for 2 seconds, after which robot arm starts moving towards the target area but stops (just before reward delivery nozzle gets exposed to the animal) and returns back to its initial position without delivering the reward (Figure 2.8 b).

2.5.4.3   Actuator Movement Type III

In type III trajectory when animal does the nose poke, first a cue LED will turn on for 2 seconds, after which robot arm starts moving towards the target area but stops and returns to its initial position, similar to Type II trajectory, after which it starts moving again towards the target area and this time, it reaches target area successfully and with a delay of 2 seconds, reward is delivered. After reward delivery, robot arm stays at target position for 5 seconds and then finally it starts moving back to its initial position (Figure 2.8 c).

2.6   Experimental Troubleshooting

Below Table 2.1 shows the list of all rodents used and the problems faced with these animals during this thesis study.

S.No	Rat ID	Performed experiments	Dopamine Signal	Problems faced during the experiment
1	DOPA-66	$(\triangle, \Psi)$	weak	° After some days dopamine signal disappeared
2	DOPA-67	$(\triangle, \Psi)$	very weak	° After some days dopamine signal disappeared ° Electrode got broken due to electrical artifact
3	DOPA-76 (rat-1)	$(\triangle, \Psi, \forall, \infty)$	prominent	°After two weeks dopamine signal gradually start to disappeared. ° Electrode got broken during further hardware optimization.
4	DOPA-77	$(\triangle, \Psi, \forall)$	prominent	°In later days, experiments were affected with noise interference. ° Electrode got broken during further hardware optimization.
5	DOPA-78			no dopamine response was observed.
6	DOPA-79 (rat-2)	$(\triangle, \Psi, \forall, \infty)$	prominent	°After two weeks dopamine signal gradually start to disappeared. ° Electrode got broken during further hardware optimization.
7	DOPA-84	$(\triangle)$	prominent	°After two weeks dopamine signal gradually start to disappeared. ° Electrode got broken during further hardware optimization.

Table 2.1: List of all rodent used during this thesis study. $(\triangle)$ unexpected reward presentation,(Ψ) reward present in response to motor movement, $(\forall)$ rewards presented with uni-directional robotic actuator movement ,(∞)rewards presented with bi-directional robotic actuator movement.

For rodent 1 and rodent 2, free food was given, and water restrictions were applied. Rodent 1 was giving prominent dopamine signals which gradually disappeared after 10 days. From rodent 2, weak dopamine signals were observed. After one week, while rodent 2 was undergoing experiment, its electrode got damaged due to electrical artifact. To be more precise and consistent from rodent 3 to rodent 7, we made some changes in the procedure for behavioral experiments. First, before starting behavioral experiment, the weight of the rodent was decreased to 85 % s and maintained throughout the experiment days. Second, instead of water restriction, food restrictions were applied due to which we observed very prominent dopamine signals except rodent 5 in which no dopamine response was observed. With Rodents 3, 4 and 6, we got very prominent dopamine signals but only with rodent 3 (rat-1) and rodent 6 (rat-2), we were successfully able to perform all experiments. We had some external noise interference issue while rodent 4 was doing experiments due to which, we had to train this animal again for nose poke. We observed that after two weeks of training we lost dopamine signals from all these animals. Further optimizations are still under way to resolve this signal loss issue.

Chapter 3

Results and Discussions

This section will demonstrate the viability of the proposed paradigm with recording from chronically implanted rats during behavioral experiments. Behavioral experiments were started after one month recovery period from the surgery day. Prior to the behavioral experiments, animal diet was restricted for up to 5-6 days in order to reduce its weight to 85% and weight was maintained. Behavioral tasks lasted for five minute continuous recordings which consisted of multiple rewarded or non-rewarded trials (only performed in paradigm where rewards were presented with bidirectional robotic actuator movement). The time at which animal does the nose poke and reward was presented were saved as time stamp in separate file. Using these time stamps, the recorded data was arranged and presented as figures which contain voltammogram at top and dopamine concentrations at bottom. Voltammogram represents current as a function of applied voltages, and dopamine concentration in nM/A. All results were arranged in such a way that button press (for unexpected reward) or nose poke time was always aligned at 10s. For all behavioral experiments, a $200\mu L$ of 30% sucrose solution was presented as reward in every rewarded trial.

The results included were obtained from various behavioral experiments performed in a chronological manner starting from reward presented unexpectedly,

reward presented in response to motor movement, reward presented with uni-directional robotic actuator movement and reward presented with bidirectional robotic actuator movement. The motivation behind the proposed paradigm was to study dopamine changes occurring while reward was associated with the movement of the robotic actuator.

3.1 Rewards Presented Unexpectedly

In this task, rewards were presented unexpectedly to the animal (rat-2). Whenever (rat-2) approached target area, reward was delivered through an external button controlled by the experimenter. A click sound was generated by solenoid valve as reward was given. In this way, (rat-2) was trained to respond to the click sound as reward predictor and whenever this click sound occurred, animal approached the target area where it received the reward. The obtained results are shown below:
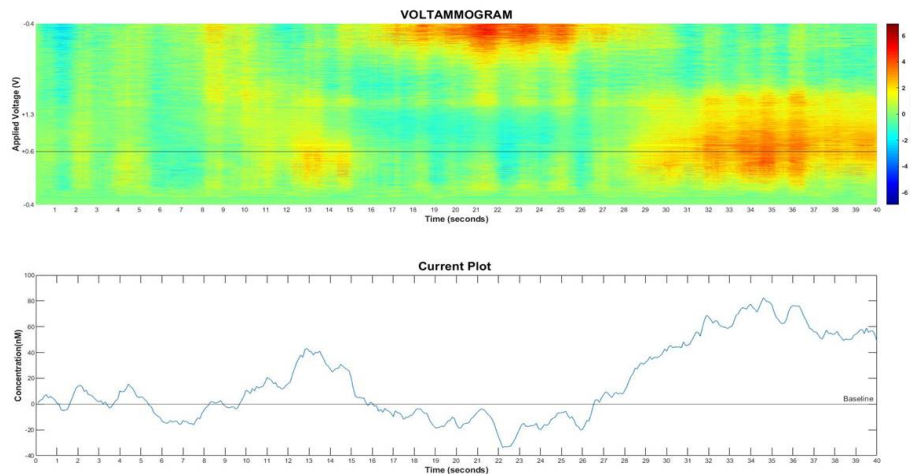
Figure 3.1: The voltammogram and dopamine concentration for single trial recorded during unexpected reward delivery (rat-2, day 01)

Figure 3.2: Current changes averaged over 10 trials in which dopamine concentration change occured during unexpected reward delivery (rat-2, day 01).

It took two days to train the (rat-2) with 100 trials per day. Figures 3.1and 3.2 represents single recording trial and the average of 10 recording trials in which highest dopamine intensity appeared, respectively recorded during unexpected reward delivery. A peak is observed in concentration graph of both figures represents change of dopamine concentration. In Figure 3.1 peak started to appear from 10seconds till 16 second followed by a small pH change. While in Figure 3.2, this peak started to appear at 10 seconds till 13 sec which appeared in the response of reward delivery through solenoid valve and is followed by long pH change. Based on the average of 10 recording trials containing highest dopamine intensity (performed by rat-2), in which unexpected reward was presented, the phasic increase in dopamine concentration was measured as 29.46 ± 8.11 nM.

In most trials, dopamine traces were observed along with noise which was expected due to movement artifacts and pH changes in active animals. Also, in some trials, a change in dopamine concentration was observed in refractory period after reward was delivered (Figure 3.1). By observing the (rat-2) response, it was assumed that since reward delivery was followed by a long refractory period, therefore in some trials when (rat-2) is highly motivated it starts licking nozzle,

this behavior of (rat-2) caused small changes in dopamine concentration during refractory period.

3.2   Rewards Presented in Response to Motor Movements

In this task animal (rat-2) was trained to perform simple motor movement task (nose poke) in order to get reward. For this purpose an IR beam sensor was used. Whenever (rat-2) performed nose poke, IR beam was cut off and trial was initiated, which was then followed by a reward predictive stimuli (cue light) with duration of 2 seconds and finally reward was delivered through solenoid valve. The obtained results are shown below:
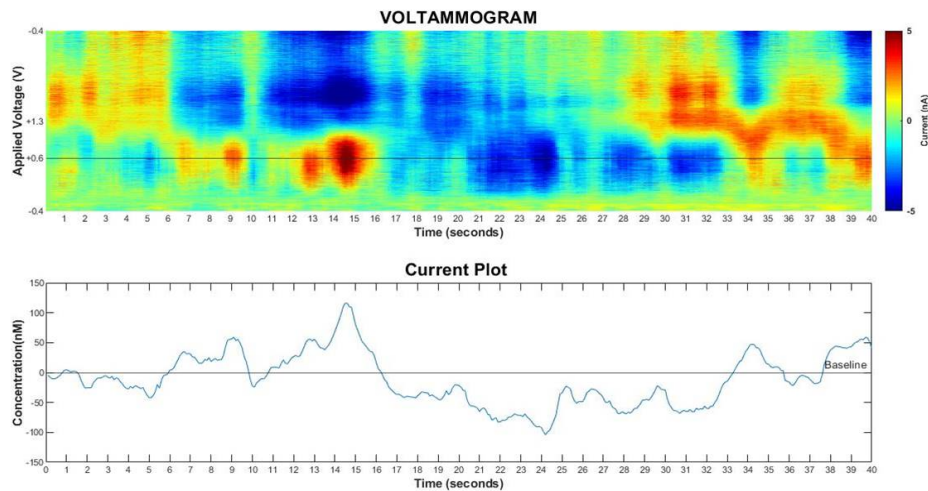
Figure 3.3: Current changes single trial in which dopamine concentration change occured during reward presentation in response to motor movement (Rat-2, Day 01).

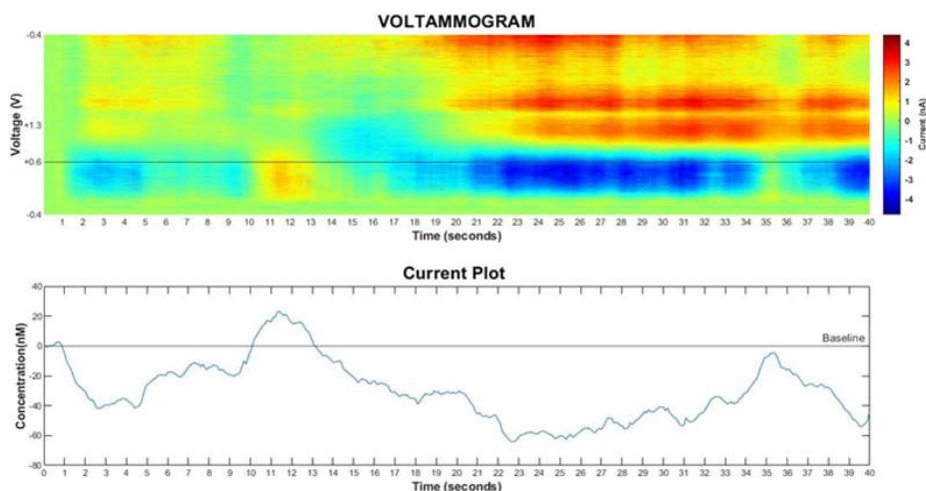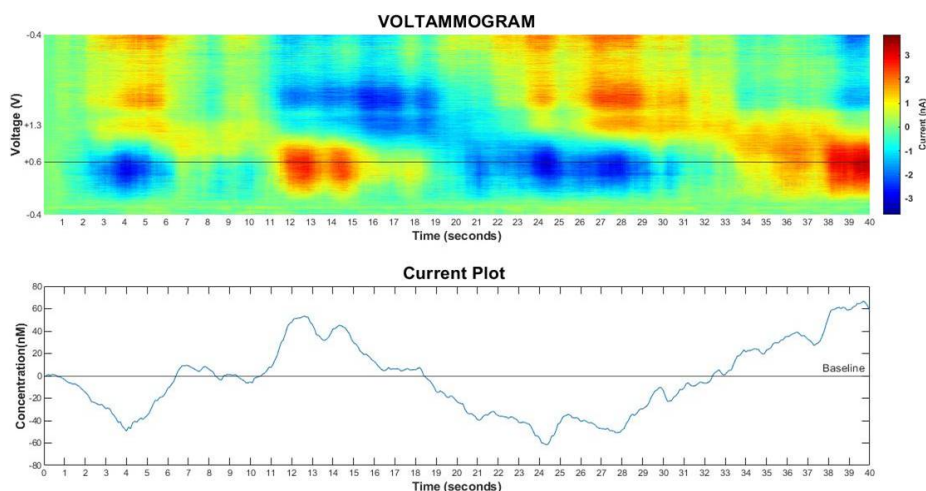Figure 3.4: Current changes averaged over 10 trials in which dopamine concentration change occured during reward presentation in response to motor movement (Rat-2, Day 01).

Figures 3.3 and 3.4 represents single recording trial and the average of 10 recording trials in which highest dopamine intensity appeared, respectively recorded during reward delivery in response to motor movements (nose poke) performed by animal at 10 seconds .In some trials dopamine peak appeared only at the time of reward delivery (Figure 3.3). but when average of 10 recording trial containing highest dopamine intensity was performed a peak is observed between 11-16 seconds in concentration graph (Figure 3.4). As condition stimulus is predictor of reward therefore peak started to appear before the reward was delivered i.e. at 12 seconds followed by an extended pH changing profile. Based on the average of 10 recording trials containing highest dopamine intensity (performed by rat-2), in which reward was presented in response to motor movement (nose poke), the gradual increase in dopamine concentration was measured as 58.43 ± 11.9 nM.

Similar to previous task, in some recording trials an increase was observed in the later part of the recording trial (refractory period). It was assumed that, this change in dopamine concentration was occurred due to nose pokes performed by

(rat-2) in refractory period.

3.3 Rewards Presented with Unidirectional Robotic Actuator Movement

In this task, whenever animals ((rat-1) and (rat-2)) performed nose poke, reward was presented in response to the unidirectional movement of robotic actuator. For this purpose nozzle through which reward was delivered was attached to a robotic actuator and initially this robotic actuator was placed beneath a transparent platform so animals were unable to access the nozzle as shown in Figure 2.6. Whenever animal did the nose poke, a reward predictive LED cue turned on for 2 seconds after which robotic actuator started moving towards the target area (where nozzle becomes exposed) within 2 seconds. After reaching target area, it will wait 2 more seconds and then reward was delivered. After this, the robotic actuator stayed at target location for 5 seconds and then it started moving back to its initial position i.e. beneath the platform. The obtained results from both animals are shown below:

Figure 3.5: Current changes in a single trial in which dopamine concentration change occurred during uni-directional robotic actuator movement (Rat-1, Day 01).

Figure 3.6: Current changes averaged over 10 trials in which dopamine concentration change occurred during uni-directional robotic actuator movement (Rat-1, Day 01).

Figure 3.7: Current changes averaged over 10 trials in which dopamine concentration change occurred during uni-directional robotic actuator movement (Rat-1, Day 04).

	Nose Poke (nM)	During Actuator Movement (nM)	Reward Delivery (nM)
Day01	4.355	12.65	222.5 ± 33.9
Day02	-10.98	9.600	63.50 ± 21.2
Day03	1.979	36.84	68.07 ± 19.3
Day04	-13.93	5.370	116.1 ± 13.9
Day05	5.800	15.78	119.6 ± 19.7

Table 3.1: Average of dopamine concentration in 10 trials in which highest concentration change occurred during uni-directional robotic actuator movement (Rat-1).

In (rat-1), it was observed that initially during his learning phase, in some trials a change in dopamine was observed during reward predictive stimulus and robotic actuator movement (Figure 3.5). But when average of 10 recording trials containing highest dopamine intensity, was performed Dopamine peak started to appear at 14 seconds when robotic actuator reached target area, this dopamine peak intensity increased when reward was delivered to the (rat-1) i.e. at 16 seconds(Figure 3.6). In later days as (rat-1) got trained, in almost every trial

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except for the initial 10 - 15 trials (which were not included to consistent results), a slight change was observed during reward stimulus and robotic actuator movement which is then gradually increasing till reward was presented i.e. at 16 seconds. Figure 3.7 represents average of 10 recording trials containing highest dopamine intensity (performed by rat-1, day 04) during reward delivery in response to unidirectional robotic actuator movement.
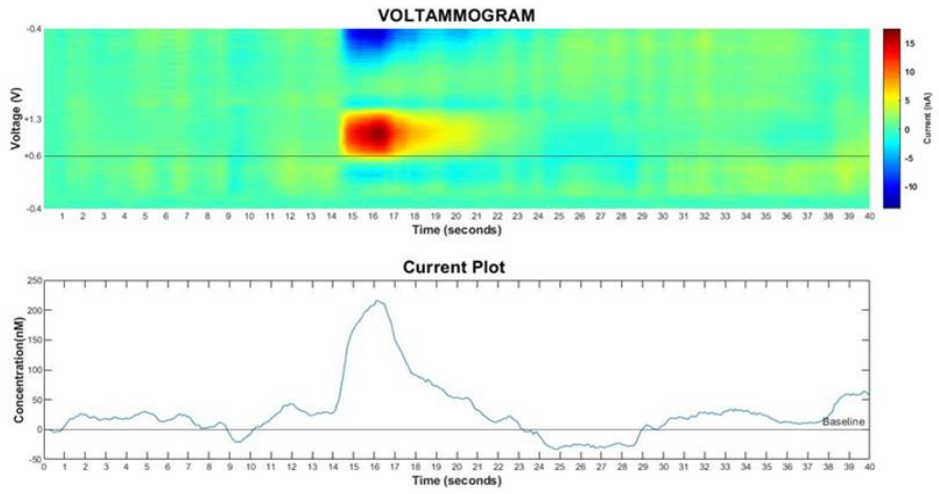

Figure 3.8: Current changes in a single trial in which dopamine concentration change occurred during uni-directional robotic actuator movement (Rat-2, Day 01).
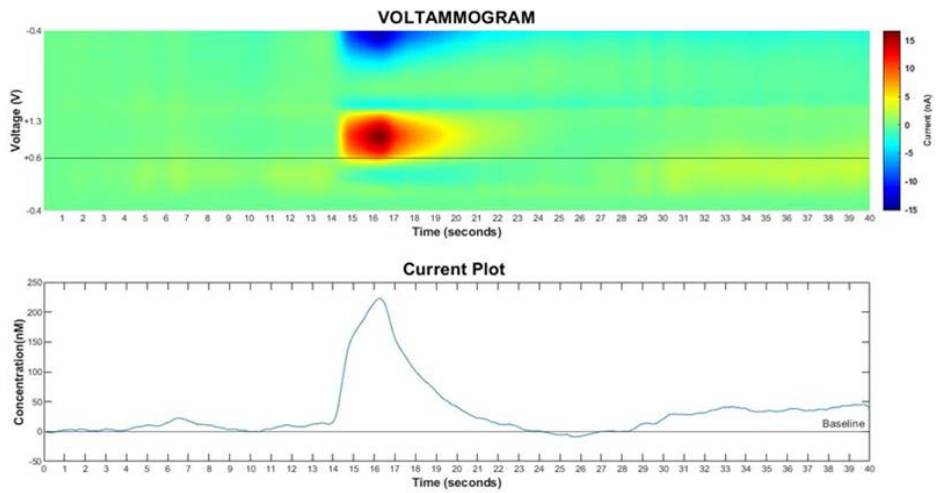
Figure 3.9: Current changes averaged over 10 trials in which dopamine concentration change occurred during uni-directional robotic actuator movement (Rat-2, Day 01).
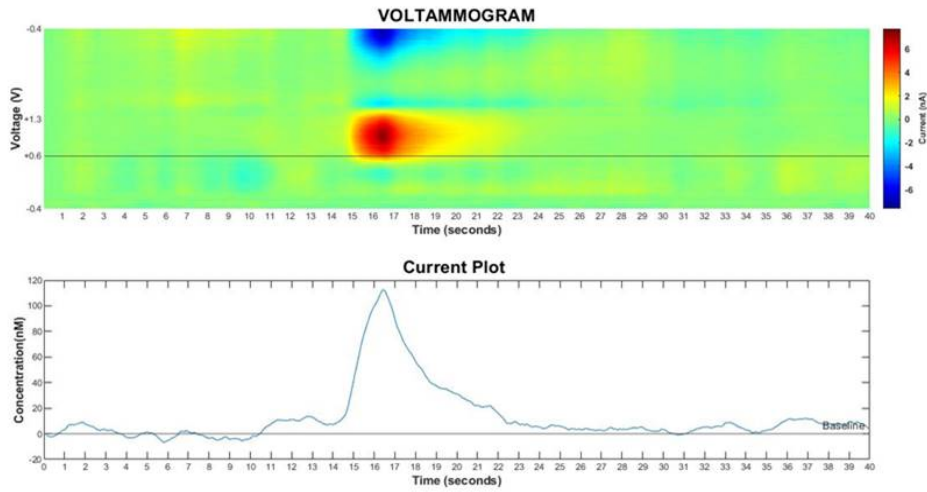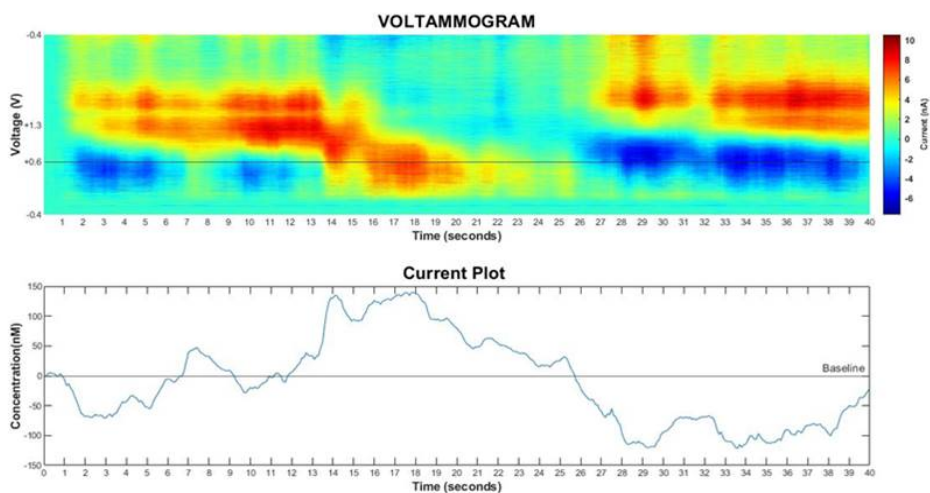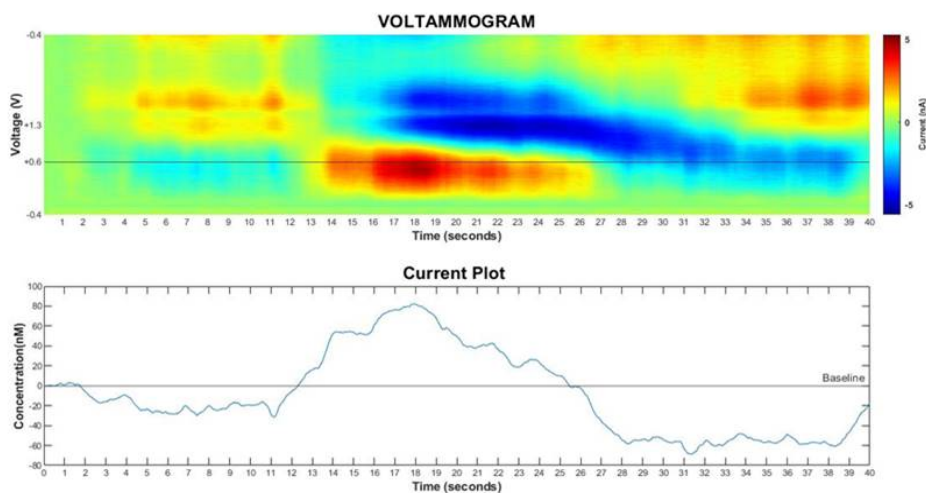

Figure 3.10: Current changes averaged over 10 trials in which dopamine concentration change occurred during uni-directional robotic actuator movement (Rat-2, Day 04).

	Nose Poke (nM)	During Actuator Movement (nM)	Reward Delivery (nM)
Day01	-40.00	10.79	84.36 ± 27.3
Day02	-4.164	37.32	69.91 ± 22
Day03	-40.90	3.067	68.51 ± 22.9
Day04	-19.19	79.00	61.98 ± 20.1
Day05	-86.28	85.39	81.38 ± 13.4

Table 3.2: Average of dopamine concentration in 10 trials in which highest concentration change occurred during uni-directional robotic actuator movement (Rat-2).

Figure 3.8 represents single recording trial performed by (rat-2) on first day of experiment. In (rat-2) a gradual increase of dopamine was observed during reward predictive stimulus and robotic actuator movement which was consistent throughout the days of observations. Figures 3.9 and 3.10 represents the average of 10 recording trials containing highest dopamine intensity performed by (rat-2) on day 01 and day 04 respectively.

In both rats, it is also observed that after reward delivery, instead of having a rapid decrease in dopamine concentration, an elongated decrease of dopamine concentration was seen, which started from 17-24 seconds in (rat-1) and from 18 to 24 seconds in (rat-2).

Table 3.1 and Table 3.2 represent the average of 10 recording trials with highest dopamine intensity for each day for (rat-1) and (rat-2) respectively. From the data it is clearly seen that when both rats does the nose poke except for few trials, dopamine concentration is in negative. It is assumed that this negative concentration is appeared due to a long refractory period of 25 seconds. From the data it is seen that initially for both rats on their first day, no dopamine was observed during the movement of robotic actuator but as the day passes, dopamine concentration becomes positive as reward was approaching (movement of robotic actuator from initial to target) towards the rats. These results illustrate the involvement of dopamine in predicting reward proximity. In order to guarantee these obtained results another paradigm was implemented which is

described in following subsection

3.4 Rewards Presented with Bidirectional Robotic Actuator Movement

In this task, reward was associated with three different movement trajectories of robotic actuator. Whenever animals (rat-1 and rat-2) does the nose poke randomly one of the three robotic actuator trajectory was selected (shown in Figure 2.7) and recording trial was performed. The probability of selected task is 40% with Task I, 30% with Task II and 30% with Task III. The purpose of this paradigm is to observe the relationship of dopamine with distant reward. The obtained results from two animals are shown below:

3.4.1 Actuator Movement Type I

The type I trajectory is similar in nature as previous paradigm, where rewards were presented in response to Unidirectional Robotic Actuator Movement. The obtained results from two animals (rat-1 and rat-2) are shown below:

Figure 3.11: Current changes in a single trial in which dopamine concentration change occurred during bi-directional robotic actuator movement type I (Rat-1, Day 01).
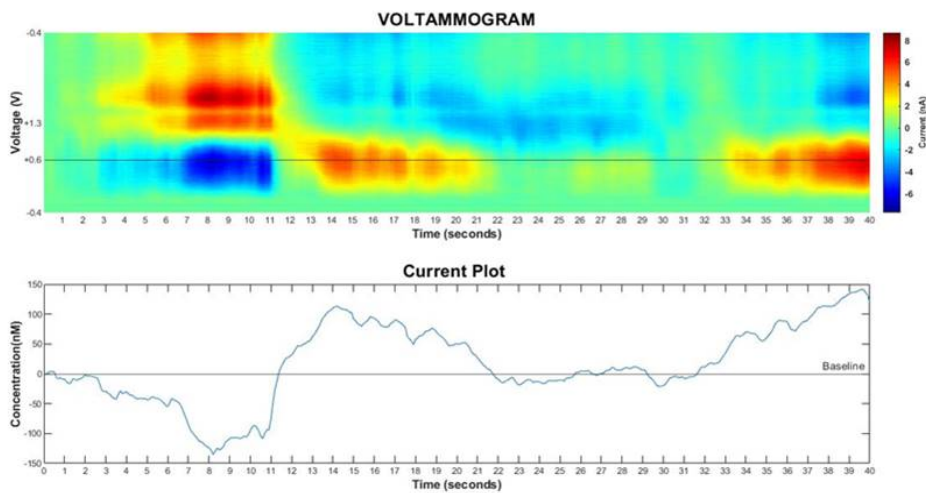

Figure 3.12: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type I (Rat-1, Day 01).

Figure 3.13: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type I (Rat-1, Day 04).

	Nose Poke (nM)	During Actuator Movement (nM)	Reward Delivery (nM)
Day01	-8.850	5.024	223.9
Day02	-13.87	11.20	196.3
Day03	4.547	12.52	134.1
Day04	2.720	7.432	18.20

Table 3.3: Average of dopamine concentration in 10 trials in which highest concentration change occurred during bi-directional robotic actuator movement type I (Rat-1).
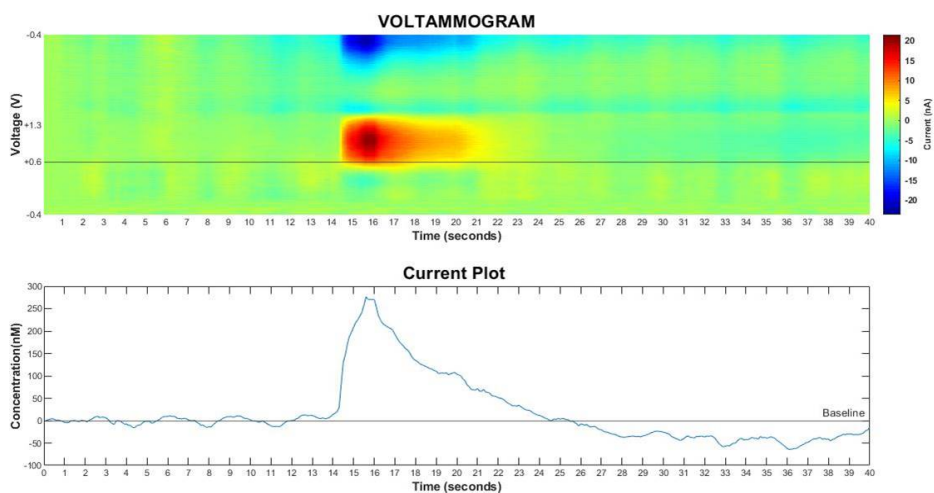
Figure 3.14: Current changes in a single trial in which dopamine concentration change occurred during bi-directional robotic actuator movement type I (Rat-2, Day 01).

Figure 3.15: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type I (Rat-2, Day 01).
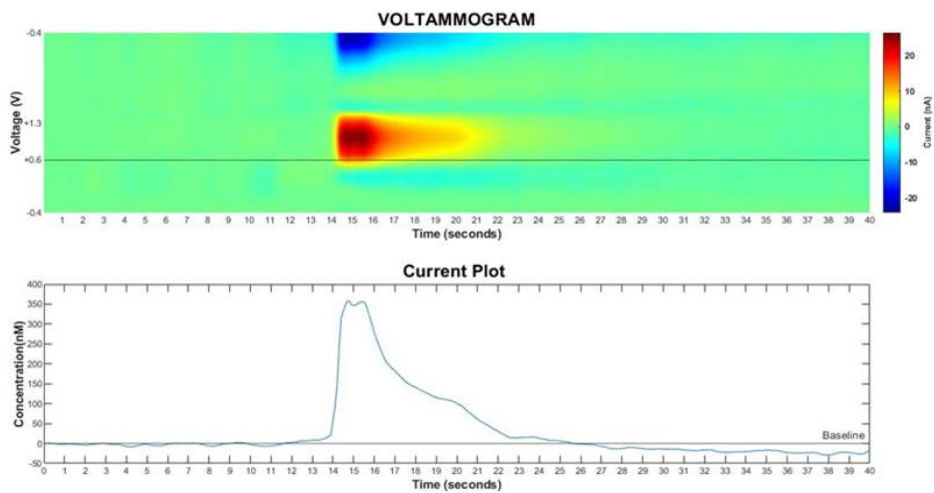
Figure 3.16: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type I (Rat-2, Day 04).

	Nose Poke (nM)	During Actuator Movement (nM)	Reward Delivery (nM)
Day01	-13.74	62.28	117.9
Day02	-16.39	16.24	88.43
Day03	9.422	15.17	84.41
Day04	9.890	18.83	58.23

Table 3.4: Average of dopamine concentration in 10 trials in which highest concentration change occurred during bi-directional robotic actuator movement type I (Rat-2).

The observation from both animals (rat-1 and rat-2) during the implementation of type I trajectory was similar to the observation in previous experiments where reward was presented with unidirectional movement of robotic actuator.

Table 3.3 and Table 3.4 represent the average of 10 recording trials with highest dopamine intensity for each day of (rat-1) and (rat-2) respectively. From the data it is observed that when animals (rat-1 and rat-2) were learning the paradigm, the dopamine concentration while performing the nose poke was negative. Later

on as both animals (rat-1 and rat-2) got trained, a small positive dopamine concentration was observed. However slight dopamine signal was observed during the forward movement of robotic actuator which was then followed by a larger dopamine signal as reward was delivered.

3.4.2 Actuator Movement Type II

In type II trajectory, when animals (rat-1 and rat-2) does the nose poke, first a reward predictive LED turned on for 2 seconds which was then followed by the movement of robotic actuator which first starts moving towards the target but before reaching the target, returns back to its initial position without delivering the reward. The purpose of such trajectory is to observe dopamine response during an unsuccessful trial as reward is going away from the animals. The obtained results from two animals (rat-1 and rat-2) are shown below:

Figure 3.17: Current changes in a single trial in which dopamine concentration change occurred during bi-directional robotic actuator movement type II (Rat-1, Day 01).
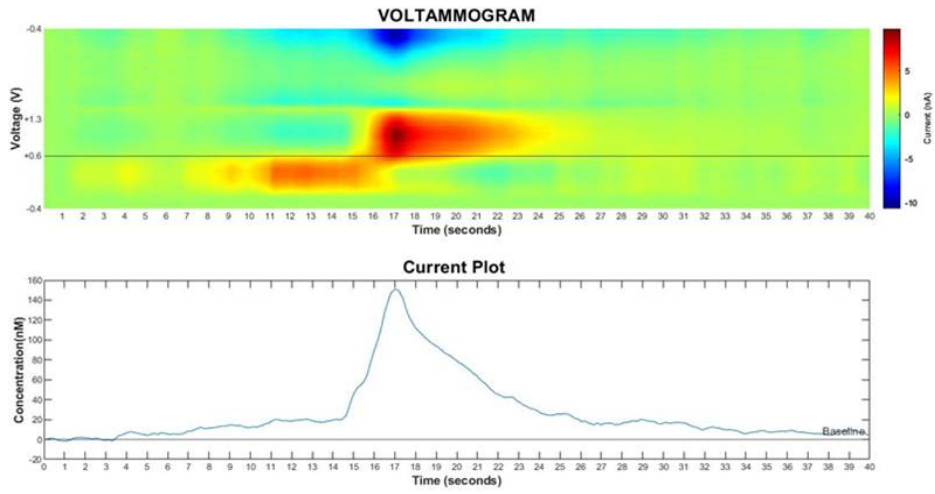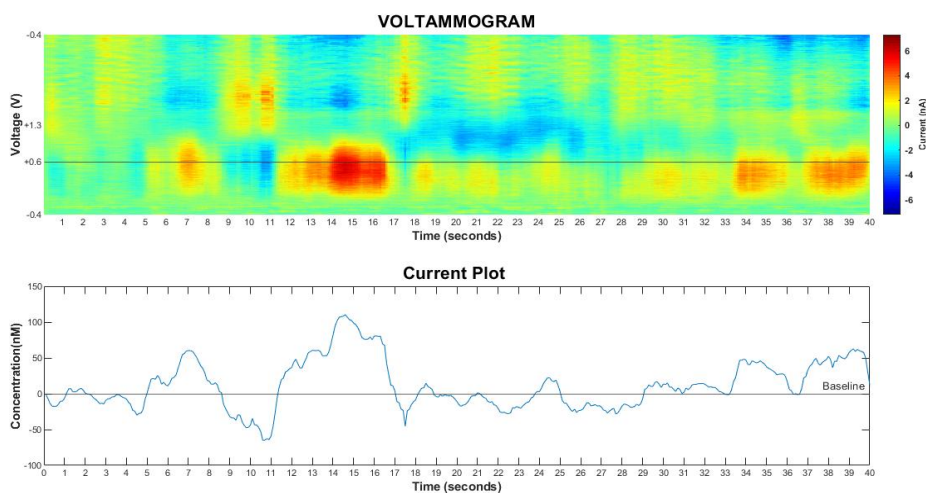
Figure 3.18: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type II (Rat-1, Day 01).
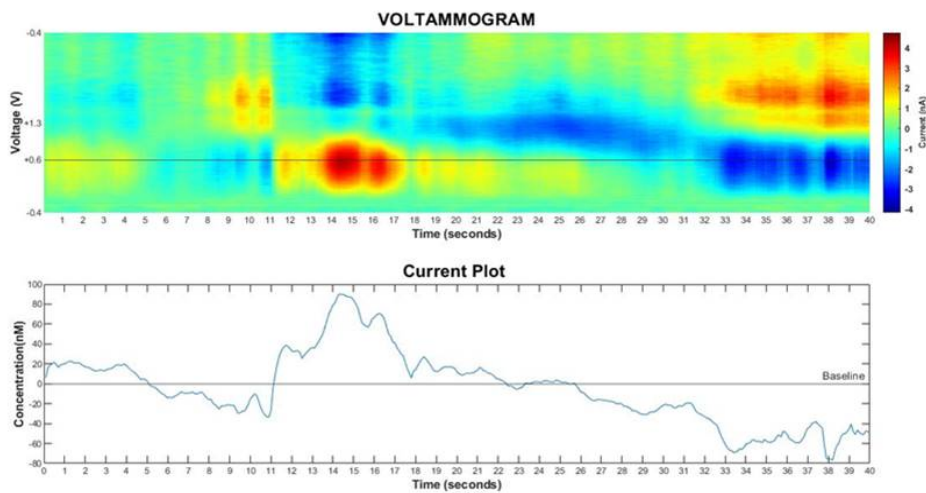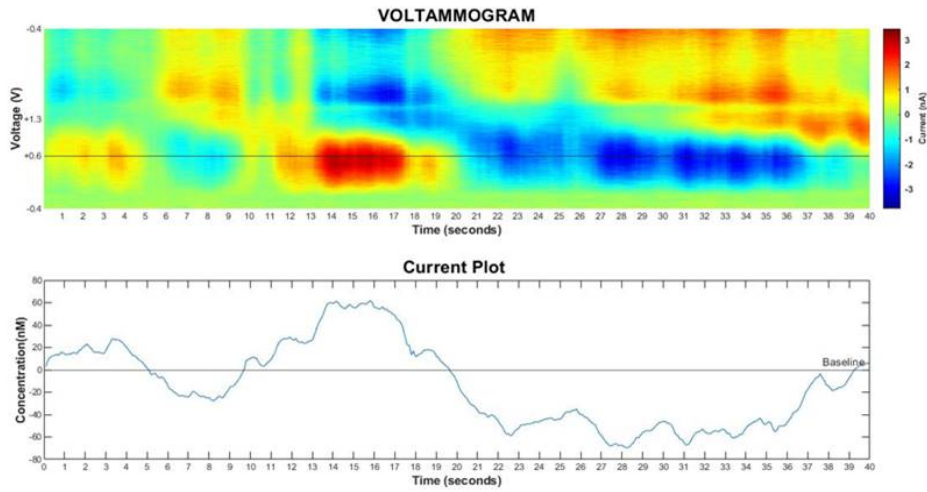

Figure 3.19: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type II (Rat-1, Day 04).

	Nose Poke (nM)	During Actuator Movement (nM)	Reward Delivery (nM)
Day01	3.478	17.60	29.72
Day02	1.540	21.40	30.40
Day03	9.030	6.398	11.42
Day04	-3.300	1.600	2.320

Table 3.5: Average of dopamine concentration in 10 trials in which highest concentration change occurred during bi-directional robotic actuator movement type II (Rat-1).

Figure 3.17 represents single trial performed by rat (rat-1) during type II trajectory in reward presentation with bidirectional robotic actuator movement. A peak is seen between 14 and 15 seconds This peak rapidly decreases after 15 seconds and remains slightly positive till the end of the recording trial. When average of 10 recording trials containing highest dopamine intensity was performed with type II trajectory figure 3.18, a peak is also clearly seen between 14 and 15 seconds. This could be due to the persistent learning phase of the (rat-1) but as he observed that robot arm reaches initial position without delivering the reward, this dopamine peak disappears quickly. As training of (rat-1) matured such responses were not observed (see Figure 3.19).
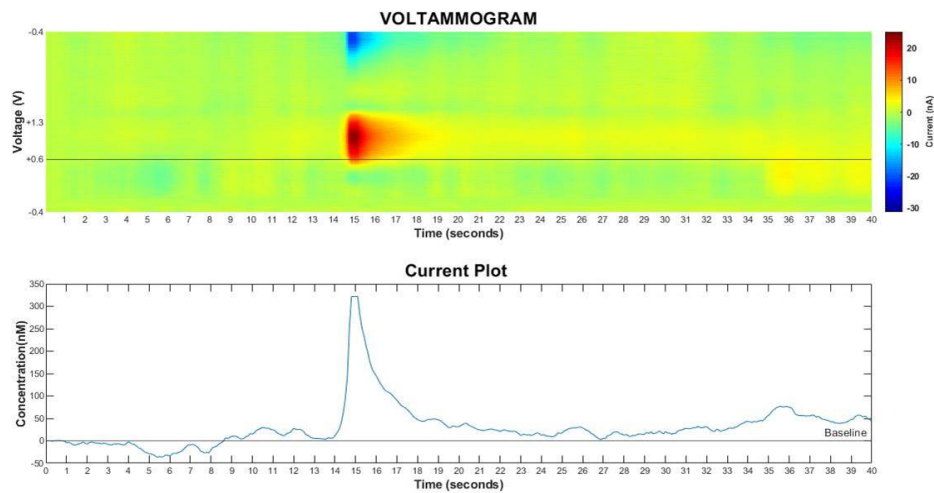
Figure 3.20: Current changes in a single trial in which dopamine concentration change occurred during bi-directional robotic actuator movement type II (Rat-2, Day 01).
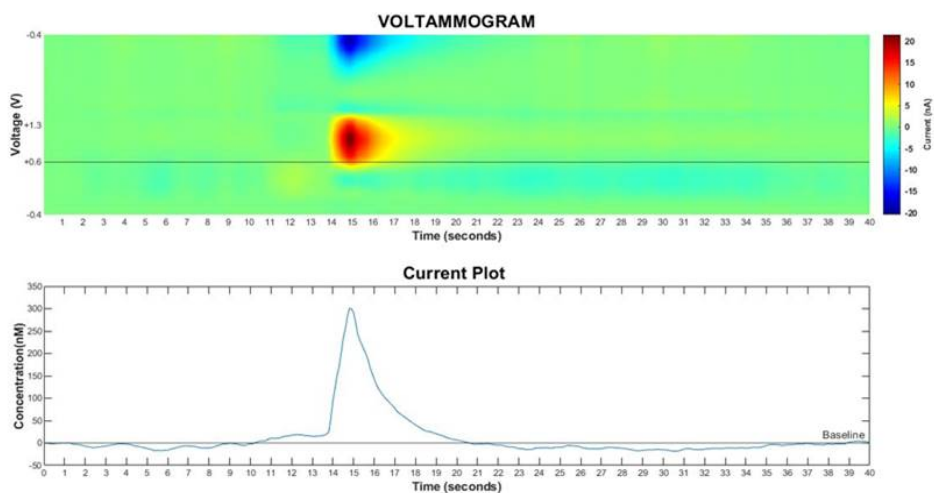

Figure 3.21: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type II (Rat-2, Day 01).
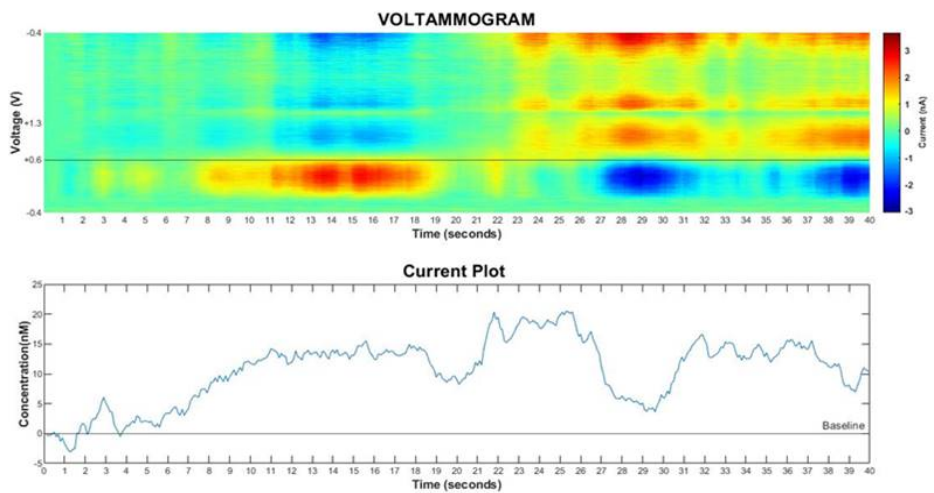
Figure 3.22: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type II (Rat-2, Day 04).

	Nose Poke (nM)	During Actuator Movement (nM)	Reward Delivery (nM)
Day01	-3.556	4.183	-16.72
Day02	3.160	20.90	39.41
Day03	7.590	16.52	-7.320
Day04	6.410	12.40	-10.40

Table 3.6: Average of dopamine concentration in 10 trials in which highest concentration change occurred during bi-directional robotic actuator movement type II (Rat-2).

For (rat-2), during the movement of robotic actuator we see slight increase but it disappears and followed by a long pH change, as the recording trial was unsuccessful (Figures 3.21 and 3.22 representing average of 10 recording trial with highest dopamine intensity). In some trials with (rat-2)small change in dopamine was observed during the refractory period. By observing behavior of (rat-2) it was assumed that these peaks appeared due to nose poke performed by the animals (rat-2) after having an unsuccessful trial (Figure 3.20). In Figures 3.12, 3.13 and 3.14, single or multiple peaks were observed in refractory period.

These peaks appeared due to nose poke performed by the animals (rat-1 and rat-2) after having an unsuccessful trial.

Tables 3.5 and 3.6 represent the average of 10 recording trials with highest dopamine intensity for each day for (rat-1) and (rat-2) respectively. From the data it is clearly seen that when robotic actuator reaches its initial position and trial ends, no larger dopamine peak is seen.

3.4.3   Actuator Movement Type III

The type III trajectory is a combination of first two trajectories i.e. when animals (rat-1 and rat-2) does the nose poke, first a cue LED will turn on for 2 seconds followed by movement of robotic actuator was in forward direction and then in backward direction similar to task II trajectory. After reaching the initial position, the robotic actuator again starts moving forward and reaches target, where after waiting for 2 seconds, reward was delivered through solenoid valve (as seen in task I trajectory). After reward delivery robot arm stays at target position for 5 seconds and then finally it starts moving back to its initial position.

The purpose of reward delivery through this trajectory was to study the changes in dopamine concentration as robotic actuator was unsuccessful in reaching target location in first attempt but it successfully reached the target in second attempt. The obtained results from two animals (rat-1 and rat-2) are shown below:
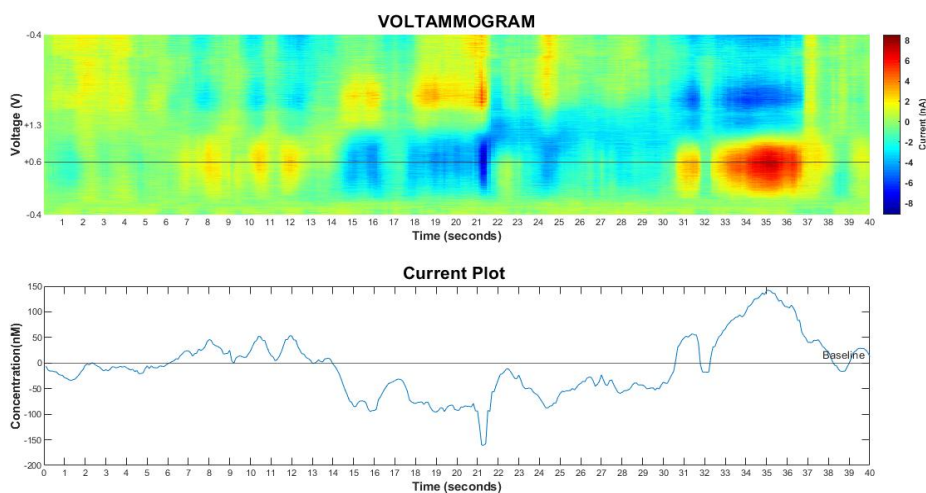
Figure 3.23: Current changes in a single trial in which dopamine concentration change occurred during bi-directional robotic actuator movement type III (Rat-1, Day 01).
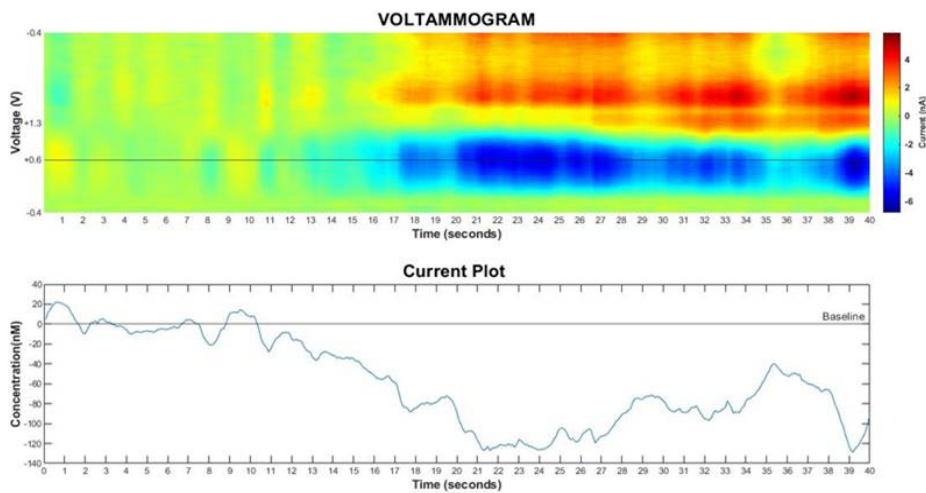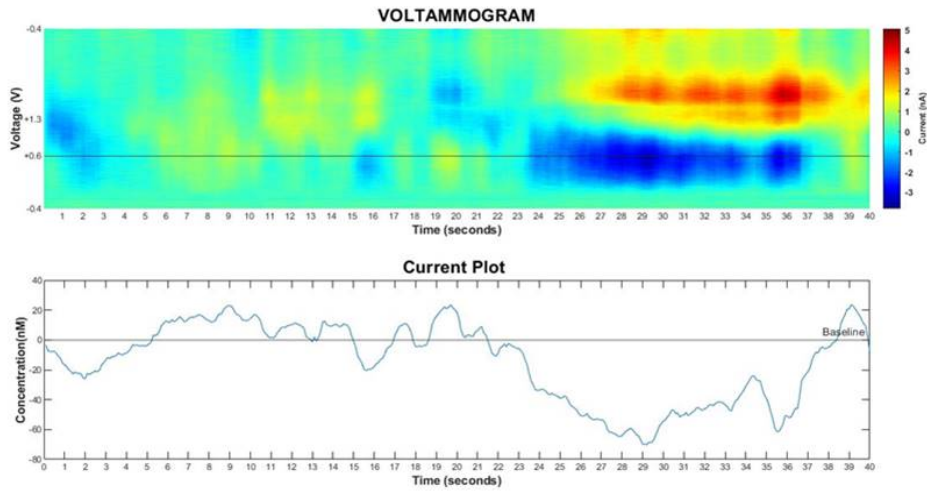

Figure 3.24: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type III (Rat-1, Day 01).

Figure 3.25: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type III (Rat-1, Day 04).

	Nose Poke (nM)	During Actuator Movement (nM)	Reward Delivery (nM)
Day01	-13.69	113.5	208.6
Day02	-4.590	101.9	188.3
Day03	-1.720	8.570	128.9
Day04	2.950	13.88	16.75

Table 3.7: Average of dopamine concentration in 10 trials in which highest concentration change occurred during bi-directional robotic actuator movement type III (Rat-1).

Figure 3.23 represent single recording trial performed by (rat-1) during reward presentation with type III trajectory of bidirectional robotic actuator movement. Two peaks were observed in response to type III trajectory of robotic actuator movement. Results obtained from the average of 10 recording trials in which highest dopamine intensity appeared (performed by rat-1) shows that when it was in learning phase, a negligible dopamine rise was seen during the nose poke, followed by two peaks. The first peak appears in response to unsuccessful attempt of robotic actuator and second peak appeared in response to second and successful

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attempt of robotic actuator movement (Figure 3.24). As (rat-1) got trained, surprisingly, only one peak was observed in response to successful attempt of robotic actuator and reward presentation (Figure 3.25).
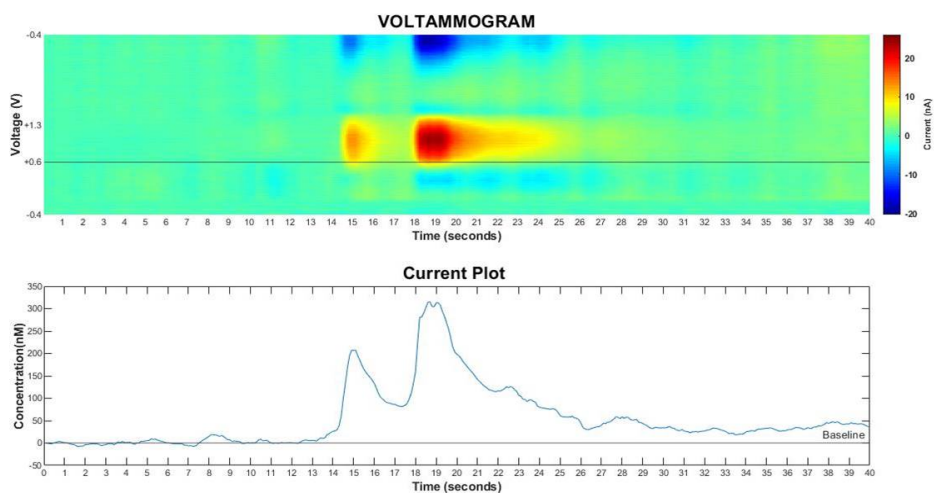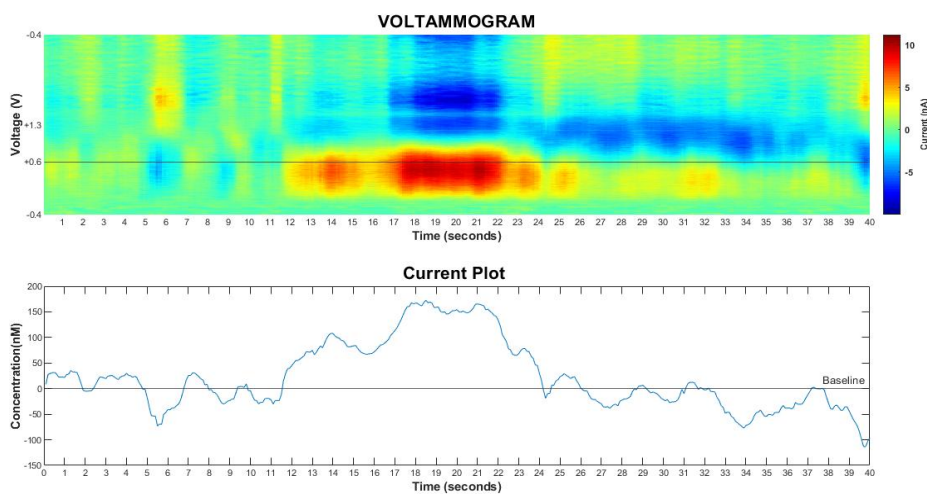

Figure 3.26: Current changes in a single trial in which dopamine concentration change occurred during bi-directional robotic actuator movement type III (Rat-2, Day 01).
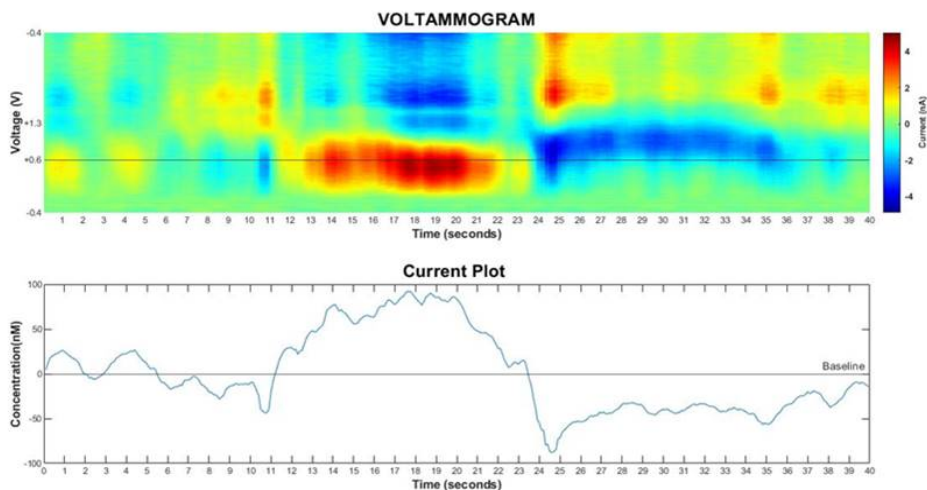
Figure 3.27: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type III (Rat-2, Day 01)..
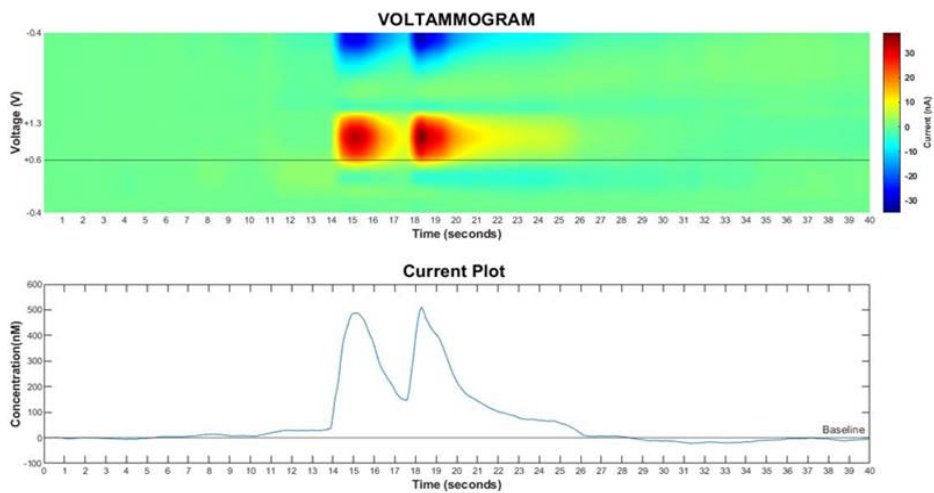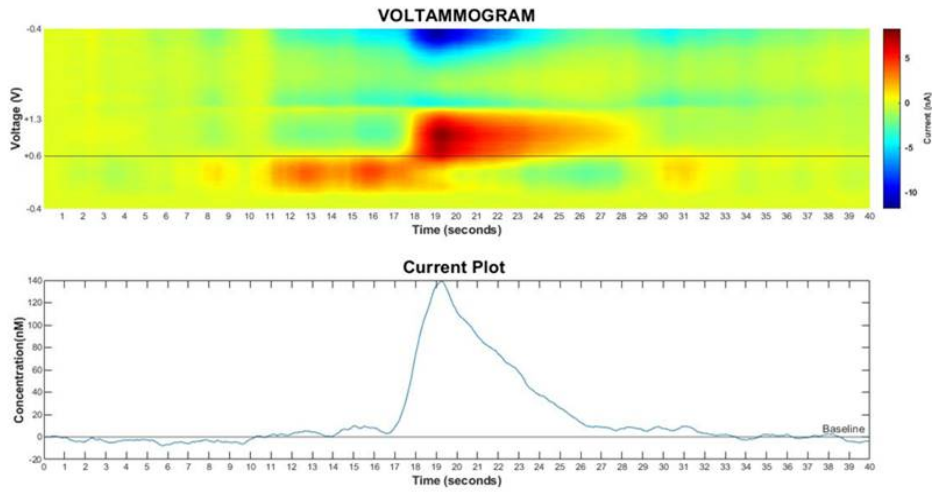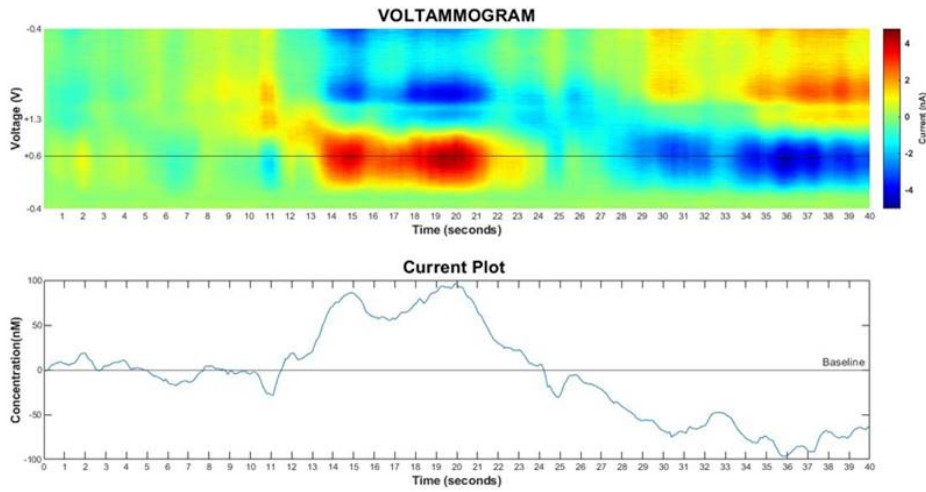
Figure 3.28: Current changes averaged over 10 trials in which dopamine concentration change occurred during bi-directional robotic actuator movement type III (Rat-2, Day 04).

	Nose Poke (nM)	During Actuator Movement (nM)	Reward Delivery (nM)
Day01	4.080	81.46	99.49
Day02	2.470	69.01	50.34
Day03	2.130	69.64	96.60
Day04	2.900	67.76	72.07

Table 3.8: Average of dopamine concentration in 10 trials in which highest concentration change occurred during bi-directional robotic actuator movement type III (Rat-2).

On the other hand with (rat-2), initially when it was in learning phase, a continuous dopamine signal was observed starting from the first attempt of robotic actuator till reward was presented (Figures 3.26 and 3.27). Later on as (rat-2) got trained, two peaks were clearly seen in response to robotic actuator movement (Figure 3.28). Table 3.7 and Table 3.8 represent the average of 10 recording trials with highest dopamine intensity for each day of rat-1 and rat-2 respectively. From the data it is observed that when both animals (rat-1 and rat-2) were learning the

paradigm, the dopamine concentration while performing the nose poke is negative. Later on as animals (rat-1 and rat-2) got trained, a small positive dopamine concentration was observed. However unlike the other two trajectories, in this trajectory a prominent dopamine concentration was observed during actuator movement and reward delivery time, except for the last two days of experiment performed by (rat-1). While performing experiments with (rat-1) we encounter some challenges. First, after two days of training a slight modification was done in the paradigm i.e. previously, time taken for robotic actuator movement in forward and backward direction is 3 seconds which is decreased to 2 seconds. The last two days of the experiments were performed with this modification. Second, after modification with the paradigm, (rat-1) was not responding the task perfectly and became demotivated (due to some external noise interference which disturbed the solenoid sound cue) due to which the results were noisy and we were unable to understand the behavior. The experiment was optimized and resumed after 10 days. As (rat-1) learned again how to perform motor movement to get reward, it was again able to undergo experiments without getting demotivated.

Apart from these problems, by observing results of multiple days from both animals (rat-1 and rat-2), it is proven that dopamine is involved in predicting proximity of reward objects. The obtained results are reproducible and are align with the timing of reward delivery.

3.5 PCR Analysis

During the experiments it was seen that due to movement artifacts and pH changes appears due to which recording trial become noisy and it is difficult to detect dopamine therefore a principle component regression (PCR) data analysis technique was applied. For this purpose a training set is generated from one recording trial performed by (rat-1) and using this training set, new concentrations are predicted. The obtained results are shown below:

Figure 3.29: PCR Analysis on same data from which training set is generate, the current plot show the normal dopamine concentration and predicted concentration is the new concentration generated with the help of training data set.

In the first result (figure 3.29) a training set is generated from the recording trial. By using this training set, new concentrations were predicted for the same recording trial. Before applying PCR to the recording trial, we can see in the current plot that after dopamine peak appears there is a small but continuous dopamine trace but when we apply PCR to the same data we can see that this trace disappeared and it only appears at reward receiving area which suggests that actually this was noise. As this result was taken from one of the earlier recordings where (rat-1) was learning to perform experiment in which reward was presented with unidirectional Robotic Actuator Movement, after applying PCR analysis dopamine only appears when reward was received by (rat-1).
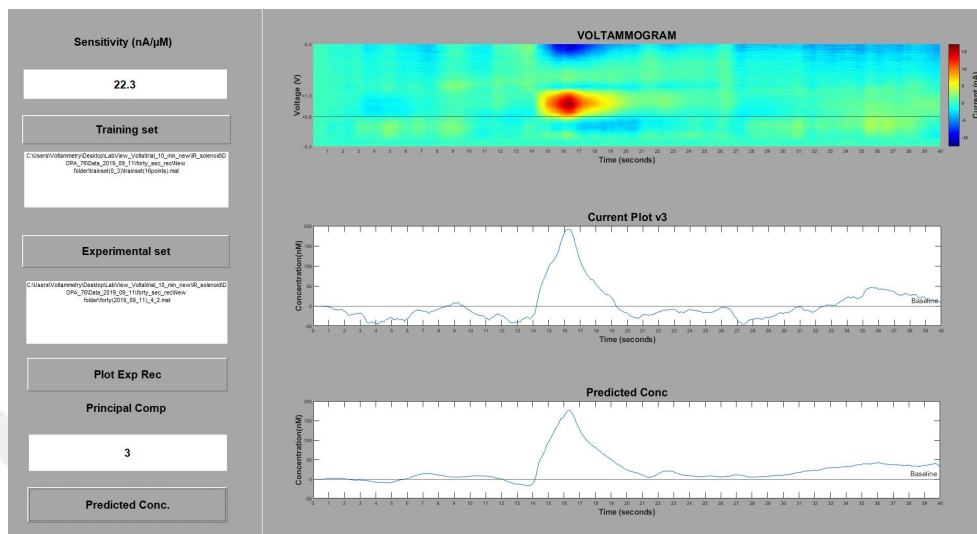
Figure 3.30: PCR Analysis on different data set. The current plot shows the normal dopamine concentration and predicted concentration is the new concentration generated with the help of same training data set which is used in Figure 3.29.

To verify the new predicted dopamine concentration, using same training set PCR analysis is applied on another recording trial of (rat-1) which was recorded at (day 11) figure 3.23. It is clearly seen that without applying PCR technique, the result is very noisy which makes difficult to identify real dopamine traces in it. After applying PCR using the same training data set which we already generated from recording trial performed on day 01, it is observed that we can clearly see the dopamine started to increase after animal does the nose poke and it becomes prominent as robot arm reaches to target area and animal gets the reward.

Chapter 4

Conclusions

Dopamine plays a pivotal role in establishing various aspects of behavior, including but not limited to, cognition, learning, memory, motor control and reward acquisition. This work studies the role of dopamine in the pursuit of approaching and receding reward cue. For this purpose a set of experiments were performed in a chronological order starting from unexpected reward delivery, reward delivery through motor movements, reward presented with unidirectional robotic actuator movement and finally reward presented with bidirectional robotic actuator movement. The robotic actuator was used to carry the reward itself toward a reward area which was accessible to the subject through a narrow slit.

The results produced from the first two set of experiments show a quick phasic response of dopamine in the nucleus accumbens as reward was presented (Sections 3.1 and 3.2). When the rats learned to perform motor movement (i.e. nose poking through a hole), dopamine response while the reward cue (i.e. the tip of the robotic actuator) approaches toward the subject was studied. For this purpose first we implemented a paradigm in which reward was presented in response to unidirectional movement of robotic actuator. From the results it is observed that as robotic actuator moves, dopamine concentration increases which becomes prominent as robotic actuator reaches the target area where the reward is delivered (Section 3.3).

To confirm the relationship between dopamine signaling and spatial proximity of the reward cue, a second paradigm was implemented in which one of three different trajectories were randomly realized by the robotic actuator in each trial. The dopamine concentration was measured to identify the dopamine release during robotic actuator movement as compared to unidirectional movement of robotic actuator. Our results indicated that as robotic actuator approaches toward the target area, increase in dopamine concentration (Section 3.4 "Task Type I and Type III") occurs and as it moves away from the target area we see a depression in dopamine concentration accompanied by pH change (Section 3.4 "Task Type II"). In conclusion, these experiments performed with a limited number of subjects have indicated a trend in the relationship between dopamine release and spatial proximity of reward. In some trials, it has been experienced that the freely moving subject also approached toward the tip of the robotic actuator when the robotic actuator starts its motion. In this sense, the distance between the reward and the subject was not completely controlled due to the movements of the subject. This situation may introduce some variance in the dopamine release during the tasks. Therefore, some modifications in the experimental setup and paradigm (e.g. implementation of head fixation) can be realized to decrease the mobility of the subjects.

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EVALUATION OF DOPAMINE SIGNALLING IN THE RAT VENTROMEDIAL STRIATUM FOR VARYING REWARD PROXIMITY