Improving Fast-Scan Cyclic Voltammetry and Raman Spectroscopy Measurements of Dopamine and Serotonin Concentrations via the Elastic Net

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Abstract

Dopamine and serotonin are two neurotransmitters known to both play a very important role in the human brain. For example, the death of dopamine producing neurons in a region of the brain known as the substantia nigra are known to cause the motor symptoms of Parkinson’s disease. Also, many antidepressants are believed to work by increasing the extracellular level of serotonin in the brain. For the first time, it is now possible to measure the release of these two chemicals at sub-second time resolution in a human brain using a technique known as fast-scan cyclic voltammetry, for example from patients undergoing deep brain stimulation (DBS) electrode implantation surgery.

In this work, we aimed to assess the feasibility of obtaining veridical dual measurements of serotonin and dopamine from substrates with mixtures of both chemicals. In the wet lab, data was collected on known concentrations of dopamine and serotonin and then used to make models capable of estimating the concentration of both chemicals from the voltammograms recorded in the patients. A method of linear regression known as the elastic net was used to make models from the wet lab data. The wet lab data was used to compare the performance of univariate and multivariate type models over various concentration ranges from 0-8000nM of dopamine and serotonin. Cross validation revealed that the multivariate model outperformed the univariate model both in terms of the linear correlation between predictions and actual values, and pH induced noise. The pH induced noise for the univariate model was 3.4 times greater for dopamine and 4.1 times greater for serotonin than the multivariate model.

Raman spectroscopy was also investigated as a possible alternative to fast-scan cyclic voltammetry. Raman spectroscopy could have several benefits over fast-scan cyclic voltammetry, including the ability to chronically implant the measurement probe into a patient's brain and make observations over a long period of time. Raman spectroscopy data was collected on known concentrations of dopamine to investigate its potential in making in vivo measurements, however this data collection failed. Therefore, simulations were made which revealed the potential of the elastic net algorithm to determine the Raman spectra of several neurotransmitters simultaneously, even when they are in mixtures and the spectra are obstructed by the noisy background. The multivariate type model outperformed the univariate type model on Raman spectroscopy data and was able to predict dopamine with an error of 805nM RMS and serotonin with an error of 475nM RMS after being trained on concentrations smaller than 5uM of both dopamine and serotonin. In addition, the original Raman spectra of both neurotransmitters was extracted from the noise and reproduced very accurately by this method.
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General Audience Abstract

The topic of this research was how to improve methods of measuring certain chemicals in the human brain called neurotransmitters. The two neurotransmitters of interest are known as dopamine and serotonin, and they were measured using a technique known as fast-scan cyclic voltammetry. The data that is recorded with fast-scan cyclic voltammetry can be difficult to interpret, and various methods have been developed to help analyze the data and determine the concentration of dopamine and serotonin from the data. Two particular methods were compared in this research, and it was found that using a model which predicts both Dopamine and Serotonin simultaneously outperforms a model which predicts them independently of each other. Also, as part of this research a new technique for measuring dopamine and serotonin was investigated. This method is called Raman spectroscopy and it uses laser light to measure the concentration of chemicals. The applicability of this new method to measuring neurotransmitters was examined and simulations were carried out to see how well the models developed for fast-scan cyclic voltammetry would work when applied to the Raman spectroscopy data.

This research is important because it enables measurements of these chemicals to be made with improved accuracy over existing methods. These chemicals are fundamental to a variety of brain disorders including Parkinson’s disease, depression, and addiction. The ability to measure their activity in the brain will lead to a deeper understanding of their function, and also allow the efficacy of various treatments for disease to be determined.
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Chapter 1: Introduction

In this chapter the motivation for this project will be explained, as well as an overview of the research goals. Then some background on neurotransmitters, positron emission tomography, functional magnetic resonance imaging, and animal models of Parkinson’s disease will be given.

1.1 Motivation

There are many functional neuroimaging techniques available to measure neural activity in the brain. However, none of the existing methods are capable of measuring the release of dopamine and serotonin in real time with good temporal resolution.

Electroencephalography (EEG) and magnetoencephalography (MEG) provide a way to directly measure the activity of neurons by detecting either the electric or magnetic field that results from current flowing through large populations of neurons. However, it is not possible to accurately measure the firing activity of dopaminergic and serotonergic neurons because they are so few in number and located deep enough in the brain that their activity is masked by other overlying brain regions.

Positron emission tomography (PET) imaging and functional magnetic resonance imaging (fMRI) imaging give an indirect measurement of neural activity by observing localized changes in blood flow to different regions of the brain. In general, PET and fMRI have much better spatial resolution than EEG and MEG (~1-2 mm), but much worse temporal resolution (~1 sec. compared to 1 msec. for EEG and MEG). As opposed to EEG and MEG it is in fact possible to measure dopamine and serotonin release with PET and fMRI through the use of contrast agents which bind to these chemicals. However, the time resolution for these methods is very poor, on the order of 10s of seconds.

Fast-scan cyclic voltammetry (FSCV) is unique in that it provides a way to measure the concentration of dopamine and serotonin in the extracellular space with sub second time resolution. Prior to 2011 [1] FSCV had never been reported in humans because it requires a probe to be placed into the neural tissue where the concentration measurements are made. Recently however, neurosurgeons have placed fast-scan cyclic voltammetry probes into the striatum of human patients who were undergoing deep brain stimulation (DBS) electrode implantation surgery. These measurements are the first of their kind, and potentially allow the concentration of dopamine and serotonin in the extracellular space to be measured with a time resolution of 100 ms. Because many of the patients from which the data was recorded have Parkinson’s disease, this data has the potential to provide a better understanding of this disease which involves the death of dopaminergic neurons. However, it also has the potential to provide insight to the function of these chemicals in a healthy human brain. Fast-scan cyclic voltammetry (FSCV) does have some drawbacks. It is extremely invasive when compared to PET or fMRI, and the probe relies on chemical reactions with the surrounding cortical tissue meaning that it can only be used temporarily. Also, it requires advanced signal processing techniques to extract useful concentration information from the data and differentiate between different neurotransmitters.

Raman spectroscopy is a possible alternative to FSCV that uses light to make chemical measurements. The main advantage of this technique is that a probe using Raman spectroscopy could be permanently implanted into a patient’s brain and allow measurement of neurotransmitter concentrations over a long period of time. The ability to continuously monitor
neurotransmitter release in the brain could be a huge asset to doctors trying to treat neurological disorders, for example to better assess drug dose requirements. Also, Raman spectroscopy has the potential to offer much better differentiation between types of neurotransmitters than FSCV and also allow the detection of chemicals that aren’t electrically active. This is because Raman spectroscopy probes the vibrational frequencies of a molecule directly using light.

1.2 Project Overview

As mentioned above, the research presented here was motivated by fast-scan cyclic voltammetry data collected in the striatum of human patients undergoing DBS surgery at Wake Forest University [1]. A prerequisite to testing for neurotransmitter levels in these data was to develop a signal processing method that could be validated in known settings. Thus the aim of my research was to develop a model which could take the recorded cyclic voltammogram as an input, and output the molar concentration of dopamine and serotonin that was present at the tip of the probe when the voltammogram was recorded. This is not at all a simple process, and much work has already been done on ways to accurately make this conversion.

For our solution to this problem, prepared concentrations of dopamine and serotonin were first prepared in the lab and cyclic voltammetry data was recorded from a probe placed in a flow cell. This data was used to make models that allow the voltammetry data collected from the patients to be converted to concentrations of dopamine and serotonin. Data was collected on 5 probes, and this data was used to make several models and compare the accuracy of each model. Once a suitable model was chosen, it was used to make predictions on the patient data.

Raman spectroscopy data was also collected in a lab at Virginia Tech using prepared concentrations of dopamine to study the feasibility of replacing the FSCV probe with a Raman spectroscopy probe. Simulated Raman spectroscopy data was also generated and used to show how the elastic net algorithm could be applied to data this type of data.

1.3 What are neurotransmitters? Why are they important?

Neurons (also known as nerve cells) are responsible for processing and transmitting information in the brain through the use of electrical and chemical signaling. One of the most important properties of neurons and what makes them different from most other cells in the body is that they can “fire”, or create an electrochemical pulse known as an action potential. This pulse quickly travels down the long neuron projection known as the axon, and can cause nearby neurons to fire as well. In this way information is passed from one neuron to the next through the central nervous system (CNS). Interestingly, heart cells are also capable of firing and creating action potentials as do neurons in the CNS. The rhythmic activity of the heart results from the discharge of excitatory impulses by cells in a region of the heart known as the sinoatrial node. These cells automatically fire in a regular pattern that results from each action potential being followed by a slow depolarization that brings the membrane to its threshold potential, thereby evoking another action potential in a repeating cycle [2].

The fastest rate at which neurons can fire is approximately 200Hz with most firing at much lower rates than this. Dopamine neuron (a specific type of neuron which this work focuses on) firing rates in mice have been observed to range from 0.8-12.7 Hz, with a mean firing rate of 4.6 Hz [3]. A typical neuron located in the cerebral cortex (the outer layer of brain tissue) receives input from a few thousand upstream neurons and passes on messages to a few thousand downstream neurons. In one cubic millimeter of cortical tissue, there are approximately one
hundred thousand neurons. This adds up to a total of approximately 40 billion neurons in the average human cortex, each with thousands of connections to other neurons. Below in Figure 1 is a diagram of two neurons in which the neuron on the left is firing and passing a message downstream to the neuron on the right.

Figure 1: Neurons Passing Messages to Each Other [4]

The connections between individual neurons are called synapses. These synapses are classified according to the type of cellular structures which make up the presynaptic and postsynaptic components. In Figure 1 the neuron on the upper right is the presynaptic neuron because it sends information to the postsynaptic neuron on the lower left. The most common synapses in the human brain and the type of synapse depicted in Figure 1 are axo-dendritic synapses where the axon of the presynaptic neuron terminates on the dendrites of the postsynaptic neuron. In other synapses found in the brain, the axon of the presynaptic cell may synapse onto a cell body, another axon, into the bloodstream, or diffusely into the adjacent nervous tissue.

The chemicals used by neurons to send messages across synapses to other neurons are known as neurotransmitters. Neurotransmitters are stored inside of a neuron in containers known as synaptic vesicles. When the membrane potential of the neuron exceeds a particular threshold value of -40 mV, the neuron fires and generates a millisecond long pulse called an action potential. When an action potential occurs in a neuron, some of the synaptic vesicles containing a
neurotransmitter move to the presynaptic membrane of the neuron and release their contents the synaptic cleft (the space between the two cells). Once released into the synapse, these neurotransmitters can attach to receptors located in the cell membrane of the postsynaptic cell. The attaching or “binding” of neurotransmitters to the receptors can either make the postsynaptic cells more likely to fire (known as an excitatory effect) or less likely to fire (known as an inhibitory effect) depending on the type of neurotransmitter and the type of receptor it binds to. This is because particular receptors admit charged particles into the cell, such as chloride (a negative ion) or sodium (a positive ion). Receptor binding can also lead to indirect effects on postsynaptic depolarization via protein cascades within the cell. Over 100 types of neurotransmitters have been discovered, though most of the compounds which function as neurotransmitters can be divided into three main chemical families: amines (a group of molecules containing molecules of carbon, hydrogen, and nitrogen), amino acids (organic compounds containing both an amino group and a carboxylic acid group), and peptides (compounds which are made up of multiple amino acids) [5].

Dopamine (DA) is the neurotransmitter which will be the main focus of this paper. Dopamine is a neurotransmitter in the amine family (the DA molecule is called a monoamine because it contains only one amine group) and is produced by a group of neurons referred to as dopaminergic neurons. There are only approximately 400,000 of these neurons in the human brain [6], and their cell bodies are concentrated in only a few locations. The two main sites of dopaminergic neuron cell bodies are the substantia nigra (SN) and the ventral tegmental area (VTA). The axons of these dopaminergic neurons extend to many other parts of the brain where they can release dopamine into the extracellular space. A large number of these dopaminergic neurons terminate in the striatum, a region of the brain, where the measurements from patients were recorded.

![Image of brain with dopamine pathways](image)

**Figure 2: The Brain’s Dopamine Pathways [7]**

The death of dopaminergic neurons in the substantia nigra is responsible for the debilitating motor impairment associated with Parkinson’s disease. Parkinsonian symptoms do not occur until 50%–80% of the dopaminergic neurons in the substantia nigra have died, and it is not clear what causes the death of these neurons in Parkinson’s disease. These neurons can also be destroyed by neurotoxins and viral infections such as encephalitis lethargica, resulting in similar movement disorders to those seen in Parkinson’s disease.

The basal ganglia comprise several subcortical nuclei or clusters of densely packed neuron cell bodies. It is composed of parts of the brain including the dorsal striatum (caudate nucleus...
and putamen), ventral striatum (nucleus accumbens and olfactory tubercle), globus pallidus, ventral pallidum, substantia nigra, and subthalamic nucleus. The leading model of basal ganglia function states that two circuits, the direct and indirect pathways, originate from two distinct populations of striatal medium spiny neurons (MSNs) and project to different output structures. Activity in the direct pathway MSNs is believed to promote movement, while activity in the indirect pathway MSNs is believed to inhibit it. The direct and indirect pathways are characterized by their different expression of dopamine receptors. D₁ (the different types of receptors are identified by a subscript) DA receptors stimulate GABAergic neurons in the presence of DA and are expressed by direct pathway MSNs whereas D₂ receptors inhibit GABAergic neurons in the presence of dopamine and are expressed by indirect pathway MSNs [8].

More is known about the function of dopamine in the CNS than most any other neurotransmitter, in particular its algorithmic or computational role. However, its exact function is still far from certain. Schultz et al. hypothesized in their 1997 paper titled A Neural Substrate of Prediction and Reward, that dopamine’s role is to signal changes or errors in the predictions of future salient and rewarding events [9]. These changes or errors in the prediction of future salient and rewarding events are called reward prediction errors. According to their paper, it has long been known that dopaminergic neurons in the VTA and SN are involved with the processing of rewarding stimuli. Some of the evidence to support this theory is that drugs like amphetamine and cocaine are addictive because they can bind to dopamine receptors – i.e. they mimic dopamine at exaggerated levels [10]. Also, mice will choose to press bars to excite dopamine neurons at the site of an electrode over other rewarding activities including food and sex. If animals are treated with dopamine receptor blockers, they learn less rapidly to press a bar for a reward food pellet.

The reward prediction error which the authors hypothesize is encoded by dopamine is delivered to various structures throughout the brain and used by them to influence the processing of predictions and to guide choice toward reward-maximizing actions. They hypothesize that in the striatum, the reward prediction error, also known as a scalar prediction signal, could have a direct effect on the choice of action to take. One reason they believe this is that in the striatum, activity relating to conditioned stimuli has been observed in several studies. The dopamine signal in the striatum is believed to encode the reward prediction error and may influence behavioral choices by modulating the level of competition of motor plans in the dorsal striatum. There is evidence for the striatal plasticity, or the ability of synapse connections to strengthen or weaken over time, after pulsatile application of dopamine. They also state that it seems reasonable to require that the prediction errors be delivered to the regions most responsible for making the predictions. This means that it is expected that there is a mechanism to couple local activity in the cortex to an enhanced sensitivity of nearby dopamine terminals to differences from baseline spike production along the parent axon. Lastly, this paper makes the point that neuromodulatory systems such as the dopamine system were so named because it was believed that they work with slower time resolution than do other connections in the brain like glutamatergic connections. While this may be true in part, this paper shows that neuromodulatory systems may also deliver precisely timed information to specific target structures.

A second important neurotransmitter which my research focuses on is Serotonin (5-HT). Serotonin is similar to dopamine in that it is a monoamine neurotransmitter and the cell bodies of the cells which produce serotonin are only located in a few specific locations in the brain. Serotonin is produced primarily by neurons in the raphe nuclei. There are 7 to 8 of these raphe
nuclei, and the serotonin neurons in the lower raphe nuclei project to the cerebellum and spinal cord while the serotonin neurons in the upper raphe nuclei spread out through the entire brain [11]. Some of these serotonergic neurons also terminate in the striatum. Serotonin is also produced in parts of the body other than the brain, and in fact about 90% of the serotonin in the body is found in the digestive track and blood platelets.

It is known that serotonin is also a very important neurotransmitter involved with depression, anxiety, panic, aggression, dominance, obsessions, punishment, analgesia, behavioral inhibition, rhythmic motor activity, feeding, and more [13]. Less is known about how it works and what its function is than is known about dopamine, partly because there are at least 15 different serotonin receptors currently known [14] (there are only 5 DA receptors [15]). Many researchers hypothesize that an imbalance in serotonin may lead to depression in certain individuals. In their 1976 paper, Asberg et al. measured the concentration of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of serotonin in 68 patients suffering from depression [16]. The concentrations formed a bimodal distribution. In the lower mode with concentrations of 5-HIAA below 15 nanograms per milliliter, there was a significant correlation between the concentration of 5-HIAA and severity of depression. This correlation was negative, with lower levels of serotonin being related to more severe depression. This led the researchers to conclude that there is a biochemical subgroup of depressive disorder, characterized by a disturbance of serotonin turnover. Today, the most common medications prescribed to treat depression are known as selective serotonin re-uptake inhibitors (SSRIs) and they work by reducing the rate at which serotonin released into the brain is reabsorbed into cells. This causes the overall level of serotonin in the extracellular space to increase.

In their 2008 paper, Dayan and Huys describe how simply predicting that something aversive will happen in the future can cause inhibition of behavior, suppression, and withdrawal [17]. They hypothesize that serotonin is involved in both the learning of these predictions of aversive events, and in the inhibitory consequences that the predictions create. In their model put forth in the paper, serotonin inhibits trains of thought that lead toward states with negative outcomes. This means serotonin can be thought of as pruning a decision tree of outcome states and choices. When 5-HT is depleted, actions that are comparatively worse lose direct inhibition that was previously preventing them and are therefore more likely to be executed.

Figure 3: Comparison of Serotonin and Dopamine Pathways [12]
One interesting theory the authors present is that not only the levels of serotonin when actions are being performed are important, but also the serotonin levels when actions were initially learned. They hypothesize that if subjects were trained while undergoing tryptophan depletion (TrD) which decreases extracellular serotonin levels, then they would explore states less when tested in a normal regime and that subjects trained while using SSRIs which increases extracellular serotonin levels, would explore states more when tested in a normal regime. Also, the authors cite several studies which showed that increasing serotonin levels on a short time scale can increase the recognition accuracy of fearful faces, indicating that negative states were not always pruned by serotonin and therefore still recognized. However, chronically increasing serotonin through the use of SSRIs yielded a decrease in the recollection of negative memories. It is unclear why a temporary decrease in serotonin and a chronic decrease in serotonin seem to produce opposite behavioral effects. In addition, the authors state that even serotonin reduction through the use of TrD does not have the same effect on all people. There are two types of 5-HT reuptake mechanisms, and the effects of TrD are more pronounced in people with less efficient reuptake mechanisms. Also, TrD can produce a relapse of depression in formerly depressed patients and those with a family history of depression more easily than normal patient populations. This may indicate that these individuals have less efficient 5-HT reuptake mechanisms than the normal population as well.

The authors model phasic (quick timescales) levels of dopamine as a reward prediction error, and they say that there is some evidence that 5-HT and dopamine are in mutual opposition in this regard. They mention some theories, including those of Deakin and Graeff [18] who suggest that serotonin rather than dopamine reports negative prediction errors based on an antagonism between serotonin and dopamine at both a behavioral and pharmacological level. In rodents 5-HT antagonizes the excitatory effects of dopamine.

In their 2002 paper **Opponent interactions between serotonin and dopamine**, Daw et al. offer that serotonin is involved in both the learning of aversive predictions and behavioral suppression that results from these predictions [19]. They say that serotonin is the most mysterious of the main vertebrate neuromodulators. Interestingly, serotonin cells do not obviously alter their firing rates in response to the sort of significant stimuli that might be expected to control behaviors typically associated with serotonin such as those listed before, anxiety, panic, aggression etc.

The authors also hypothesize that the dorsal raphe serotonin system and the ventral tegmental and substantia nigra dopamine system may act as mutual opponents. In their model, the tonic (slowly changing) serotonergic signal reports the long-run average reward rate, tonic dopamine reports the long-run average punishment rate, and that the phasic (rapidly changing) serotonin might report an ongoing prediction error for future punishment. However, other evidence shows that this dopamine serotonin interaction is much more complicated than simple opposition. An illustration of this complexity is that the rise of 5-HT caused by SSRIs has overall pro-dopaminergic effects [17].

### 1.4 PET Imaging of Dopamine

Positron Emission Tomography (PET), is an imaging method which provided some of the first measurements of the DA system within the living human brain. During a PET scan, a radioactive tracer is injected into the subject. There is a waiting period to allow the isotope to build up in the tissue of interest, then the subject is placed into the PET scanner. While in the scanner, the radioactive tracer isotope undergoes positron emission decay and emits a positron. This positron travels a short distance through the tissue before interacting with an electron and...
being annihilated. This positron-electron interaction creates a pair of gamma photons. Since each photon created has nonzero momentum, conservation of momentum means that they must travel in opposite directions. Detectors placed around the perimeter of the PET scanner detect these two photons as they strike the detector ring almost simultaneously [20].

When a pair of photons interact with the ring of detectors it can be inferred that the location of the positron-electron interaction lies on the line between the detector pairs as shown in Figure 4. Using image reconstruction algorithms, the concentration of the radioactive isotope in each voxel can be computed resulting in a 3D concentration map. To recap, first some type of molecule tagged with a radioactive tracer is injected into the body. After allowing enough time for the tracer to disperse through the body, a PET scan allows a 3D map of where the molecule ended up to be generated.

The PET measurements of the DA system are indirect, and rely on the use of radiotracers to label molecules such as DA receptors, DA transporters, and DA precursors. A ligand is a substance that forms a complex with a biological molecule, an example of a specific type of ligand is a neurotransmitter such as dopamine which binds to a receptor. In PET imaging, ligands are often radiolabeled with a radioactive isotope. Raclopride is a ligand that can be labeled with a radioactive carbon-11 atom and is often used in PET imaging because it binds to some types of dopamine receptors. Once ligands are radiolabeled they are then injected into the tissue under study and bind with their corresponding receptor. In PET imaging, often the ligand-receptor binding kinetic properties are measured, but presynaptic dopamine turnover can be measured as well. Unfortunately, PET scans are slow and can require up to two hours to complete. PET can also require the sampling of arterial blood for molecular studies, which is mildly invasive and painful.

As an example of how PET imaging and labeled ligands can be used to study the dopaminergic system, Cicchetti et al. administered a chemical known as 6-OHDA to rats and monitored the degradation of dopaminergic neurons using PET imaging of presynaptic dopamine transporters [22]. 6-OHDA is a neurotoxin that causes dopaminergic cells in the substantia nigra...
to die. The results of 6-OHDA lesioning are symptoms that closely resemble those of Parkinson’s disease. The specific ligand used in this study to bind to dopamine transporters was \([^{11}\text{C}]\)-CFT. The \([^{11}\text{C}]\) at the beginning of the name means that carbon-11 is the radioactive isotope used to tag the CFT molecule. CFT is a dopamine reuptake inhibitor that is an analog of cocaine and easily binds to dopamine transporters. Their study revealed a significant decrease in the binding parameters of \([^{11}\text{C}]\)-CFT in the striatum after 6-OHDA lesioning. The \([^{11}\text{C}]\)-PK11195 ligand was also administered in parallel to measure the inflammatory response of the brain. The \([^{11}\text{C}]\)-PK11195 ligand attaches to activated microglia, cells whose job it is to provide immune defense in the central nervous system. This ligand allowed them to determine that inflammation is a significant component of progressive dopaminergic degeneration that can be monitored by PET imaging.

A recent article by Le Foll et al. [23] also shows how PET imaging can be used to monitor the occupancy of two different types of dopamine receptors in the same experiment. They test the drug buspirone to see how well it binds to D3 receptors compared with D2 receptors with hopes that it could be used to treat drug addiction. Previous findings suggested that buspirone binds to D3 receptors much more readily than it does to D2 receptors and also that it disrupts psychostimulant self-administration in preclinical models. D3 receptors were chosen as targets for drug addiction treatment because preclinical and post-mortem human brain studies have found that D3 receptor levels are elevated after chronic exposure to drugs of abuse and also PET data has shown D3 receptor levels to be higher in individuals who abuse stimulants. A labeled ligand of \([^{11}\text{C}]\)-(+)-PHNO was used to test whether the buspirone binds to D3 receptors more than D2 receptors. Amounts of \([^{11}\text{C}]\)-(+)-PHNO binding in the dorsal striatum reflect D2 receptor availability while in other regions including the hypothalamus and substantia nigra it reflects D3 receptor availability. Their hypothesis was that buspirone did more readily bind to D3 receptors, and therefore a selective decrease in \([^{11}\text{C}]\)-(+)-PHNO binding in D3 rich areas relative to D2 areas would be observed. Magnetic Resonance (MR) structural images were also acquired to aid region of interest delineation of the PET images. The cerebral cortex was used as a reference region for the PET images. These previous two studies give indications as to how PET imaging can and has been used to study the body’s dopamine system. In the first study, the labeled ligand bound itself to a dopamine transporter protein and another labeled ligand used bound itself to activated microglia cells. In the second study, the labeled ligand used bound itself to D2 and D3 dopamine receptors.

The most common protein classes studied with PET are neuroreceptors, ligand transporters, and enzymes. PET currently works best when changes in protein density are very large (>50%). Some radiotracers are sensitive to the receptor occupancy while others are not. As an example, there are several sensors for the 5-HT system which appear to be insensitive to the amount of endogenous 5-HT being released. This has been tested by administering drugs that alter serotonin levels in the brain and seeing that these sensors do not show a change in receptor density. In general, before a PET signal change can be attributed to a protein density change it is necessary to perform additional testing to ensure that the binding of the radiotracer to the protein is not affected by other chemicals or neurotransmitters that can also bind to the protein and compete with the radiotracer.

Despite the success of PET imaging with dopamine, only a few other neuroreceptor systems have demonstrated success in measuring endogenous fluctuations. There is a need to design a new generation of radiotracers with faster protein association. This could allow multiple releases of neurotransmitters with a high time resolution to be monitored. Current imaging-tracer
techniques only allow for detection of a single neurochemical release over a relatively long period of time. These studies mentioned in this section show the great variety of measurements that are possible to make with PET imaging. The main drawback to PET imaging however is the time required to take a scan. PET imaging can be used to monitor changes in the dopamine system that take place over the course of several minutes or hours, but monitoring phasic dopamine levels over the course of seconds or even fractions of second is not currently possible.

1.5 fMRI Imaging of Dopamine

Magnetic Resonance Imaging (MRI) is another type of imaging modality similar in some ways to PET imaging. However instead of using radiolabeled molecules and radiation detectors as does PET, MRI relies on large magnetic fields to partially polarize the nuclear spins of atoms within a patient. The spins are then excited with a properly tuned pulse of radio frequency radiation. When the atoms relax from this radio frequency (RF) excitation, they emit a small amount of radio frequency information which can be detected and used to infer the structure of the cross section of tissue present. This allows brain anatomy to be studied non-invasively in great detail.

Approximately 20 years ago it was discovered that MRI imaging could be made sensitive to brain activity as well as brain anatomy. The magnetic resonance (MR) signal is not sensitive directly to neuronal activity, but can detect changes in blood flow which occur as an indirect result of neural activity [24]. When neuronal activity in a particular part of the brain increases, the body sends an oversupply of oxygenated blood to that area. This causes the oxygen level of blood in the region surrounding the neuronal activity to increase, a change that can be detected by the MRI machine because oxygenated hemoglobin and deoxygenated hemoglobin have different magnetic signatures. This process of imaging neuronal activity based on blood flow is called functional MR imaging (fMRI) and the signal it detects is called the blood oxygenation level dependent (BOLD) signal. The hemodynamic response imaged by BOLD signals begin approximately two seconds after neural stimulation and peak after four to six seconds. Also, the hemodynamic response is thought to encode the total cellular activity within a region rather than single cell activity. The BOLD signal is believed to represent the local field potential during neural stimulation [25].

It is possible to image brain structures in the dopamine system such as the subthalamic nucleus (STN) and the substantia nigra (SN) using the BOLD signal and fMRI, but there are many limitations to doing this [26]. These structures are very small, and their location can vary considerably from one person to the next. Their size is less than the spatial resolution of most fMRI scanning sequences. Hollander et. al. show that fMRI signals reported in previous studies of these structures are likely a mixture of signals from the STN, SN, and the surrounding tissue instead of resulting from only one structure. They recommend that the standard smoothing strategies used for fMRI imaging should be avoided altogether when trying to study the activity of small subcortical nuclei. They further recommend that individual anatomical masks should be used based on a structural scan of the individual.

In the article Detection of dopaminergic neurotransmitter activity using pharmacological MRI: correlation with PET, Microdialysis, and Behavioral Data [27] the use of fMRI to measure dopamine neurotransmitter activity was investigated. The dopamine system has high regional specificity, unlike the glutamatergic system, with most of the receptors being located in the frontal cortex and basal ganglia. Also, the dopamine system in animals can be selectively
destroyed using 6-hydroxydopamine. This allows it to be determined if the fMRI response measured in this experiment is due to dopamine.

Rats were used for this study, with microdialysis probes placed into the striatum of the rats. Control and lesioned rats were used, with 6-OHDA injected to kill at least 90% of the dopaminergic neurons in the lesioned rats. All MR measurements were performed in a 4.7T GE Omega CSI imager.

Either the drug D-amphetamine or CFT was injected intravenously while fMRI images were acquired or 90-180 minutes post injection. In these fMRI experiments the hemodynamic response being measured is caused by drug administration rather than task dependent activation and are therefore termed pharmacological MRI (phMRI) experiments. PET measurements were also acquired on the same animals after being scanned by phMRI. Dopamine transporters were imaged using carbon-11 labeled CFT as a tracer.

Their results showed that both amphetamine and CFT administration cause an increase in BOLD signal activity observed through phMRI imaging. Furthermore, these signal increases were localized to regions of the mouse brain known to be high in dopaminergic receptor density.

The authors admit that there are some potential problems with such an experiment, mainly because the metabolic response to the neurotransmitter binding rather than the binding itself is being observed by the phMRI, whereas in PET imaging, the actual binding of the ligand (in this case CFT) to the receptor is what is being observed. Also, for phMRI experiments, a large dose of the drug is necessary, and in the case of amphetamines, such large doses have the potential to activate other monoamine neurotransmitter systems such as serotonin. This means that the response seen may not be only a function of dopamine. Lastly, if the phMRI hemodynamic response is only indirectly coupled to the neurotransmitter release, then the signal observed may not provide useful information about the actual neurotransmitter release.

It was also observed that the BOLD response on the ipsilateral side of the brain was greatly diminished for those that underwent 6-OHDA administration. It is clear that this response was due to endogenous dopamine because Gd-DTPA (a MRI contrast agent used to map out blood flow) administration revealed that there was no change in relative cerebral blood volume (rCVB) or relative cerebral blood flow (rCBF). This means that the 6-OHDA only affected the dopamine system and not the vascularization or the hemodynamics on the side that was lesioned. The degeneration of the dopamine system on the ipsilateral side could also be seen by using [11C]-labeled CFT and PET imaging. They conclude that the phMRI response observed was due to release of dopamine in the striatum.

Parkinson’s disease doesn’t occur until approximately 80% of the dopaminergic neurons die. This means it may be possible to use phMRI (MRI along with administration of CFT) as a prescreening for the early stages of Parkinson’s disease. The authors do mention that it is still unclear if the metabolic response is due to the presynaptic uptake of dopamine or the postsynaptic stimulation of D1 and D2 receptors.

In their 2010 paper Mikhail Shapiro [28] introduced the development of a MRI contrast agent for imaging dopamine which could someday be used in humans for Parkinson’s disease diagnosis. This method would work differently from the phMRI technique previously mentioned in which the BOLD response to a drug such as CFT is monitored. Instead of imaging the changing magnetic properties of the blood in the brain, the magnetic properties of the contrast agent itself are what is imaged. The researchers state that MRI is capable in ideal circumstances of providing information at high spatial (<100 um) and temporal (~1s) resolutions from living specimens. BOLD imaging is based on hemoglobin, and this can be thought of as endogenous
oxygen-sensitive MRI contrast agent present in blood. However, this provides only a slow and indirect readout of neural activity because of the complexity of neurovascular coupling. They worked to develop a dopamine sensitive MRI contrast agent by modifying the bacterial cytochrome P450-BM3 (BM3). A cytochrome is an iron containing protein. When a ligand binds to a particular site on this protein, it leads to a drop in the MRI signal enhancement. However, as the protein naturally occurs, dopamine does not easily bind to its receptor site. To make the protein able to bind to dopamine, the researchers used an absorbance-based screen and a technique known as directed evolution to shift the specificity of BM3 away from its natural ligand and towards dopamine. Directed evolution is a molecular engineering method that uses rounds of mutation and selection to generate proteins with novel functionality. This approach of modifying proteins through directed evolution could be generalized to create probes for other targets besides dopamine. After creation of the DA sensitive BM3 sensor, it was injected into the brain of living rats. Then to test for the ability to measure endogenous DA a solution with elevated K+ levels was injected as K+ is known to release large amounts of DA into the striatum. The DA sensitive BM3 was injected into one hemisphere and the unmodified BM3 was injected into the other to ensure that the observed MR signal changes were only due to dopamine. The K+ injections produced a discernable signal decrease of up to 3% near the DA sensitive BM3 injection site. B

The researchers believe that these contrast agents will allow functional neuroimaging based on direct detection of neuronal events rather than hemodynamic changes. Also, exogenous delivery of macromolecules such as BM3 to large regions of animal brains should be possible, possibly through the use of transgenic subjects. Because of their small size, BM3-based DA sensors might sample synaptic dopamine better than voltammetry or microdialysis probes. With appropriate targeting it could become synapse specific and offer a combination of spatial coverage and precision that is currently inaccessible to other methods and potentially well suited to the types of studies of dopaminergic function that we are interested in.

Another way to indirectly assess dopamine and serotonin in fMRI is by using tasks known to elicit signals in their respective nuclei. This is currently most appropriate for dopamine since its computational correlates are well documented. For example in the article Testing the Reward Prediction Error Hypothesis with an Axiomatic Model [29], rewards were defined as stimuli that elicit approach behaviors, include subjective feelings of pleasure during consumption, and lead to reinforcement of cues and actions. There are a variety of brain structures both cortical and subcortical which make up the brain’s reward circuit, but the striatum lies at the core of these circuits. Many fMRI experiments have attempted to understand how the striatum contributes to reward processing. In this article, an approach known as the axiomatic approach which is rooted in economic theory is used to formally test the entire class of RPE models on neural data. They show that fMRI measurements of the striatum, medial prefrontal cortex, amygdala, and posterior cingulate cortex satisfy necessary and sufficient conditions for the entire class of RPE models. The authors state that although parameterized temporal difference approaches have been shown to account for electrophysiological data very well, regression approaches cannot in principle falsify the hypothesis that dopamine-related activity encodes some kind of RPE signal and therefore cannot formally test this hypothesis. Caplin and Dean [30] recently examined the necessary and sufficient properties of any RPE signal. They showed that any RPE signal must possess three critical features. The authors then devised an fMRI experiment where the BOLD response was measured in subjects’ brains as they played
monetary lotteries for real money. Subjects played a lottery with two prizes available +$5 and -$5 at a variety of probabilities 0-100% in 25% increments.

They performed two analyses. First, they used the traditional regression-based analysis. This required that several assumptions about reward and expectation were made. They assumed that reward is a linear function of monetary reward with no change in slope at the origin. They also assumed that predicted reward was equal to the utilities of the prized weighted by their objective probabilities. This requires that the RPE signal is then proportional to the difference in dollars between the outcome received and the lottery’s expected value. They also had to hypothesize that the BOLD response would follow the canonical two-gamma hemodynamic impulse response function. With these parameterizations, they found that BOLD activity in the striatum (including the nucleus accumbens, putamen, and caudate) was significantly correlated with RPE at the p<0.001 uncorrected level. At p<0.01 BOLD activity in the medial prefrontal cortex was also correlated with the RPE term. However, other candidate areas were not correlated significantly including the anterior insula, amygdala, and posterior cingulate cortex. According to the authors, this correlation cannot however tell us whether or not the data are compatible with the RPE hypothesis. It is impossible to tell from this data analysis whether the reason no significant correlation is observed was because of a fundamental mismatch between the actual signal and the model, or if our current measurements are simply not sensitive enough to tell. They further state that plotting the average BOLD response to positive and negative outcomes from the three two-prize lotteries showed that the hemodynamic responses to outcomes in the amygdala, posterior cingulate cortex, and also the medial prefrontal cortex do not at all resemble the canonical hemodynamic response.

The axiomatic approach used was fairly unique because it required no assumptions about the nature of the unobservable subjective variables like reward and expectation. Instead of looking for correlations with specific RPE models, it tests approaches critical to the entire RPE model class. They showed that BOLD activity in the anterior insula falsifies the axiomatic model for RPE and therefore cannot encode RPEs. However, the anterior insula almost completely satisfied an alternate model for RPE absolute value or salience. There are several theories which suppose dopamine neurons and dopamine-related activity may encode salience in addition to or instead of RPEs. A recent electrophysiological study found neurons in the dorsolateral substantia nigra that increase activity in response to both unexpected appetitive and aversive events.

A disadvantage of the axiomatic approach is that regions of interest must be specified manually by the experimenter. With regression-based methods, candidate RPE areas can be identified through a whole brain analysis. They state that future research could establish whether the quantity of dopamine release in these areas measured with electrochemical methods satisfies the axiomatic model, directly testing the linkage between RPE representations and dopamine. Given the still to be determined function of serotonin this approach has not been attempted to identify serotonergic modulation of cortical regions.

1.6 Animal models of PD & dopamine dysfunction

Models of neurotransmitter dysfunction present in cases of pathology offer insights into the normative roles of neurotransmitters. In turn models of neurotransmitter pathology can be used to mimic the human condition. These models can either be in vitro or in vivo. In vitro models (meaning in glass tubes) are performed outside of a living organism. As research progresses in vivo (meaning in living animals) models are eventually used. This means that it is necessary to have a way of reproducing the disease, in this case Parkinson’s, in a living animal.
In their article *Animal Models of Parkinson’s Disease*, Fabio Blandini and Marie-Therese Armentero [31] describe some of the more common animal models used to study Parkinson’s disease. Parkinson’s disease is characterized by the death of dopaminergic neurons in the substantia nigra, therefore animal models of the disease must somehow kill the dopaminergic neurons in these locations. The first PD animal models developed relied on using pharmacological agents which selectively kill dopaminergic neurons in the substantia nigra. There are several agents currently in use, each with their own benefits and drawbacks. There is no perfect agent which creates an exact model of PD, but different agents are able to recreate different aspects of the disease.

Pharmacological agents can be broadly divided into two categories, those which are systemically administered, meaning that they do not need to be injected directly into the animal’s brain, and those that are locally administered, meaning that they must be directly injected into the animal’s brain.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a systemically administered pharmacological agent. The ability of MPTP to selectively kill dopaminergic neurons was discovered accidently when young drug users from Northern California began exhibiting parkinsonism. Further investigation revealed that an intravenous injection of a narcotic containing MPTP had caused the symptoms. MPTP is transported across the blood brain barrier and converted by monoamine oxidase B into 1-methyl-4-phenylpyridinium ion (MPP+). This is carried by the dopamine transporter into the dopaminergic neurons of the substantia nigra (SNpc). Once in the neurons, it blocks the complex 1 activity in the mitochondria and prevents them from converting ADP to ATP.

A newer model of PD comes from chronic IV injection of rotenone to rats. Rotenone easily crosses the blood brain barrier and can enter dopaminergic neurons without relying on the DAT as does MPP+. Once in the cell, it blocks complex 1 activity. This causes the degeneration of dopaminergic neurons and Lewis body (LB)-like cytoplasmic inclusions containing ubiquitin and α-synuclein. Lewis bodies are neuronal inclusions containing structurally altered neurofilament and are always found in the substantia nigra and other brain regions in Parkinson’s disease [32]. This is unique among pharmacological agents, as most do not create LB-like inclusions.

Paraquat is an herbicide with a structure very similar to that of MPP+. However, the way in which paraquat works is very different than MPP+. Paraquat is a charged molecule, and therefore is not able to cross the blood brain barrier. It must use a neutral amino acid transporter to cross the barrier. In addition, unlike MPP+ paraquat does not significantly block complex 1 activity.

6-hydroxydopamine (6-OHDA) was the first animal model of PD ever generated. 6-OHDA is a hydroxylated analog of DA with a high affinity for DAT. 6-OHDA is not able to cross the blood brain barrier, so local injection is necessary. After being injected, 6-OHDA is transported by DAT into the cell where it oxidizes and forms hydrogen peroxide. It can also accumulate in the mitochondria and block complex 1 activity.

In a recent 2015 study by Morales et al. [33], 6-OHDA was used to generate a selective animal model of Parkinson’s disease. The authors state that there is increasing evidence that degeneration of the dopamine neurons in Parkinson’s disease starts in the axon and synaptic terminal of the cell and progresses back along the axon to the cell body in the substantia nigra. They state that at the onset of PD, more than 70% of DA, and more than 50% of the tyrosine hydroxylase, DA transporter, and vesicular monoamine transporter proteins have been lost in the
striatum. Despite these large losses in the striatum, less than 30% of DA cells in the substantia nigra are lost compared to age matched controls. 6-OHDA is the most common method of studying this dying-back degeneration. 6-OHDA is injected into the striatum and taken into the dopamine neuron by dopamine transporters. However, injecting the 6-OHDA into the striatum causes damage to many types of cells, not only dopamine cells. This occurs because of the penetration by the needle and the hydrostatic pressure caused by the injection of solution. Previous work had explored the possibility of reducing the damage to non-dopamine neurons by injecting the solution into the brain ventricle.

The researchers injected either a single dose of 6-OHDA into the lateral ventricle, or vehicle solution without the 6-OHDA. Four rats were also injected with 6-OHDA in the striatum. The 6-OHDA injection into the striatum caused dopamine neuron axons to kill around the injection site, but also caused unspecific cell death. A cavity of about 150-400 um diameter around the injection locus was found. 6-OHDA perfusion into the lateral ventricle induced a lateral denervation (loss of dopamine cell axons) of the striatum. This occurred quickly, with a loss of synapses observable only 4 hours after the perfusion in regions near the ventricle. Over the following weeks, the denervated striatum was colonized by new axons arriving from the edge of the denervation area. Six months after 6-OHDA administration, the denervated areas were repopulated with new dopamine synapses. The denervated areas never regained the original high synaptic density observed before lesioning however. The authors say that animal models for PD are often presented with a specificity problem. Peripheral administration of some drugs such as MPTP, paraquat, and rotenone are able to degenerate dopamine neurons without inducing unspecific damage linked to the injection procedure. However, they also affect the DA cell bodies and can cause non-dopaminergic toxicity in brain tissue.

Overall, we can see that imaging and animal models provide tools to study neurotransmitters in the brain and that much development has focused on dopamine. Less approaches have been developed for the study of serotonin and very few approaches are designed to assess both transmitters in concert. We will pursue this latter goal in the following chapters.

**Chapter 2: Background**

In this chapter to methods of measuring neurotransmitter concentrations will be discussed. The first is fast-scan cyclic voltammetry, a technique that has been used to measure neurotransmitter release in living rodents for several decades and only within the last decade has been used to measure neurotransmitter release in living human patients. We will also discuss some of the limitations of fast-scan cyclic voltammetry and how another technique known as Raman spectroscopy could possibly be used to circumvent these limitations.

### 2.1 Fast-Scan Cyclic Voltammetry

As it has been shown, both PET and fMRI imaging can be used to study the release of neurotransmitters in the human brain. However, to monitor neurotransmitter release with time resolution on the order of seconds, both of these methods are unsuitable. Methods which rely on electrochemical probes are invasive, but allow the measurement of neurotransmitter release on sub second timescales. Electrochemical methods are used to study chemical reactions that take place at the interface of an electrode and a solution [34].

Ralph Adams is credited as being the first person to implant a carbon microelectrode in the brain of a rat to measure in vivo concentrations of catecholamine neurotransmitters and their
metabolites in the extracellular fluid [35]. Catecholamine neurotransmitters are a family of neurotransmitters including dopamine and norepinephrine which happen to be electrochemically active. However, it was quickly discovered that catecholamines and their metabolites are not the only substances in the brain that will oxidize at a carbon electrode. Therefore, care must be taken to ensure that the electrochemical signals observed are actually from the target chemical and not another substance in the brain. To overcome this problem of selectivity encountered with electrochemistry, a method known as microdialysis has been used instead [35]. In microdialysis, small molecules are sampled from the extracellular fluid of the brain by passing a perfusion fluid through the hollow fiber of a dialysis membrane. This method has very good chemical specificity. However, electrochemical techniques cannot be matched by microdialysis in their ability to offer higher spatial and temporal resolution. Also, microelectrodes are smaller than microdialysis probes and therefore do not cause as much damage to the brain when they are implanted as do microdialysis probes. Naturally occurring transients in dopamine concentration are not observable with microdialysis because of the temporal blurring associated with the diffusion across the dialysis membrane. Electrochemical techniques are able to provide unique information on the operational characteristics of neuronal systems in the intact living brain.

Broadly speaking, voltammetry is the part of electrochemistry which deals quite generally with the determination and interpretation of current-voltage curves [34]. Cyclic voltammetry is a potential sweep method where the potential is swept from an initial potential to a final potential and then returned to the initial potential, usually at the same sweep rate. The technique provides voltammetric (current-voltage relationship) information about the substance being detected which can help researchers differentiate between different chemicals which are reacting with the probe. Cyclic voltammetry is the fastest of the electrochemical analysis techniques. When the sweep rate is exceptionally high, this method is referred to as fast-scan cyclic voltammetry (FSCV).

Fast-scan cyclic voltammetry can be used to measure the concentration of chemicals including dopamine and serotonin. It relies on the fact that both dopamine and serotonin are electroactive chemicals which can be oxidized in the presence of an external voltage source. For our experimental setup, a carbon fiber probe is connected to a voltage source which quickly sweeps the voltage of the probe tip up and down in a triangular waveform. When the voltage of the carbon fiber tip exceeds the voltage of the solution by a certain threshold, both dopamine and serotonin will oxidize and give up electrons which are absorbed into the carbon fiber tip. As the voltage of the carbon fiber tip is reduced these electrons can be reabsorbed by the oxidized dopamine and serotonin. The movement of these electrons creates a current which can be measured. In Figure 5 shown below, this oxidation current can be seen as a peak for high concentrations of dopamine.
All electrochemical measurements have a nonzero background signal (illustrated in Figure 5) and according to Michael and Borland this makes it almost impossible to use electrochemical measurements to measure static concentrations of a chemical because it is usually impossible to know what fraction of the current being measured is due to the charging current and what fraction is due to the presence of the target substance [35]. Instead, it is much more common to use electrochemical techniques to monitor dynamic events.

In the article *Phasic dopamine release in the rat nucleus accumbens symmetrically encodes a reward prediction error term*, FSCV was used to measure the release of dopamine in the nucleus accumbens core of rats while they perform a probabilistic decision making task [36]. In the article they mention the work of Rutledge et al. [29] in using axioms to test signals in the brain for RPE equivalence. They found that during tasks in which humans received probabilistic rewards, the BOLD signal in the nucleus accumbens meets necessary and sufficient criteria for encoding RPEs. However, the nature of the BOLD signal observed by fMRI makes it unclear how this activity is related to the extracellular dopamine concentration. Therefore, this study reports FSCV measurements of dopamine release in the nucleus accumbens core of rats performing a simple set of behavioral tasks. They also show that dopamine release, unlike firing rates, can encode positive and negative reward prediction errors on a symmetric scale. To perform the conversion from the voltammetry data to the dopamine concentration, first the average of the past 10 seconds was subtracted as background. Principle components regression was then used to convert this background subtracted current into a concentration of dopamine.

Recently, researchers used FSCV to measure the release of dopamine in the striatum of a human patient [1]. This patient suffered from late-stage Parkinson’s disease and was undergoing elective surgery for DBS-electrode implantation into the subthalamic nucleus. The carbon fiber microelectrode was placed into the right caudate nucleus. The patient (who was awake for this procedure) then played a stock market trading game. FSCV measurements with a scan rate of 10 Hz were collected.

In an article just released, researchers from this same group expanded their human study from one patient to a group of 17 patients [37]. These patients were also undergoing deep brain stimulating electrode implantation for the treatment of Parkinson’s disease. The patients played a
sequential investment game while recordings of dopamine were made in the striatum. An elastic-net based approach was developed to allow measurements of extremely low levels of dopamine. This is important because levels in the striatum of Parkinson’s disease patients are at abnormally low levels because of the disease.

The researchers found that the dopamine release in the striatum combines two different error signals: a reward prediction error and a counterfactual prediction error term. That is, the dopamine levels in the striatum encode the difference between actual reward and expected reward along with encoding the difference between what was earned and what could have been earned.

2.2 Raman Spectroscopy

Another method of measuring dopamine and serotonin in the brain which shows great potential is Raman spectroscopy. To obtain the Raman spectra of a chemical sample a laser with a narrow linewidth at a particular wavelength is shone onto the sample. Photons reflect from the sample, most with the same energy as they were emitted (elastic scattering). However, a very small number of the photons manage to exchange some energy with the chemical bonds between atoms in the molecule as they interact with it and are reflected with a different amount of energy than they were emitted from the laser. This is known as Raman scattering. The amount of energy gained or lost by the photon gives information as to the type of chemical bonds present in the sample. A typical setup for Raman spectroscopy is shown below in Figure 6.

![Raman Spectroscopy Setup](image)

**Figure 6: Raman Spectroscopy Setup**

Confocal Raman imaging is when a Raman spectrometer is coupled to a standard optical microscope. This provides rapid, detailed and accurate neurotransmitter analysis and allows for millisecond time resolution of biochemical dynamics [38]. In a 2012 article titled *Detection and Monitoring of Neurotransmitters - a Spectroscopic Analysis*, confocal Raman imaging was used to collect the spectra of several common neurotransmitters. They mention that although neurotransmitter release can be measured through electrochemical techniques such as amperometry, fast scan cyclic voltammetry, and chromatograph, optical techniques have the additional advantage of being able to provide information about the composition of tissue at the molecular level. Also, they mention that while carbon fiber microelectrodes are much smaller than probes previously used for microdialysis, their chemical stability is affected by the biological environment and they are long-term degradable. With Raman spectroscopy, a non-
degradable optical fiber sensing probe can be used. This probe can be produced with very small dimensions. Optical detection approaches which rely on fluorescence or luminescence do have the advantage of providing a very strong signal, however they are not able to easily determine the composition of a complex mixture of neurochemicals as is possible with Raman. Also, Raman allows for the chemical dynamics to be monitored with millisecond time resolution.

For their experiments, dopamine, adenosine, and serotonin were used to prepare solutions of 100 uM concentration in deionized water. The solutions were placed onto glass slides and allowed to diffuse together. Also, dopamine was additionally placed onto a block of gelatin (to simulate brain tissue). An alpha 300 WITec confocal Raman system using a 532 nm excitation with a Nd:YAG laser was used.

In a recent article titled *A low background Raman probe for optical biopsy of brain tissue*, researchers attempted to design a probe for use in the removal of brain tumors. The probe they design has several advantageous features, including the fact that a conventional stereotactic brain biopsy needle is the only part of the probe that enters the brain. The lenses, filters, and fiber optics are all mounted at the end of the needle outside of the brain. An excitation wavelength of 830nm is used to minimize the sample fluorescence. Also, fluorescent background caused by silica is an issue that affects most Raman probes. It is possible to subtract this fluorescent background in software, though this presents some problems. It may confound statistical analysis being performed on the spectra and also as the total number of background counts is increased, the shot noise is also increased, leasing to a reduced signal to noise ratio. Shot noise is a consequence of the discrete nature of photons. Since the arrival of photons at a pixel of the detector is an independent random process, it follows a Poisson distribution. With a Poisson distribution, the variance (noise) is proportional to the mean [39].

**Chapter 3: Materials and Methods**

In this section an overview of the how the wetlab data was collected for the fast-scan cyclic voltammetry probes will be given. Next, how the wetlab data was used to make models capable of predicting dopamine and serotonin concentrations will be discussed. Then the univariate model type and the multivariate model type will be discussed. Lastly the failed collection of Raman data will be discussed, along with possible reasons for its failure.

**3.1 Fast Scan Cyclic Voltammetry Overview**

The flow chart below gives a high level overview of the processes which comprise making fast-scan cyclic voltammetry measurements as in the study by Kishida et al. [37]. My work in this thesis only comprises two of the processes, namely collecting flow cell cyclic-voltammetry on known dopamine and serotonin concentrations and making a predictive model from flow cell data.
In behavioral study, this model would then be applied to patient fast-scan cyclic voltammetry data and used to obtain a time series of the estimated dopamine and serotonin concentration at 100 ms intervals over the course of the operating room data collection. These time series of dopamine and serotonin would then be compared with the concurrently recorded patient behavioral data to look for any interesting correlations. However, this work does not apply the models to any patient data, it just examines the performance of the models on known concentrations of neurotransmitters recorded in the wet lab.

In a current behavioral study by Kishida et al. [37] an entirely different set of probes is used to make the models that the set of probes used to collect data in humans. Therefore, we wished to use the same experimental setup and make predictions on probes that did not go into making the predictive models. It should be noted that a possible alternative approach would be to use the same probes to make the predictive models and collect patient data. To do this, it would be necessary to characterize how the sterilization procedure changes the probes’ response. However, the change due to sterilization is likely much smaller than the differences in probe construction. Another possibility is that the probe could be placed into a known sterilized solution by the neurosurgeon immediately before being placed into the patient’s brain. This would provide useful information about the probe’s response at the time the data was collected and would make it easy to determine if this response had changed after sterilization.

3.2 Flow Cell Data Recording

The probe’s response to known concentrations of dopamine and serotonin was needed before any predictions could be made on the patient data. A flow cell (Figure 8) was used to measure the carbon fiber microelectrode’s response to known concentrations of dopamine and serotonin.
as well as the response to changes in the solution’s pH. This wetlab data can be thought of as calibrating data.

Figure 8: Schematic of Flow Cell (Probe Tip Shown in Insert)

Solutions were prepared beforehand and placed into a microplate. These solutions were then injected into the right side of the flow cell using a syringe and fast scan cyclic voltammagrams were recorded from the microelectrode.

To make these known solutions of varying concentrations, dopamine and serotonin were mixed with a phosphate-buffered saline (PBS) solution. The PBS solution was used because its osmolarity and ion concentrations match those of the human body.

Table 1: Composition of Phosphate-Buffered Saline Solution

<table>
<thead>
<tr>
<th>Salt</th>
<th>Concentration (mmol/L)</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>137</td>
<td>8.0</td>
</tr>
<tr>
<td>KCl</td>
<td>2.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>10</td>
<td>1.42</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.8</td>
<td>0.24</td>
</tr>
</tbody>
</table>

As shown in Figure 8, the microelectrode was connected to a headstage amplifier (Axon Instruments, CV-7B/EC) operated in voltage clamp mode (Figure 9). This means that the voltage of the microelectrode’s tip was swept over a preset waveform while the current flowing into or out of the microelectrode’s tip was recorded.
The voltage of the microelectrodes tip was swept in a triangular waveform from -0.6V to +1.4V and then back to -0.6V. This triangular voltage waveform lasted for 10ms and was repeated with a frequency of 10Hz. During the 90ms for which there was no triangular voltage waveform being applied, the probe’s voltage was held at -0.6V. This waveform is shown below in Figure 10.

The headstage was connected to a signal amplifier (Axon Instruments Multiclamp 700B). This signal amplifier was connected to an analog-to-digital converter (Axon Instruments Digidata 1440A) which was connected via USB to a laptop.

There were four datasets collected in the flow cell. One dataset consisted of trials where the concentration of dopamine was varied from 0-8000 nM in 100 nM increments (80 total trials), one where the concentration of serotonin was varied from 0-8000 nM in 100 nM increments (80 total trials), one where the pH level was varied from 6.8-7.8 in increments of 0.2 (6 total trials), and one where a random concentration of dopamine (0-8000 nM) and serotonin (0-8000 nM) along with a random pH level (6.8-7.8) were chosen for each trial (40 total trials).

The CV data recorded for each of these trials lasted for 65 s, and 250 uL of the solution was injected into the flow cell using a syringe during the first 5 s. After the injection of the solution, there is some transient behavior caused by the movement of the solution. CV recordings from the 50-60 s time window were used to train the model and the remaining portion of the trial was discarded to ensure that the transient behavior had decayed to a small enough level.

Below is a plot which shows how the varying levels of dopamine and serotonin affected the derivative of the cyclic voltammagram (Figure 11). This plot could just as easily have shown how varying levels of dopamine and serotonin affected the undifferentiated cyclic voltammagram. However, our model was trained on the derivative of the voltammagram because the derivative amplifies the oxidation and reduction peaks and tends to filter out the background charging current which is comprised of lower frequency components.
The inset in both figures shows the peaks caused by the oxidation of dopamine and serotonin. There is also an obvious peak where the reduction of dopamine and serotonin occur, but this is not shown in an inset. It is evident that while the two oxidation peaks are very similar, they are not identical. These small differences are what the model relies upon to differentiate between the presence of dopamine and serotonin, and make predictions on the two substances.

![Figure 11: Overlay of Applied Voltage Waveform, Raw Current Signal, and Derivative of Signal for Varying Dopamine and Serotonin Levels](image)

The four datasets (dopamine only, serotonin only, pH only, and random mixtures) were collected for five probes. Microscope images of these five probes are shown below. These five probes were only used to collect flow cell data, not patient data.

### 3.3 Probe Characterization

The carbon fiber microelectrodes were constructed to have the same outside dimensions as intraoperative electrophysiology electrodes that are used for functional mapping during DBS electrode implantation. They are constructed by hand in the lab using a microscope to guide the assembly.

The components of the probe are:
1. Polyimide-coated stainless steel guide tube
2. 360 µm OD fused-silica capillary - 271.5-mm long
3. 90-µm OD fused-silica capillary - 20-mm long
4. 7-µm OD carbon fiber tip

The carbon fiber tip was attached to a silver wire which ran through the center of both fused-silica capillaries. The stainless steel guide tube was coated with an insulating polyimide coating, but approximately one millimeter of the stainless steel tube was left uncoated. This uncoated portion acted as the reference electrode and the carbon fiber tip acted as the active electrode.

The length of the carbon fiber microelectrode tips varied considerably among the five probes, and this caused a lot of variation in the background waveform of each of the probes (Figure 12).
It was desired to characterize how much the background current of a probe could vary from day to day. To do this, the probes’ responses to PBS only were recorded on multiple days. Below is a plot showing the waveform of PBS only recorded. The color indicates which probe the recording was made from. There were four types of collections done for each probe and each collection included a measurement of only PBS. Most collections were performed on different days, and all of these PBS measurements on a single probe give an idea of how much the background can vary over time.
Figure 13: Overlay of all PBS waveforms over all trials (top) and standard deviation of waveform at each time index over all trials (bottom).

It is evident from this plot that on average, the standard deviation of the background waveform is approximately 10 nA, though it exceeds 100 nA near the switching point. This standard deviation represents the amount that should be expected for the background of a probe to vary from one day to the next and from one experimental data collection to the next (the long term variance).
To characterize the additive white Gaussian noise (AWGN) of the measurements, the same analysis was performed except that only one 10 second collection of the PBS waveform was included for each probe. Based on the assumption that the background current varies slowly, it can be assumed that these standard deviation measurements give a good estimate of the amount of AWGN only. It appears that the standard deviation of the AWGN is approximately 0.1-0.2 nA, except near the transition region where it peaks to around 1.1 nA. This analysis shows that the variation in the background current is approximately 2 orders of magnitude larger than the AWGN. However, the fact that the background current varies slowly means that it doesn’t have as large of an affect if the measurement times are short compared to the rate of this background variation.

The approximate length of each carbon fiber tip was measured from these microscope images. Also, the sum of the absolute value of the average background voltammagram for each of the five probes was computed. When this sum was plotted as a function of the probe length, there was a clear positive relationship between the two (Figure 15).
Figure 15: Total charge as a function of probe length (right) and sensitivity as a function of probe length (left). The length of the shortest probe was normalized to 1.

All probes have an oxidation peak sensitivity of between 0.2 and 2.2 nA/100 nM. When this sensitivity is plotted as a function of the relative probe length, it is clear that the relationship is also positive (longer probes have a higher sensitivity to the chemicals).

3.3.1 Length Based Calibration Schemes

Because the length of the probe and the size of the PBS only waveform response were found to have such a strong relationship, this indicates that it may be possible use a probe’s measured length to scale the waveforms from that probe and produce more accurate predictions. This was not done for any of the subsequent analysis in this thesis, though it shows promise as something that should be investigated in the future. The main reason why this was not done in this thesis is that current electrochemical work in human patients [37] doesn’t perform any scaling and it was desired to recreate these same findings and produce results which could estimate the accuracy of unscaled predictions.

3.4 Making the Predictive Model

The next step after collecting the wetlab data was to produce a supervised learning algorithm – in other words to generate models capable of making predictions on patient data using known concentration training data. First a generative model of the data will be described. This model is not used to make concentration predictions, but it does do a good job of breaking contribution to the measured signal from different sources of signal and noise.

3.4.1 Generative Model

A generative model is capable of making simulated data which closely resembles that seen from probe collections. The generative model that will be used

\[ I(t) = B(t) + S(t) + \epsilon(t) \]
\( I(t) \)- The observed current waveform at time \( t \).
\( S(t) \)- The additional current caused by the oxidation and reduction of dopamine and serotonin.
\( B(t) \)- The background charging current at time \( t \). It is assumed that this is slowly varying stochastic process.
\( \epsilon(t) \)- Measurement noise. It is assumed that this is additive white Gaussian noise with a mean of 0 and some standard deviation.

This is depicted graphically below. The total current measured is modeled as the sum of three independent signals.

![Graph showing individual contributions to the generative model](image)

**Figure 16: Individual Contributions to the Generative Model**

Another assumption made about this model is that \( S(t) \), the additional current caused by the oxidation and reduction of dopamine and serotonin is equal to the oxidation shape vector for dopamine multiplied by the concentration of dopamine plus the oxidation shape vector for serotonin multiplied by the concentration of serotonin. In other words there is a linear mapping between the concentration of serotonin and dopamine and \( S(t) \).

The problem of determining chemical concentrations from the recorded current waveforms was framed as a supervised machine learning task. The objective then became to determine a function that mapped cyclic voltammetry current waveforms to concentrations of dopamine and serotonin.

### 3.4.2 Univariate Model

In the paper *Subsecond dopamine fluctuations in human striatum encode superposed error signals about actual and counterfactual reward* [37], a univariate linear model was applied. To
apply this model, the assumption was made that this mapping between the dependent variable (time derivative of current waveform) and the independent variables (concentration of dopamine) could be modeled by a general linear model of the form $Y = X\beta$ where $X$ is the raw voltammogram or the derivative of the voltammogram and $Y$ is the concentration of dopamine. The $\beta$ is a column vector that predicts dopamine (or serotonin depending on the whether the dopamine or serotonin labels are used).

### 3.4.3 Multivariate Model

It was desired to generate predictions of serotonin and dopamine at the same time and increase the specificity of the probe (so that dopamine is not confused for serotonin and vice versa). To do this, the assumption was made that this mapping between the dependent variable (time derivative of current waveform) and the independent variables (concentration of dopamine and serotonin) could be modeled by a general linear model of the form $Y = X\beta$ where $X$ is again the raw voltammogram or the derivative of the voltammogram and $Y$ is the concentration of dopamine and the concentration of serotonin (Figure 17). $\beta$ is a matrix consisting of two column vectors; one to predict dopamine and one to predict serotonin. This corresponds to our assumption about $S(t)$ in the generative model.

![Figure 17: General Linear Model for Predictions](image)

Once the predictive model was chosen, the task then became to estimate the two column vectors comprising $\beta$ and which map derivatives to dopamine and serotonin concentrations.

### 3.4.4 Estimating $\beta$ Through Elastic Net Linear Regression

There are multiple ways to estimate these model parameters. A method called elastic net regression was used [40] [41]. Elastic net regression is identical to least-squares estimation with the addition of a penalty for both the $l_1$ norm and $l_2$ norm of the columns in the $\beta$ matrix. Elastic
net regression has several important advantages over least-squares estimation. Both the $l_1$ norm and $l_2$ norm penalties help to prevent overfitting by penalizing extreme values of $\beta$. However, the $l_1$ norm has the additional capability to perform feature selection by setting some of the elements of $\beta$ to 0. The parameter $\lambda$ determines the weight of the $\beta$ penalty terms and the parameter $\alpha$ determines the relative weight of the $l_1$ norm and $l_2$ norm penalties. If $\alpha$ is set to 0 then this is just $l_1$ norm reconstruction which is also commonly referred to as Lasso regression.

As shown in Figure 16, we have hypothesized that $S(t)$ (the additional current caused by the oxidation and reduction of dopamine and serotonin) is sparse in the time domain. Since the electrode voltage is also changing as a piecewise linear function of time it is also sparse in the voltage domain. This means that the $l_1$ norm’s tendency to create sparse $\beta$ vectors could be advantageous in this situation. In some cases the performance of $l_1$ norm reconstruction can be improved by the addition of an $l_2$ norm penalty [40] [41]. If there are a group of variables which are highly correlated, then the of $l_1$ norm reconstruction will tend to only select one variable from the group, and it does not care which one is selected. The $l_2$ norm penalty term can help in the selection of multiple variables from a group of correlated variables.

When $Y$ is a single scalar response variable, the elastic net algorithm looks to find the $\beta$ which minimizes.

$$\min \frac{1}{2} \| Y - X\beta \|^2 + \lambda \alpha \| \beta \|_{l_1} + \frac{\lambda (1 - \alpha)}{2} \| \beta \|_{l_2}$$

Since both the dopamine and serotonin concentrations are being predicted, $Y$ is not a scalar but a vector with two entries. For the case where $Y$ is a vector, the elastic net algorithm looks to find the $\beta$ which minimizes.

$$\min \frac{1}{2} \| Y - X\beta \|_F^2 + \lambda \alpha \sum \| \beta_k \|_2 + \frac{\lambda (1 - \alpha)}{2} \| \beta \|_F^2$$

Here the $F$ subscript stands for the Frobenius norm. This norm is calculated by squaring each element of a matrix, summing these squared elements, then taking the square root of the sum. Though the form when the response variable is a vector looks slightly different than the case when the response variable is a scalar, it is easy to see that the vector form reduces to the scalar form as the number of elements in the vector is reduced to one.

When $\beta$ is a column vector (the case when $Y$ is a scalar) each row of $\beta$ is just a scalar. The $l_2$ norm of a scalar is equivalent to the absolute value of the scalar.

$$\sum \| \beta_k \|_2 = \sum |\beta_k|$$

Therefore this is just the sum of the absolute value of the elements of $\beta$ or the $l_1$ norm of $\beta$. The term

$$\| \beta \|_F^2$$

likewise becomes the $l_2$ norm of $\beta$ when $\beta$ is a column vector.

The algorithm used to calculate $\beta$ is shown below. It works by calculating one row of $\beta$ at a time, cycling through all of the rows until convergence is achieved.
1. Initialize $\beta = \beta_0, R = Y - X\beta_0$.
2. Iterate until convergence: for $k = 1, \ldots, p$
   a. Update $R_{-k}$ by
      $$R_{-k} = R + X_k\beta_k.$$ 
   b. Update $\beta_k$ by
      $$\beta_k \leftarrow \frac{1}{\|X_k\|^2 + \lambda(1 - \alpha)} \left(1 - \frac{\lambda\alpha}{\|X_k^TR_{-k}\|^2} \right) X_k^TR_{-k}.$$ 
      Note that if $\|X_k^TR_{-k}\|^2 \leq \lambda\alpha$, then $\beta_k \leftarrow 0$.
   c. Update $R$ by
      $$R = R_{-k} - X_k\beta_k.$$ 

Below is a plot showing the applied voltage, a raw voltammogram, the derivative of this voltammogram, and two $\beta$ vectors for dopamine calculated with different values of $\alpha$. It is evident that the higher value of $\alpha$ generates a more sparse model because it emphasizes the $l_2$ norm penalty on the $\beta$ vector. In fact, the majority of the elements in the $\beta$ vector are 0 for $\alpha = 1$. With $\alpha = 0.1$, there are many fewer elements of the $\beta$ vector set to 0.

![Figure 18: Applied Voltage, Measured Current, Derivative of Measured Current, and Beta Vectors for Two Alpha Values](image)

Below is a plot which shows the dopamine and serotonin $\beta$ vectors overlaid on an example voltammogram derivative. This plot shows how the $\beta$ vector for dopamine and the $\beta$ vector for serotonin each pick out different parts of the waveform.
3.5 Using the Model to Make Predictions

To make predictions, the $\beta$ vector (matrix for the multivariate model) was simply multiplied by the observed voltammgram. If the observations are broken apart as in the generative model, three terms are left over.

$$Y = X\beta = (I)' \beta = (S + B + \epsilon)' \beta = (S' + B' + \epsilon') \beta = S'\beta + B'\beta + \epsilon' \beta$$

In the paper by Kishida et al., a slightly more involved method of model predictions were used. Calibration data was obtained in the range of 0-8000nM, but instead of using all of this data to find one value of $\beta$, the data was instead broken up into several dozen models, each trained on a subset of the data. These subsets had mean concentrations which progressively increased. This was done based on the assumption that the waveform for dopamine is not constant as the concentration was increased. Therefore, given a particular voltammgram taken at some unknown concentration, predictions were made using each of the $\beta_1, \beta_2, \ldots, \beta_N$ predictive models. Each model $\beta_i$ resulted in a prediction $pred_i$. The distance between each of the predictions and the means of the data which produced $\beta_i$ was computed, $pred_i - \mu_i$. The model with the minimum distance between the mean of the data and the prediction was chosen and this prediction used as the overall prediction. Put simply, models whose predictions fell within the range of concentrations used to make the predictions were preferred. We did not impose such constraints but instead tested whether our probes were able to predict concentrations across large ranges for both DA and 5-HT.

3.6 Failed Raman Spectroscopy Data Acquisition

Next we turn our attention to Raman spectroscopy, a measurement technique that measures the same information as fast-scan cyclic voltammetry (concentrations of chemicals in solution), but in an entirely different way. Raman spectroscopy data was recorded at Virginia Tech, but the acquisition failed. No spectra were observed that could be attributed to dopamine or serotonin. Even though the acquisition failed, we will talk about how Raman spectroscopy data was recorded, preprocessed, and analyzed, as well as some possible reasons that the acquisition may have failed.

3.6.1 Raman Spectroscopy Apparatus
It was wished to examine the possibility of designing a Raman spectroscopy probe that would measure dopamine and serotonin concentrations in a similar fashion to how the FSCV probe made measurements. To do this, Dr. Leng, a professor at Virginia Tech was contacted. He provided access to a WiTec confocal Raman imager. The microscope had two different lasers, one operating at 532 nm and the other operating at 785 nm. It was decided to use the 785 nm excitation wavelength even though the Raman response at longer wavelengths is much lower than at shorter wavelengths. The Raman response varies as the fourth power of the wavelength so it would be expected that signals at 532 nm would be stronger than those at 785 nm by a factor of \((785/532)^4 = 4.741\). The reason for choosing 785 nm in spite of the reduced Raman scattering is that the eventual goal was to make measurements in a living human brain, not on a solution in a glass flow cell. In many biological tissues, the dominating factor is tissue fluorescence. This fluorescence can completely overwhelm the Raman spectra, but longer wavelengths minimize the fluorescence of tissue. Weak Raman signals can be compensated for by longer scan times, higher laser powers, or better probe design. However, if the fluorescence is overwhelming the Raman spectra, there is little that can be done.

3.6.2 Raman Data Acquisition.

It was unclear what type of spectra would be observed from dopamine dissolved in PBS solution. Also, it was unclear how sensitive this Raman spectrometer would be. It was first decided to use concentrations of dopamine in PBS ranging from 0 nM up to 10000 nM. This range was chosen simply because the FSCV dopamine concentrations used to calibrate the probe was chosen to be in the range of 0 nM to 8000 nM.

The following concentration ranges were prepared and data was collected on each. To test the machine, a small amount of undiluted 100 mM dopamine was placed into a quartz capillary tube. This produced the following spectra
There was an obvious peak around 750 l/cm which was assumed to be from dopamine. Next, the planned sequence of dopamine and serotonin collections were performed. The data collected is shown in the table below.

**Table 2: Raman Spectroscopy Data Collected**

<table>
<thead>
<tr>
<th>PBS</th>
<th>DA + PBS</th>
<th>5HT + PBS</th>
<th>DA + 5HT + PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>stock PBS solution</td>
<td>1nM - 22mS integration time, 1000 spectra collected</td>
<td>1nM - 22mS integration time, 1000 spectra collected</td>
<td>500nM - 22mS integration time, 1000 spectra collected</td>
</tr>
<tr>
<td>DA + PBS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10nM - 22mS integration time, 1000 spectra collected</td>
<td>10nM - 22mS integration time, 1000 spectra collected</td>
<td>500nM - 22mS integration time, 1000 spectra collected</td>
<td>500nM - 22mS integration time, 1000 spectra collected</td>
</tr>
<tr>
<td>100nM - 22mS integration time, 1000 spectra collected</td>
<td>100nM - 22mS integration time, 1000 spectra collected</td>
<td>1000nM - 22mS integration time, 1000 spectra collected</td>
<td>1000nM - 22mS integration time, 1000 spectra collected</td>
</tr>
<tr>
<td>1000nM - 22mS integration time, 1000 spectra collected</td>
<td>1000nM - 22mS integration time, 1000 spectra collected</td>
<td>10000nM - 22mS integration time, 1000 spectra collected</td>
<td>10000nM - 22mS integration time, 1000 spectra collected</td>
</tr>
<tr>
<td>100nM - 1S integration time, 1 spectrum collected</td>
<td>100nM - 1S integration time, 1 spectrum collected</td>
<td>500nM - 1S integration time, 1 spectrum collected</td>
<td>500nM - 1S integration time, 1 spectrum collected</td>
</tr>
<tr>
<td>100nM - 1S integration time, 1 spectrum collected</td>
<td>100nM - 1S integration time, 1 spectrum collected</td>
<td>500nM - 1S integration time, 1 spectrum collected</td>
<td>500nM - 1S integration time, 1 spectrum collected</td>
</tr>
</tbody>
</table>
The data was taken back for analysis. An integration period of 22 ms was used, and 1000 of these integration periods were collected for each sample. Because of the particular Raman spectrometer used, over a 22 ms integration period the intensity at each wavelength was a whole number and corresponded to the “counts” value of the CCD. The CCD counts value represents the number of electrons produced by the incoming photons. The amount of noise made it impossible to see any spectra with such low concentration ranges and short integration periods.

![Figure 21: A Comparison of the Data from One Integration Period (Top), and Data Integrated over 1000 of these Periods (Bottom)](image)

After averaging over all 1000 collection periods, some peaks become visible above the noise floor. The reason that the noise floor shrinks as the number of periods averaged over increase can be explained as follows. There are 1024 spectral bins $b_1, b_2, \ldots, b_{1024}$. Call the CCD count over one collection period at time $t$ for bin $b_i$ $c_{it}$. Call the mean of $c_{it}$ as $t$ varies $\mu_i$ and the standard deviation $\sigma_i$. The central limit theorem tells us that if

$$C_i \overset{\text{def}}{=} \frac{1}{T} \sum_{t=1}^{T} c_{it}$$

Then $C_i$ approaches a normal distribution with mean $\mu_i$ and standard deviation $\frac{\sigma_i}{\sqrt{T}}$. The plot below shows that the variance of a particular bin can vary wildly.
The fact that some bins have very large variances is caused by artifacts that occurred during the data collection. An example of one such artifact which occurred in a collected integration period is shown below, and is likely caused by a cosmic ray hitting the detector.

To remove these artifacts, all counts $c_{lt}$ for which $|c_{lt} - \mu_l| > 10\sigma_l$ were found. For each of these counts we set $c_{lt} = \mu_l$. The new variances are shown below.
It is evident that after the removal of these infrequent artifacts, the variance is practically constant over all wavelengths of the spectrometer. All of the means were then recalculated \( \mu_i \) for this artifact corrected spectra. These post correction \( \mu_i \)'s are plotted below.

This gives us a very accurate picture of what the actual Raman spectra of PBS looks like. You can see a number of distinct peaks caused by molecular resonances. There were no additional peaks in the Raman spectra observed in data collected for samples containing
dopamine or serotonin. The reason that no additional peaks were observed on samples containing dopamine or serotonin is likely because the signal was too small and not visible above the noise floor. A concentration of 100 mM dopamine only produced a peak approximately 3 counts above the background. The next highest concentration collected was 10 uM, meaning that we would expect this peak to be only $3\times 10^{-4} = 0.0003$ counts above the background if the peak height scales linearly with the concentration of the chemical present.

It was decided that more data collections should be made using even higher concentrations of dopamine in an attempt to clearly see the spectra of dopamine. Also, it was discovered that the background of the spectra measured slowly increased over the course of the experiment as if the equipment was “warming up”. Thus in a second data collection process, the concentrations were collected in a random order to account for this time varying property of the background. Five collection sets were acquired, 0-200 nM, 0-1.1 uM, 0-2454.5 uM, and only 2454.5 uM. It was thought that these concentration ranges would produce clearly visible peaks (0.073635 counts above the background at 2454.5 uM). However, as will be shown in the results section, no peaks were visible.

## Chapter 4: Results

We sought to test and compare the performance of a univariate model (which actually consists of two models, a model which only predicts dopamine and a model which only predicts serotonin) and a multivariate model (consisting of one model which predicts both neurotransmitters simultaneously) in predicting solutions of dopamine and serotonin.

### 4.1 Cross Validation

The data was collected on five probes. For each of these five probes there were 81 concentrations of dopamine collected, 81 concentrations of serotonin, 6 pH ranges, and 41 collections with a random mixture of dopamine and serotonin with a random pH level, for a total of 209 trials for each probe.

A technique known as cross-validation was used to assess the performance of each model type. To perform cross-validation the dataset was partitioned into three sets, training, validation, and testing. Three probes were placed into the training set and used to generate the models. One of the remaining two probes not used for training was used for validation. The alpha and lambda parameters of the model were varied to produce the minimum prediction error on the validation probe. Finally, the model along with the alpha and lambda value were used to make predictions on the test probe and see how well the model performed. This method of cross validation does a good job of predicting how well the models will perform on new data because the test probe was not used create the model.

With 5 probes there are 20 ways the data can be partitioned into training, validation, and testing. For each of the 5 probes chosen as the test probe, there are 4 remaining probes that can be chosen for the validation probe. We evaluated the performance of the univariate and multivariate models over all 20 partitions and calculate the average performance of each type of model.

### 4.2 Univariate Model
The univariate models are actually two models (one for dopamine and one for serotonin). Each model was trained over a grid of alpha values (0 to 1 in increments of 0.1) and lambda values (0 to 100 in increments of 1). For each of the training probes (starting with 1 in Figure 26) we show the predictions of dopamine and serotonin as well as the beta vector generated for each of the 4 possible validation probes. In the figure showing predictions you can see how the probe chosen to validate on affected the predictions. Also, two plots which show how the models confused dopamine and serotonin are shown. The first of these confusion plots shows what dopamine predictions look like for solutions in which there was no dopamine, only an increasing amount of serotonin. The second confusion plot shows the same thing, but for serotonin predictions.

<table>
<thead>
<tr>
<th>Testing on Probe 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Alpha: 0.675</td>
</tr>
<tr>
<td>Average Lambda: 53.5</td>
</tr>
<tr>
<td>Variance Alpha: 0.188</td>
</tr>
<tr>
<td>Variance Lambda: 2558.8</td>
</tr>
</tbody>
</table>

In Figure 26 you can see how the univariate models did at predicting probe 1. The dopamine predictions have a positive offset of around 1000-2000 nM, and both dopamine and serotonin predictions appear to have a smaller slope than the actual slope of the prepared concentrations. In Figure 27 you can see that the beta vectors are all fairly similar, and they are also quite sparse.

![Figure 26: Probe 1 Predictions](image-url)
In Figure 28 you can see that most of the models seem to interpret an increase in serotonin levels as a decrease in dopamine levels. Also, in Figure 29 you can see that an increase in dopamine is causes an increase in serotonin predictions at low dopamine concentrations and a decrease in serotonin predictions at high dopamine concentrations.
In Figure 30 it is evident that the vertical offset is much lower than for probe 1. Also, most of the predictions appear to be very accurate, though there is a lot of variation. For both dopamine and serotonin, validating on probe 1 seemed to produce the worst predictions. In Figure 31 you can see that the beta vector produced by validating on probe 1 has many more nonzero entries than the other beta vectors.
Confusion plots for dopamine predictions (Figure 32) and serotonin predictions (Figure 33) on probe 2 show that as the alternate chemical varies concentration, the predicted chemical varies little. This means that these predictions are less likely to confuse a change in dopamine for a change in serotonin and vice versa.
Testing on Probe 3
Average Alpha: 0.55
Average Lambda: 62
Variance Alpha: 0.173
Variance Lambda: 2336.3

Testing on probe 3 produced very poor results. You can see that the dopamine predictions hardly changed at all as the actual concentration varied in Figure 34. The serotonin predictions...
varied more, but there was a large offset error. The betas in Figure 35 are also very noisy, with many nonzero entries.

The confusion plots in Figure 36 and Figure 37 indicate the models do not confuse the chemicals easily, but this is just because the output of the models is always very close to 0 regardless of the actual concentration.
Figure 36: Increasing Serotonin Confusion Plot

Figure 37: Increasing Dopamine Confusion Plot

<table>
<thead>
<tr>
<th>Testing on Probe 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Alpha: 0.925</td>
</tr>
<tr>
<td>Average Lambda: 49.5</td>
</tr>
<tr>
<td>Variance Alpha: 0.1880</td>
</tr>
<tr>
<td>Variance Lambda: 1666.3</td>
</tr>
</tbody>
</table>

The predictions on probe 4 are extremely good. In Figure 38 there is almost no vertical offset error, and the slope is very close to the true concentration slope. The beta vectors in Figure 39 all
look very similar, with peaks corresponding the oxidation and reduction regions of the voltammogram.

The confusion plots in Figure 40 and in Figure 41 also show the near ideal behavior of this mode. There is a clear distinction between the two chemicals.

Figure 38: Probe 4 Predictions

Figure 39: Betas used to Predict Probe 4
Figure 40: Increasing Serotonin Confusion Plot

Figure 41: Increasing Dopamine Confusion Plot

<table>
<thead>
<tr>
<th>Testing on Probe 5</th>
<th>Average Alpha: 0.575</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average Lambda: 64.75</td>
</tr>
<tr>
<td></td>
<td>Variance Alpha: 0.1092</td>
</tr>
<tr>
<td></td>
<td>Variance Lambda: 2236.9</td>
</tr>
</tbody>
</table>
The predictions on probe 5 are also good, but not as good as those for probe 4. In Figure 42 it is apparent that the predictions validated with probe 3 are an outlier, the rest are very similar. In Figure 43 it is evident that the betas of the prediction with probe 3 being used to validate are also very different, with almost all nonzero values and huge amplitudes.

Figure 42: Probe 5 Predictions

Figure 43: Betas used to Predict Probe 5

The confusion plots of Figure 44 and Figure 45 show that all models have good specificity, though there is a strange peak in the serotonin predictions near trial 10 for the serotonin confusion plot.
In Table 3 you can see a summary of the univariate model performance. Ccoef DA is the Pearson correlation coefficient between the actual dopamine concentration and the predicted concentration for the test probe averaged over all possible validation/training probe combinations. RMS DA represents the root mean squared error between the actual dopamine...
concentrations and the predicted concentrations. Noise DA is the variance of predicted dopamine as the pH level is changed with no dopamine or serotonin present. %Var exp Da is the percent of variation in the actual level of dopamine explained by the predicted dopamine levels. The corresponding parameters for 5HT are defined in the same way.

Table 3: Summary of Univariate Model Performance

<table>
<thead>
<tr>
<th>Test Probe</th>
<th>Ccoef DA</th>
<th>Ccoef 5HT</th>
<th>RMS DA (nM)</th>
<th>RMS 5HT (nM)</th>
<th>Noise DA (nM^2)</th>
<th>Noise 5HT (nM^2)</th>
<th>%var exp DA</th>
<th>% var exp 5HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9502</td>
<td>0.9058</td>
<td>1363.3</td>
<td>1669.0</td>
<td>1.4442e+05</td>
<td>2.3165e+05</td>
<td>0.7628</td>
<td>0.6724</td>
</tr>
<tr>
<td>2</td>
<td>0.9409</td>
<td>0.9720</td>
<td>1905.7</td>
<td>826.5</td>
<td>3.6860e+05</td>
<td>1.3306e+06</td>
<td>0.5298</td>
<td>0.8965</td>
</tr>
<tr>
<td>3</td>
<td>0.9103</td>
<td>0.9512</td>
<td>2512.7</td>
<td>2160.5</td>
<td>5.9938e+04</td>
<td>2.2164e+05</td>
<td>0.3660</td>
<td>0.4561</td>
</tr>
<tr>
<td>4</td>
<td>0.9681</td>
<td>0.9707</td>
<td>764.4</td>
<td>668.9025</td>
<td>9.8329e+05</td>
<td>7.7320e+05</td>
<td>0.9295</td>
<td>0.9413</td>
</tr>
<tr>
<td>5</td>
<td>0.9607</td>
<td>0.8635</td>
<td>1474.2</td>
<td>1767.7</td>
<td>3.0129e+06</td>
<td>9.4110e+06</td>
<td>0.8093</td>
<td>0.6843</td>
</tr>
<tr>
<td>Average</td>
<td>0.94604</td>
<td>0.93264</td>
<td>1604.053</td>
<td>1418.52</td>
<td>9.1382e+05</td>
<td>2.3936e+06</td>
<td>0.67948</td>
<td>0.73012</td>
</tr>
</tbody>
</table>

Multivariate Model

The multivariate model performance was evaluated next. This model tries to minimize the squared dopamine error and the squared serotonin error simultaneously.

<table>
<thead>
<tr>
<th>Testing on Probe 1</th>
<th>Average Alpha: 0.85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Lambda: 31.25</td>
<td>Variance Alpha: 0.1870</td>
</tr>
<tr>
<td>Variance Lambda: 1601.5</td>
<td></td>
</tr>
</tbody>
</table>

In Figure 46 you can see that the multivariate model had similar performance in predicting probe 1 as did the univariate model. It does appear that the predictions of the multivariate model have smaller spikes though. Figure 47 shows the beta vectors for the multivariate models which look similar to the univariate betas. However, if the multivariate model sets a beta to a nonzero value for dopamine, it must do the same for serotonin because of the algorithm used.
In Figure 48 and Figure 49 you can see that the confusion plots for the multivariate model are almost identical to the univariate model confusion plots for probe 1.
Figure 48: Increasing Serotonin Confusion Plot

Testing on Probe 2
Average Alpha: 0.55
Average Lambda: 5.25
The predictions for probe 2 are better than the predictions for probe 1, except when validation probe 1 is used as shown in Figure 50. The betas shown in Figure 51 have a very smooth appearance that is not very sparse except for the betas for validation on probe 1 which are very spiky looking.

Figure 50: Probe 2 Predictions

Figure 51: Betas used to Predict Probe 2
The confusion plots in Figure 52 and Figure 53 show that at low concentrations the models have poor specificity, but the specificity improves as the concentration of the chemical of interest increases.

**Figure 52: Increasing Serotonin Confusion Plot**

**Figure 53: Increasing Dopamine Confusion Plot**

[Testing on Probe 3]
The predictions for probe 3 are very poor, as they are for the univariate model. Figure 54 shows the poor sensitivity of dopamine predictions and the offset of the serotonin predictions. Figure 55 shows that the betas are also quite noisy looking in the sense that the location of nonzero elements appear to be somewhat random.

Figure 54: Probe 3 predictions

Figure 55: Betas used to Predict Probe 3
The dopamine predictions have a slightly negative slope as the serotonin concentrations increase as can be seen in Figure 56. The serotonin predictions in Figure 57 are fairly flat, but have a vertical offset.

**Figure 56: Increasing Serotonin Confusion Plot**
Figure 57: Increasing Dopamine Confusion Plot

Testing on Probe 4
Average Alpha: 0.675
Average Lambda: 46.25
Variance Alpha: 0.2580
Variance Lambda: 2431.5
The predictions on probe 4 are very good for the multivariate model as can be seen Figure 58. The betas are also very clean looking as can be seen in Figure 59. It appears that there are two large positive peaks in the first half of the waveform for serotonin and only one for dopamine.

![Figure 58: Probe 4 Predictions](image1)

![Figure 59: Betas used to Predict Probe 4](image2)
The confusion plots in Figure 60 and Figure 61 show that despite a few horizontal offsets, the dopamine predictions are not heavily affected by the serotonin concentrations and the serotonin concentrations are not heavily affected by the dopamine concentrations.

Figure 60: Increasing Serotonin Confusion Plot
Figure 61: Increasing Dopamine Confusion Plot

<table>
<thead>
<tr>
<th>Testing on Probe 5</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Alpha: 0.575</td>
<td></td>
</tr>
<tr>
<td>Average Lambda: 2.25</td>
<td></td>
</tr>
<tr>
<td>Variance Alpha: 0.1825</td>
<td></td>
</tr>
<tr>
<td>Variance Lambda: 2.250</td>
<td></td>
</tr>
</tbody>
</table>
The predictions for probe 5 are shown in Figure 62 and the betas are shown in Figure 63. The predictions look fairly accurate, as well as the betas except for when probe 3 is used for validation.

![Figure 62: Probe 5 Predictions](image1)

![Figure 63: Betas used to Predict Probe 5](image2)

The confusion plots shown in Figure 64 and Figure 65 show that all models have good specificity. Even the predictions when validating on probe 3 are have fairly good specificity.
In Table 4 we have listed the summary of the multivariate model performance. These are the same parameters are reported for the univariate model performance.
Table 4: Summary of Multivariate Model Performance

<table>
<thead>
<tr>
<th>Test Probe</th>
<th>Ccoef DA</th>
<th>Ccoef 5HT</th>
<th>RMS DA (nM)</th>
<th>RMS 5HT (nM)</th>
<th>Noise DA (nM^2)</th>
<th>Noise 5HT (nM^2)</th>
<th>% var exp DA</th>
<th>% var exp 5HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9673</td>
<td>0.9244</td>
<td>1369.4</td>
<td>1659.2</td>
<td>2.1555e+04</td>
<td>1.8704e+05</td>
<td>0.7471</td>
<td>0.6500</td>
</tr>
<tr>
<td>2</td>
<td>0.9445</td>
<td>0.9734</td>
<td>18043</td>
<td>1012.3</td>
<td>4.3936e+05</td>
<td>5.1064e+05</td>
<td>0.5607</td>
<td>0.8708</td>
</tr>
<tr>
<td>3</td>
<td>0.9191</td>
<td>0.9437</td>
<td>2395.1</td>
<td>2176.4</td>
<td>2.4672e+04</td>
<td>2.6905e+05</td>
<td>0.3615</td>
<td>0.2414</td>
</tr>
<tr>
<td>4</td>
<td>0.9702</td>
<td>0.9725</td>
<td>754.9904</td>
<td>699.0870</td>
<td>1.3479e+06</td>
<td>5.7749e+05</td>
<td>0.9313</td>
<td>0.9379</td>
</tr>
<tr>
<td>5</td>
<td>0.9611</td>
<td>0.8689</td>
<td>1467.3</td>
<td>2266.6</td>
<td>1.5914e+06</td>
<td>1.4071e+06</td>
<td>0.7205</td>
<td>0.6779</td>
</tr>
<tr>
<td>Average</td>
<td>0.95244</td>
<td>0.93658</td>
<td>1558.218</td>
<td>15627.2</td>
<td>6.9909e+05</td>
<td>5.9026e+05</td>
<td>0.66422</td>
<td>0.7156</td>
</tr>
</tbody>
</table>

Looking at the predictions of the multivariate and univariate models we can see that the performance of the two is almost identical in our test with the multivariate model having a higher linear correlation coefficient and the univariate model explaining a slightly higher percentage of the variance. Interestingly though, the noise of the multivariate model calculated on the pH only trials is much lower than the univariate model. This is really what sets the two models apart. The multivariate model seems to be less affected by noise factors such as pH as shown in Figure 66.

![Figure 66: Comparison of Noise Model Sensitivity Analysis](image)

The next analysis performed was intended to determine the relative sensitivity of the univariate and multivariate models. To determine this 10%, 50%, and then 90% of the betas were zeroed and then used to make predictions. It was also desired to see how the sensitivity of these models varied as a function of the concentration of chemical present. Therefore, the concentration ranges were divided into low (where concentrations of dopamine or serotonin were...
from 100 to 1000 uM), medium (where concentrations of dopamine or serotonin were from 1100 to 4000 uM), and high (where concentrations of dopamine or serotonin are at least 4100 uM).

**Univariate Model Performance**

### 10% zeroed

<table>
<thead>
<tr>
<th></th>
<th>Ccoef DA</th>
<th>Ccoef 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low (100-1000 uM)</strong></td>
<td>0.1116</td>
<td>0.1917</td>
</tr>
<tr>
<td><strong>Medium (1100-4000 uM)</strong></td>
<td>0.9220</td>
<td>0.3354</td>
</tr>
<tr>
<td><strong>High (4100-8000 uM)</strong></td>
<td>0.5769</td>
<td>0.5160</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>RMS DA</th>
<th>RMS 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low (100-1000 uM)</strong></td>
<td>7.3402e+03</td>
<td>2.0802e+04</td>
</tr>
<tr>
<td><strong>Medium (1100-4000 uM)</strong></td>
<td>7.3019e+03</td>
<td>2.2392e+04</td>
</tr>
<tr>
<td><strong>High (4100-8000 uM)</strong></td>
<td>7.7071e+03</td>
<td>2.2701e+04</td>
</tr>
</tbody>
</table>

### 50% zeroed

<table>
<thead>
<tr>
<th></th>
<th>Ccoef DA</th>
<th>Ccoef 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low (100-1000 uM)</strong></td>
<td>0.2349</td>
<td>0.0676</td>
</tr>
<tr>
<td><strong>Medium (1100-4000 uM)</strong></td>
<td>0.7088</td>
<td>0.6050</td>
</tr>
<tr>
<td><strong>High (4100-8000 uM)</strong></td>
<td>0.2794</td>
<td>0.8359</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>RMS DA</th>
<th>RMS 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low (100-1000 uM)</strong></td>
<td>3.4374e+04</td>
<td>1.6591e+04</td>
</tr>
<tr>
<td><strong>Medium (1100-4000 uM)</strong></td>
<td>3.4292e+04</td>
<td>1.7342e+04</td>
</tr>
<tr>
<td><strong>High (4100-8000 uM)</strong></td>
<td>3.2731e+04</td>
<td>1.8377e+04</td>
</tr>
</tbody>
</table>

### 90% zeroed

<table>
<thead>
<tr>
<th></th>
<th>Ccoef DA</th>
<th>Ccoef 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low (100-1000 uM)</strong></td>
<td>-0.0051</td>
<td>-0.1646</td>
</tr>
<tr>
<td><strong>Medium (1100-4000 uM)</strong></td>
<td>0.7171</td>
<td>-0.2188</td>
</tr>
<tr>
<td><strong>High (4100-8000 uM)</strong></td>
<td>0.7588</td>
<td>-0.5498</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>RMS DA</th>
<th>RMS 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low (100-1000 uM)</strong></td>
<td>9.0627e+03</td>
<td>2.1827e+04</td>
</tr>
<tr>
<td><strong>Medium (1100-4000 uM)</strong></td>
<td>7.9866e+03</td>
<td>2.4450e+04</td>
</tr>
<tr>
<td><strong>High (4100-8000 uM)</strong></td>
<td>6.3909e+03</td>
<td>2.8570e+04</td>
</tr>
</tbody>
</table>

**Multivariate Model Performance**

### 10% zeroed

<table>
<thead>
<tr>
<th></th>
<th>Ccoef DA</th>
<th>Ccoef 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low (100-1000 uM)</strong></td>
<td>-0.0156</td>
<td>0.1516</td>
</tr>
<tr>
<td><strong>Medium (1100-4000 uM)</strong></td>
<td>0.9570</td>
<td>0.2101</td>
</tr>
<tr>
<td><strong>High (4100-8000 uM)</strong></td>
<td>0.6487</td>
<td>0.5377</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>RMS DA</th>
<th>RMS 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low (100-1000 uM)</strong></td>
<td>4.0006e+03</td>
<td>1.8082e+03</td>
</tr>
</tbody>
</table>
The preceding sensitivity analysis revealed that at some point zeroing more of the betas does not further increase the RMS error. This is because the mean offset of the model is not included in the betas which can be zeroed. When all of the other betas are zeroed the predictions will just be a constant equal to the mean of the training data. Figure 67 shows that the RMS error decreased for the univariate DA and the multivariate 5HT when the percentage was increased from 50% zeroed to 90%.
Simulated Raman Spectroscopy Data

As stated before, there was no visible signal from dopamine or serotonin the data collected. It is unclear why this occurred, as the highest concentrations data was collected on should have in theory produced visible peaks. In the paper titled “Detection and Monitoring of Neurotransmitters - a Spectroscopic Analysis” [38] Raman spectroscopy is performed on dopamine, serotonin, and adenosine. An excitation wavelength of 532 nm was used. 100 μM concentrations were prepared of each. There are several peaks visible in the measured spectra of each chemical from this study. Even taking into account the fact that signals are approximately 4.7 times greater at 532 nm than they are at 785 nm, it would seem that there should be peaks seen at the high concentration ranges our data was collected at as well.

It is unlikely that the wrong concentration was mixed and placed into the quartz flow cell. A more likely cause is that either the focal plane of the microscope wasn’t aligned with the interior of the flow cell causing the spectra of the quartz flow cell to be seen instead of the spectra of the solution inside of the flow cell, or that the Raman scattering was weaker at this wavelength than expected from theory.

Whatever the cause, no data allowing the spectra of dopamine or serotonin to be determined was obtained from the Raman data collection at Virginia Tech. Therefore, in order to try out the elastic net algorithm on Raman spectroscopy data, it was necessary to make simulated Raman spectra. To make these simulated Raman spectra, a model very similar to that of the generative model made for FSCV data was used. It was assumed that the total observed spectra was equal to the background spectra, the AWGN noise, and the spectra from dopamine and serotonin. The spectra from dopamine and serotonin was assumed to be proportional to the concentration of each.
There were a few important differences however between the two models. For one, the background current was not assumed to vary for the Raman model as it was with the FSCV model. This assumption appears to be fairly accurate based on the Raman data collected. There is a horizontal shift in the background which slowly varied during the course of the experiment, but the actual shape of the background spectra does not appear to vary. The height of the peaks was based off the results presented by Manciu et al. [38]. The spectral contribution of 1uM dopamine is shown in Figure 69 and the contribution of 1uM serotonin is shown in Figure 70.
Using the above spectra for dopamine and serotonin, the simulated data was generated in three concentration ranges spanning 3 orders of magnitude.

- Low – 0, 0.05uM, 0.25uM, and 0.5uM
- Medium – 0, 0.5uM, 2.5uM, and 5uM
- High – 0, 5uM, 25uM, and 50uM

In the following set of figures you can see what a simulated spectra for the low, medium, and high concentration ranges would look like (i.e. no visible peaks). The dopamine and serotonin spectra are scaled according to the amount of each present and then noise and the background PBC spectra are added in.

Low (dopamine on top, serotonin on bottom) - 0.5uM spectra
Figure 71: 0.5uM Dopamine Spectra

Figure 72: 0.5uM Serotonin Spectra

Medium (dopamine on left, serotonin on right) - 5uM spectra

Figure 73: 5uM Dopamine Spectra
Now we use these simulated Raman spectra to train univariate models and multivariate models and see how they perform at low, medium, and high concentration ranges.

**Univariate Model**
The univariate model was trained first. You can see the performance of this model both by examining the betas and the table.

Low – 0, 0.05uM, 0.25uM, and 0.5uM
The best alpha was found to be 0 (corresponding to a pure $l_1$ norm penalty) through cross validation and the best lambda was found to be 1.927. At these low concentration ranges, the model fails to identify any of the Raman peaks at all is thus unable to predict concentrations at these low ranges.

![Image](image1.png)

**Figure 77: Low Concentration Dopamine Beta**

![Image](image2.png)

**Figure 78: Low Concentration Serotonin Beta**

Medium - 0, 0.5uM, 2.5uM, and 5uM
The best alpha was found to be 0.4 through cross validation and the best lambda was found to be 0.1549. At these concentration ranges the model appears to find more than half of the Raman spectral peaks present in the signal.
High - 0, 5uM, 25uM, and 50uM
The best alpha was found to be 0 and the best lambda was found to be 37.479. At this highest range the model is able to detect practically all of the Raman spectral peaks.
The univariate performance results are summarized in the following tables. These tables describe the same Ccoef and RMS parameters as were used with the fast-scan cyclic voltammetry section. The cross correlation coefficient is also reported, with Cross Correlation Coefficient DA/5-HT representing the correlation between dopamine predictions and the actual value of serotonin. The Cross Correlation Coefficient 5-HT/DA represents the correlation between serotonin predictions and the actual value of dopamine present.

<table>
<thead>
<tr>
<th></th>
<th>Ccoef DA</th>
<th>Ccoef 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>-0.5639</td>
<td>-0.0226</td>
</tr>
<tr>
<td>Medium</td>
<td>0.8808</td>
<td>0.9536</td>
</tr>
<tr>
<td>High</td>
<td>0.9993</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Cross Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA/5-HT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cross Correlation Coefficient 5-HT/DA</td>
</tr>
<tr>
<td></td>
<td>RMS DA</td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
</tr>
<tr>
<td>Low</td>
<td>0.1819</td>
</tr>
<tr>
<td>Medium</td>
<td>1.0104</td>
</tr>
<tr>
<td>High</td>
<td>1.1152</td>
</tr>
</tbody>
</table>

**Multivariate Model**

Low – 0, 0.05uM, 0.25uM, and 0.5uM
The best alpha was 0 and the best lambda was 4.564 as determined by within sample leave one out cross validation.

![Figure 83: Low Concentration Dopamine Beta](image1)

![Figure 84: Low Concentration Serotonin Beta](image2)

Medium – 0, 0.5uM, 2.5uM, and 50uM
The best alpha was 1.0 and the best lambda was 0.1.660.
Figure 85: Medium Concentration Dopamine Beta

Figure 86: Medium Concentration Serotonin Beta

Figure 87: High Concentration Dopamine Beta

High – 0, 0.5uM, 2.5uM, and 50uM
The best alpha was 0.8 and the best lambda was 0.381.
As can be seen in Figure 89, the univariate and multivariate models again have similar performance characteristics. It seems that especially with this simulated Raman spectroscopy data the transition from a readable signal to one too corrupted by noise is very fast.
Chapter 5: Conclusion

This work extends the work on fast-scan cyclic voltammetry by testing whether mixtures of two substances with similar oxidation and reduction profiles can be recovered using a supervised learning approach. Previous studies only reported relative changes in dopamine by observing a qualitative shift in the oxidation and reduction curves. I have shown that it is possible to obtain quantitative concentrations of dopamine and serotonin at the same time with only one measurement with certain error tolerances.

5.1 Summary of work

Monoamine neurotransmitters such as dopamine and serotonin have been extensively studied in animals and humans, however their exact role and function is still poorly understood. For the first time, fast scan cyclic voltammetry measurements have been recorded from the striatum of human patients who were playing a probabilistic decision making task. These measurements contain information about the release of neurotransmitters including dopamine and serotonin that have never been studied with such high time resolution in humans. I developed, advanced signal processing and machine learning techniques to test whether this sort of patient data could be interrogated for concentration transients of both dopamine and serotonin.

Confocal Raman spectroscopy data was collected on prepared samples of dopamine and serotonin in vitro. This data revealed that very high concentrations of both are necessary to be detected with standard Raman spectroscopy. Also, the same elastic net signal reconstruction and regression techniques that were applied to the FSCV data were applied to the Raman data. With
simulated data, it was shown that these techniques are able to determine individual Raman spectra from a noisy mixture of sources and convert a Raman spectrum into concentrations of each mixture component present.

The most commonly chosen value of alpha was 1.0 for both fast-scan cyclic voltammetry data as well as Raman spectroscopy data as determined by cross validation. This corresponds to the Lasso regression analysis method. A few alphas were found to be lower values, but most were still near 1.0. This is likely caused by the fact that both signals are sparse and alpha values close to 1.0 force more of the beta elements to be 0.

The performance of the univariate and multivariate models was very similar on the fast-scan cyclic voltammetry data. However, the multivariate model appeared to reduce the signal noise caused by pH changes.

5.2 Future Directions

In the article Fourier transform Raman spectroscopy [42] published in 1986, the author states that one of the biggest problem with the routine application of Raman spectroscopy is the interference caused by fluorescence of impurities or of the sample itself. One solution is to use long-wavelength near-infrared lasers such as a Nd/YAG laser operating at 1.064 um. The longer wavelength means that fluorescence is drastically reduced. However, two new problems arise. First, the Raman signal at this longer wavelength is approximately 1/16 of the signal present at 514.5 nm because it decreases in proportion to the fourth power of the wavelength. Also, available detectors in this region are much noisier than photomultipliers. Eight years later Mizuno et al. [43] used Fourier transform Raman spectroscopy to obtain high quality Raman spectra of human brain tissues and several kinds of brain tumors. The authors mention that Raman spectroscopy is one the most powerful non-invasive probes for investigating biological materials at the molecular level. They mention two major disadvantages of Raman spectroscopy, interference by fluorescence of the tissue (mentioned in the 1986 article above) and the decomposition of biological materials caused by the heating of the laser beam. They decide to use Fourier transform Raman spectroscopy because it overcomes the mentioned two disadvantages. Acceptable spectra were obtained in the range of 400-2970 cm^-1. In the article by Katz et al. [44], a method known as compressed sensing is applied to Fourier-transform Raman spectroscopy. The compressed sensing techniques they apply allow for a considerable reduction in the acquisition time. They demonstrate the technique on coherent anti-Stokes Raman scattering spectroscopy and show that it is possible to resolve sparse vibrational spectra using <25% of the Nyquist rate samples in single pulse CARS experiments. They say that in FT spectroscopy, a set of N equally spaced time-domain measurements are made. Raman spectra are sparse, which means that compressed sensing techniques can be applied to reduce the number of samples necessary. The CARS data they looked at was composed of N=281 equally spaced measurements. They chose M<N points at random and used them as the input for a CS reconstruction algorithm. They found that M~>60 samples was all that was needed to accurately resolve the Raman lines. Therefore, it is proposed that compressed sensing and Fourier transform Raman spectroscopy be combined to make a probe capable of measuring neurochemical levels in vivo. The probe would operate in the infrared region of 1.064 um allowing for the fluorescence of brain tissue to be almost completely eliminated. Because detectors are difficult to manufacture which are sensitive to this long wavelength, Fourier transform Raman could be used to allow a single element detector to be used instead of the detector arrays and CCD arrays needed for traditional spectrometers. To reduce collection time to sub-second timescales, compressed
sensing techniques would be used to sample the Raman spectra over a small subset of randomly spaced Fourier coefficients. L1-norm reconstruction techniques could then be used to recreate the Raman spectra from a very small number of Fourier components. Raman spectra are perfect for compressed sensing strategies because the spectra are very sparse. Except for shifts at a few discrete molecular resonances, the Raman spectra are zero at most wavenumbers.

References


