THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF VETERINARY AND BIOMEDICAL SCIENCES

# INVESTIGATING DOPAMINE UPTAKE IN MOUSE BRAIN SYNAPTOSOMES VIA CHRONOAMPEROMETRY

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Spring 2010

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biology
with honors in Veterinary and Biomedical Sciences

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## Abstract

Dopamine and serotonin are monoamine neurotransmitters implicated in a suite of physiological functions. Although both serotonin transporters (SERT) and dopamine transporters (DAT) show high affinity for their preferred substrates, recent research has shown that each transporter is not entirely selective, with both allowing some promiscuity of transport (Faraj *et al.,* 1994; Pan *et al.,* 2001; Schmidt and Lovenberg, 1985; Baganz *et al.,* 2008). The serotonin and dopamine neurotransmitter systems have been shown to play overlapping roles in many physiological functions (Murphy *et al.,* 1998; Torres *et al.,* 2003). For example, recent research has shown that drug reward pathways and locomotor behavioral pathways depend on synergy between the serotonin and dopamine neurotransmission systems, as opposed to being solely controlled by the dopamine system, as was once thought.

The goal of this thesis research was to gather preliminary data concerning additional interactions between these systems. Homogenized frontal cortex and striatal brain tissue from mice lacking one functional copy of the serotonin transporter gene (SERT +/- mice) was used to investigate dopamine uptake monitored via chronoamperometry. However, dopamine uptake was difficult to characterize, and was observed in only a few of the synaptosomal preparations. In frontal cortex synaptosomes, four preparations were deemed to have successful uptake. The average dopamine uptake rate was 3.6 nmol/g tissue-min after treatment with 1 μM dopamine, with a standard error of 0.6 nmol/g tissue-min. Striatal synaptosomes had a similarly low success rate, with two solutions showing

successful uptake. These two preparations were treated with different concentrations of dopamine, so no average rate of uptake was calculated. One synaptosomal solution treated with 1 μM showed an uptake rate of 5.0 nmol/g tissue-min, while the solution treated with 1.5 μM dopamine showed an uptake rate of 1.9 nmol/g tissue-min. In the future, similar dopamine uptake experiments will continue to be performed in brain tissue from SERT +/- mice, as well as with tissue from SERT +/+ and SERT -/- mice. The ultimate goal of this project is to determine the ability of SERT to modify dopamine uptake by either promiscuous uptake of dopamine or by compensatory alterations in DAT expression. Furthermore, this study will also seek to determine whether dopamine that has been cleared from solution is lost from synaptosomes during the process of vacuum filtration.

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## Acknowledgements

First, I would like to thank my thesis advisor, Anne Andrews, for allowing me to join her neuroscience group in September of 2008. Thank you for taking a chance on me and allowing me to work in your laboratory for these past two years. Your guidance has helped me to grow as both a scientist and a student.

Also, I would like to extend a special thanks to Brendan Beikmann for guiding all of my research work and being a constant source of help throughout my entire tenure in the Andrews Group. In addition, thanks to other members of the lab including Tracy Gilman, Yogesh Singh, Stephanie Altieri, Jason Ochroch, Daniel Ramos, and Lauren Sawarynski for providing me with periodic advice and assistance.

Finally, I would not have any of the opportunities I have today without my parents, Ed and Bonnie, and my brother, Gary. I am very fortunate to have such an incredible and loving family. Thank you for your unwavering support, even in light of the crazy things I do.

# Introduction

Monoamine neurotransmitters are molecules that are characterized by the presence of one amino group connected by a two-carbon chain to an aromatic ring structure (Hyland, 2008). One common monoamine neurotransmitter is 5-hydroxytryptamine (5-HT), more commonly referred to as serotonin, which is found in the enterochromaffin cells of the gastrointestinal tract (Ormsbee and Fondacaro, 1985; Schofield *et al.,* 1967), in blood platelets (thrombocytes) (Vatassery *et al.*, 1981), in lymphocytes (Gordon and Barnes, 2003), and in the central nervous system (CNS) (Coleman, 1973).

Serotonin is synthesized in a biochemical pathway beginning with the essential amino acid L-tryptophan. This precursor is converted to 5-hydroxy-L-tryptophan (5-HTP) by the enzyme tryptophan hydroxylase (L-tryptophan-5-monooxygenase), which is the rate-limiting step in this pathway (Leathwood, 1987). 5-hydroxy-L-tryptophan is then converted to serotonin by the enzyme 5-hydroxytryptophan decarboxylase (aromatic L-amino acid decarboxylase) (Kizer and Chan, 1963). The major product of the breakdown of serotonin is 5-hydroxyindoleacetic acid (5-HIAA), which is generally excreted in the urine (de Jong *et al.,* 1983). This breakdown is catalyzed by monoamine oxidase (MAO), which is generally found bound to outer mitochondrial membranes (Sabol *et al.,* 1998), and aldehyde dehydrogenase, which is found in both the cytosol and mitochondria (Crabb, 2004).

The importance of serotonin continues to grow in light of new research showing that it is functionally relevant in all major bodily organ systems. More specifically, the versatility of serotonin is exemplified by the suite of physiological functions it is involved

in, such as the regulation of mood and anxiety (Lesch, *et al.,* 1996), blood clotting and hemostasis (Dorofeev and Khnychev, 1970), bowel motility (Shepherd, 1963), aggression and impulse control (Zubieta and Alessi, 1993), ejaculatory latency (de Jong, *et al.,* 2006), bladder control (Ramage, 2006), feeding (Tecott and Abdallah, 2003), circadian rhythms (Liu and Borjigin, 2006), memory and learning (McEntee and Crook, 1990), emesis (Minami *et al.,* 2003), endocrine function (Collu *et al.,* 1974), muscle activity (Jeffrey *et al.,* 1991), pain (Sommer, 2004), and sexual behaviors (Murphy *et al.,* 1998). Serotonin has also received much attention in recent years due to the expansion of knowledge suggesting that its modulation is implicated in the treatment of mood and anxiety (Lesch *et al.,* 1996), eating and sleep disorders (Stunkard *et al.,* 2009), substance abuse (Bankson and Yamamoto, 2004), and some neurodegenerative diseases (Morgan *et al.,* 1987), making this neurotransmitter system a key pharmacological target.

Since serotonin plays such an integral role in normal bodily functions, large changes in serotonin levels can be devastating. Mice genetically altered to be deficient in serotonin were found to have breathing difficulties, grew tired easily, were relatively small and weak (Sze *et al,.* 2000), were more aggressive and inattentive to their progeny as mothers (Alenina *et al.,* 2009), and had decreased longevity compared to mice with normal levels of serotonin, often dying of heart failure (Cote *et al.,* 2003). In humans, it has been postulated that insufficient brain serotonin levels may cause sudden infant death syndrome (SIDS), the leading cause of infant mortality in the United States (Patterson *et al.,* 2006). However, an excess of serotonin activity can cause serotonin syndrome (serotonin toxicity), which is characterized by involuntary muscular contraction or twitching (Sternbach, 1991), hyperreflexia and hyperthermia (Gillman, 1999), uncontrollable sweating, heavily dilated

pupils, tachycardia (Hegerl *et al.,* 1998), anxiety, agitation, and confusion (Isbister *et al.* 2007). A common cause of serotonin toxicity is the combination of multiple serotonergic agents with different mechanisms of action. For example, toxicity can be caused by combining monoamine oxidase inhibitors (MAOIs) with selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine (Prozac), fluvoxamine (Luvox), paroxetine (Paxil), escitalopram (Lexapro), or sertraline (Zoloft) (Isbister *et al.,* 2007). Serotonin toxicity is often a surprise medical diagnosis due to the perceived safety of the SSRI class of drugs (Isbister *et al.,* 2004).

The serotonin transporter (SERT or 5-HTT) controls or limits the actions of serotonin. This transporter is an integral membrane protein that contains 12 transmembrane domains. It is part of a larger superfamily of sodium- and chloride-dependent neurotransmitter symporters that also includes transporters of GABA, glycine, norepinephrine, dopamine, betaine, taurine, proline, and creatine (Chen *et al.,* 2004). As such, the main function of SERT is to control the levels of extracellular serotonin by removing it from the extracellular space via sodium-dependent recycling (reuptake) (Murphy *et al.,* 2004). It is encoded by the *SLC6A4* gene, which is located on the 17$^{th}$ chromosome in humans (Gelernter *et al.,* 1995).

The human SERT (*SLC6A4*) gene is modulated by a polymorphic element (alternate DNA base sequence) that is approximately one thousand base pairs upstream of its initiation site in the serotonin-transporter-linked polymorphic region (5-HTTLPR) (Lesch *et al.,* 1996). The polymorphism consists of a 43 base pair insertion, which is referred to as the “l” or long allele, or lack thereof, which is referred to as the “s” or short allele (Lesch *et al.,* 1996). The short variant of this polymorphism has reduced transcriptional efficacy,

which results in decreased expression of SERT (Lesch *et al.,* 1996). Specifically, comparison of the polymorphic alleles revealed that the innate activity of the long allele is approximately three times higher than the SERT promoter with the deletion (Heils *et al.,* 1996). A 40% reduction in SERT is thereby seen in a little less than 70% of the Caucasian human population. These numbers are highly variable between different ethnic groups, though, with the "s" allele being observed at a frequency of 25% in African Americans, 40% in European Americans, and 80% in Japanese (Gelernter *et al.,* 1997; Singh *et al.*, 2010). A reduction in expression has been linked to increased anxiety (Lesch *et al.,* 1996), neuroticism (Golimbet *et al.,* 2001), and stress-related depression (Caspi *et al.,* 2003) in both men and women.

In addition to the aforementioned problems with imbalances of serotonin, other medical problems such as alcoholism (Lovinger, 1999), clinical depression (Owens and Nemeroff, 1994), obsessive-compulsive disorder (OCD) (Insel *et al.,* 1985), and hypertension (Lebrec, 1990) are specifically associated with irregularities in SERT function. Recent research has also shown that social anxiety disorder is associated with a higher affinity of serotonin for SERT in the thalamus and striatum of patients measured by $^{123}$I-β-(4-iodophenyl)-tropane single photon emission computed tomography (SPECT) due to a decreased extracellular serotonin concentration near the transporter, a high amount of SERT, or from a combinatory effect of these two phenomena (Van Der Wee *et al.,* 2008). Furthermore, SERT is a key target for pharmacologic drugs and drugs of abuse. The selective serotonin reuptake inhibitors (SSRIs) are antagonists of SERT, extending the spatial and temporal duration of serotonin in the extracellular fluid by blocking reuptake of this neurotransmitter. Although the mechanism is not completely understood, it is thought

that serotonin remaining in the synaptic cleft for an increased period of time allows for repeated stimulation of the serotonin postsynaptic receptors, and that this in turn improves mood (Gobbi *et al.*, 1997). This group of drugs is the single most frequently prescribed group used to treat depression, OCD, alcoholism, and generalized social anxiety disorder (Murphy *et al*., 2004). Hallucinogenic drugs such as 2,5-dimethoxy-4-iodoamphetamine (DOI) and lysergic acid diethylamide (LSD) have varying effects on the serotonin system including changing intracellular signaling pathways (Wise, 1996), altering neuron firing patterns (Star *et al.,* 2008), and altering gene expression (Nichols and Sanders-Bush, 2001). Hallucinogens have a wide range of effects on the body and are powerful at extremely low doses (Wise, 1996). Other drugs of abuse such as 3-4-methylenedioxymethamphetamine (MDMA or Ecstasy) also target SERT. The link between MDMA and the serotonergic system is exemplified by the fact that mice that have SERT function absent or reduced due to targeted mutagenesis have reduced or absent locomotor stimulation (hyperactivity) in response to MDMA administration (Bengel *et al*., 1998).

Dopamine, much like serotonin, is a monoamine neurotransmitter. It is grouped along with the neurotransmitters epinephrine and norepinephrine into a family called the catecholamines, all of which are water-soluble, contain both a catechol ring and amine side chain, and are derived from tyrosine (Ben-Jonathan and Hnasko, 2001). As a neurotransmitter, dopamine is crucial for organisms to carry out smooth, controlled movements (Kish *et al.,* 1988). Dopamine is also integral to the execution of well-learned movements without having to explicitly think about performing the task, including crucial items like walking and writing (Lange *et al.,* 2006). Degeneration of dopaminergic neurons in the substantia nigra results in Parkinson's disease, which is manifested as a loss of

control of these routine movements (Kish *et al.,* 1988). In addition, the dopaminergic system has been shown to be important in certain cognitive abilities such as memory (Browman *et al*., 2005), attention (Heijtz *et al.,* 2007), and problem solving (Mukhin and Adrianov, 1982). Dopamine is also implicated in the reinforcement of activities (Holroyd and Coles, 2002), reward-seeking behavior (Wise, 2004), and motivation to perform certain activites (Depue and Collins, 1999). This neurotransmitter system enables humans to label objects or events in their environment as pleasurable, such as food and drink, or sexual activity (Giuliano and Allard, 2001). It is hypothesized that the brain gauges it expectations for reward, and when these expectations are surpassed, the firing of some dopaminergic neurons increases, which increases the desire and motivation to repeat these actions in the future (Arias-Carrion and Poppel, 2007). This hypothesis is supported by the actions of the drugs nicotine, cocaine, and amphetamines, all of which directly or indirectly lead to an increase in the firing of dopaminergic neurons in the brain (Spanagel and Weiss, 1999). Unfortunately, long-lasting neuroadaptive mechanisms that develop during habitual drug abuse may lead to the highly addictive nature of the aforementioned drugs, and the relative inability of addicts to abstain from use (Kuhar *et al.,* 1991; Spanagel and Weiss, 1999).

The function of DAT, and to a lesser extent, the other monoamine transporters, can be blocked by cocaine and methamphetamines. However, the mechanisms by which each of these drugs achieves this inhibition is fundamentally different. Cocaine causes a direct blockade of DAT function by binding the transporter and reducing the rate of transport. The tricyclic CNS stimulant mazindol (Sanorex) reduces DAT function in a similar way (Kilty *et al.,* 1991). Amphetamines, on the other hand, exert similar effects by triggering a

signal cascade inside neurons. One hypothesis is that this cascade signals through either protein kinase C (PKC) or MAP kinase pathways, which causes the eventual internalization of DAT, instead of direct binding to the transporter (Kahlig *et al.,* 2004).

As well as functioning as a neurotransmitter, dopamine can also be considered a neurohormone, which is a hormone produced and released by neurons. The main neurohormonal function of dopamine is to inhibit the secretion of prolactin (luteotropic hormone) from the anterior pituitary gland (Agu *et al.,* 1986). Prolactin is an important hormone in the process of lactogenesis (Parsons and Nicoll, 1971), which fills the mother's breast with milk (Neville *et al.,* 2001). Dopamine with this function can be alternately referred to as prolactin-inhibiting factor (PIF), prolactin-inhibiting hormone (PIH), or prolactostatin (Ben-Jonathan and Hnasko, 2001; Parsons and Nicoll, 1971).

Since the dopamine neurotransmission system is so complex, dysfunctions in the system can have broadly ranging, and sometimes devastating, effects. For instance, loss of dopaminergic innervation from the substanatia nigra to the striatum results in the movement disorder known as Parkinson's disease (PD). The common clinical symptoms of Parkinson 's disease are tremors (Krack *et al.,* 2004), bradykinesia (Berardelli *et al.,* 2001), rigidity (Bettucci *et al.,* 1991), postural instability (Morris, 2000), autonomic irregularities (Brooks *et al*., 1990), behavioral and sensory abnormalities (Scott *et al.,* 1984), gait difficulty (Morris, 2000), dystonia (Poewe *et al.,* 1988), and pain (Goetz *et al.,* 1985), all of which become worse as the disease progresses. A keen grasp of these symptoms is important for physicians to have, since there is no definitive test for Parkinson's disease (Jankovic, 2007). Other common pathological states, such as schizophrenia (Swerdlow and Koob, 1987), autism (Ernst *et al.,* 1997), and attention deficit hyperactivity disorder

(ADHD) (Dougherty *et al.,* 2000), are similarly associated with dysfunction in the dopamine neurotransmission system.

The essential amino acid tyrosine (or phenylalanine, which is converted to tyrosine) is the starting point in the synthesis of dopamine. In the first step of this pathway, tyrosine is converted to L-3,4-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase (tyrosine 3-monooxygenase). L-3,4-dihydroxyphenylalanine is then decarboxylated by aromatic L-amino acid decarboxylase (dopa decarboxylase) to form dopamine (Elsworth and Roth, 1997). An important part of this pathway is the fact that L-DOPA, which is also the precursor of epinephrine and norepinephrine, can cross the blood-brain barrier, whereas dopamine cannot. As such, L-DOPA has been used as a therapy to reverse the biological deficits incurred as a result of dopamine-responsive dystonia (DRD) and in Parkinson's disease (Casseron and Genton, 2005; Elsworth and Roth, 1997).

Dopamine is primarily cleared from the extracellular fluid and into neurons by the dopamine transporter (DAT). As such, removal of dopamine by DAT terminates its function. The rate at which dopamine clearance takes place can have profound effects on an organism, as evidenced by the fact that mice lacking DAT have severe cognitive impairment (Miller *et al..* 2001), poor spatial abilities (El-Ghundi *et al.,* 1999), hyperactivity (Gainetdinov *et al.,* 1999), and irregular motor control (Ralph *et al.,* 2001). This phenotype highly resembles the effects of ADHD in humans (Gainetdinov *et al.,* 1999). DAT, along with SERT, is a part of the integral transmembrane superfamily of sodium- and chloride-dependent neurotransmitter symporters. Accordingly, DAT has a high sequence homology (~50%) with SERT (Murphy *et al.,* 2004). The mechanism for reuptake by DAT is by co-transporting two sodium ions and one chloride ion into neurons with dopamine, using the

gradients formed by the sodium/potassium ATPase pump. Although similar to the mechanism of DAT, SERT co-transports serotonin into neurons with only one sodium ion and one chloride ion (Torres *et al*., 2003).

The gene encoding DAT has a variable number tandem repeat (VNTR) DNA sequence, or a sequence where a nucleotide pattern is repeated in adjacent fashion a varying amount of times, towards its 3' end. The varying number of times this sequence repeats has been shown to be associated with the basal expression level of the transporter (Michelhaugh *et al.,* 2001), and has also been associated with several disorders (Miller and Madras, 2002). First, a linkage between bipolar disorder and the highly polymorphic VNTR of DAT has been demonstrated (Greenwood *et al.,* 2006). Specifically, these data show that genetic differences in the regions of exon 9 through exon 15 of the dopamine transporter gene are of particular clinical significance (Greenwood *et al.,* 2001). In addition, research has shown that variability in this genetic sequence can make withdrawal symptoms from alcohol worse in some individuals, making them particularly vulnerable to alcoholism (Sander *et al.,* 1997). Alcoholics experiencing significant withdrawal symptoms showed a markedly higher prevalence of a nine-repeat allele in the region of interest (Sander *et al.,* 1997; Ueno *et al.,* 1999). Finally, a ten-repeat allele of the tandem repeat sequence polymorphism showed a small, but significant, role in individual genetic predisposition to ADHD (Yang *et al.,* 2007).

# Chapter 1. Dopamine Uptake in Frontal Cortex and Striatal Synaptosomes

## Introduction

Synaptosomes (detached synapses) are neuronal liposomes that were discovered in 1958, and have been increasing in popularity as a model for study in cellular biology ever since this time (Whittaker, 1993). Specifically, synaptosomes are isolated nerve terminals (Booth and Clark, 1978). The preparation of functional synaptosomes is generally begun by homogenizing brain tissue, spinal cord tissue, retinal tissue, sympathetic ganglia, or myenteric plexus in a buffering solution that is isoosmotic with the bodily fluid of the animal from which the tissue is derived (Whittaker, 1962). Brain or spinal tissues are generally the most conducive to synaptosomal preparation due to the abundance of nerve terminals and varicosities in these regions (Jonakait *et al.,* 1979; Whittaker, 1993). After homogenization, synaptosomes are purified by multiple steps of fractionation performed by centrifugation (Whittaker, 1962).

Despite the processing of the neural tissue into synaptosome preparations, these structures, if prepared correctly, undergo resealing of the plasma membrane (Whittaker, 1962). Membranes reseal spontaneously to achieve a state of maximal stability in an aqueous environment, with hydrophilic portions of the membrane facing the aqueous intracellular and extracellular environments, and hydrophobic portions of the membrane shielded from interactions with water (Steinhardt *et al.*, 1994). Synaptosomes also retain the ability to respire (Kauppinen and Nicholls, 1986), take up oxygen and nutrients such as glucose (Bradford and Thomas, 2006), maintain a membrane potential by eliminating

intracellular sodium ions while accumulating intracellular potassium ions (Scott and Nicholls, 1980), and produce cellular ATP energy via mitochondria (Lai and Clark, 1976). In addition, these structures still contain the molecular machinery necessary for the release of neurotransmitter in a calcium-dependent fashion (Turner *et al.,* 1993). They can also uptake and store neurotransmitter much like normal neural tissue (Whittaker, 1962). One of the major advantages of using a synaptosome system in research is, therefore, eliminating the complexity of working with synapses *in situ* without the loss of realistic neural communication systems (Whittaker, 1962). Synaptosomes also are advantageous to use because they are relatively easy to prepare in a standard laboratory setting. Additionally, the prevalence of this model system in the scientific literature allows for greater ease when comparing results across different studies and laboratories. Finally, when using synaptosomes to study neurotransmitter uptake, the process of uptake can be analyzed independently of neurotransmitter release and diffusion, allowing for tighter experimental specificity and control not afforded while studying intact tissues (Perez *et al.,* 2007).

However, there are a few disadvantages to this preparation. For instance, a primary disadvantage is in the inability to differentiate between chemical species in intact synaptosomes, as opposed to those from contaminants in the preparation such as free mitochondria, myelin, or membrane fragments (Leblanc, 2002). In addition, distinguishing between neuronal and glial populations has not been possible (Leblanc, 2002). Finally, synaptosomal membranes are weaker than normal cellular plasma membranes (Goodkin and Howard, 1973), which means they must be treated with special care during experiments to not rupture them.

Chronoamperometry, along with constant potential amperometry, differential pulse voltammetry, rotating disk electrode voltammetry, and fast cyclic voltammetry, is one of the most commonly employed analytical electrochemical techniques used to study signaling in neurons (Perez *et al.,* 2007). However, the use of chronoamperometry to measure uptake in synaptosomes, as in this study, is rare. To measure dopamine, a carbon fiber electrode is positioned in a synaptosomal solution and held at a zero resting potential as compared to a Ag/AgCl reference electrode (Gerhardt *et al.,* 1984). A drift current measurement is then taken for 100 ms. Then the potential is stepped to +0.70 V, which exceeds the dopamine oxidation potential, and is held there for 100 ms (Daws and Toney, 2007). This square wave voltage step produces a change in current recorded by the electrode that decays over time (Daws *et al.,* 2005). The initial current charges the electrode and is permitted to decay for 20 ms before the oxidation current is sampled and integrated for 80 ms (Daws and Toney, 2007). The potential is then held at 0 V for 100 ms, which is below the dopamine oxidation potential, causing dopamine to be reduced. Finally, the electrode is allowed to rest for 700 ms to decrease the amount of products that will adsorb to the electrode surface (Dawes and Toney, 2007).

One advantage of chronoamperometry is that the current that is measured by the working electrode is integrated over a long period of time relative to the application of the electrical potential, which gives a more favorable signal to noise ratio when compared to other electrochemical techniques (Faulkner, 1983). Similarly, the high frequency at which recordings are made enhances signal to noise ratios because even small changes in current due to certain chemical species can be detected relative to potentially large background currents (Dawes and Toney, 2007). This high resolution is amenable to measuring small,

but biologically important, changes in neurotransmitter transporter function (Perez and Andrews, 2005).

One issue with the use of chronoamperometry is that the current that is detected by carbon fiber electrodes is not discriminate between the monoamines and their metabolites (Brazell *et al.,* 1987), which leads to overestimated and inaccurate values of current. Similarly, limited information about the identity of the electrolyzed species can be obtained from chronoamperometric measurements (Cheng and McCreery, 1977).

Neurotransmitter uptake kinetics have been assessed by many different methods over the years, and radiochemical analysis has proven to be one of the most prevalent and well-tested. The use of this technique, which involves labeling neurotransmitters with radioisotopes, has been used in studies involving brain synaptosomes (Perez *et al.,* 2006), brain slices (Korpi, 1982), and cell cultures (Enerback and Jarlstedt, 1975). However, recent research has shown that the filtration process used in radiochemical analysis leads to drastically underestimated levels of serotonin uptake in synaptosomes (Perez *et al*., 2006). This inaccuracy was determined experimentally by ensuring complete uptake of serotonin using chronoamperometry and then using a synaptosomal filtration process similar to that of a radiochemical analysis experiment (Perez *et al*., 2006). After filtration and analysis using high performance liquid chromatography (HPLC), it was determined that 75% of the serotonin that was originally present in the synaptosomes was subsequently detected in the filtrate due to relative weakness of the synaptosomal membranes (Perez *et al*., 2006). Additionally, radiochemical methods afford little sensitivity for determining subtle but important alterations in uptake, like that which occurs between SERT +/+ and SERT +/– mice (Perez *et al.,* 2007). For these reasons, the

use of chronoamperometry, rather than radiochemical analysis, was preferred in this study of the uptake of dopamine into synaptosomes.

The areas of the brain that were homogenized in this experiment and eventually prepared into synaptosomes were the frontal cortex and striatum. The frontal cortex is responsible for what is generally termed "executive functions" associated with higher mental processes (Roca *et al.,* 2009). These executive functions include, but are not limited to, choosing between a suite of actions to determine which will be most favorable (Seitz *et al.,* 2000), determining similarities between events or responses (Axelrod *et al.,* 1992), and gauging the response to social reactions to certain behaviors (Eslinger *et al.,* 1992). It has been hypothesized that these executive functions evolved as a cognitive control response to the more elaborate sensory and motor systems that developed in humans and other higher thinking organisms, which provide more flexibility in actions, and, thus, more room for errors (Miller and Cohen, 2001). Damage to this region can have highly variable effects including losses in cognitive flexibility and spontaneity (Benton, 1968), loss of problem solving ability (Channon, 2004), difficulty in speech (Crinion *et al.,* 2003), lack of inhibition and social competence (Konishi *et al.,* 2003), losses in creativity (Carlsson *et al.,* 2000), and motor difficulties (Hernandez *et al.,* 2002). These changes generally lead to marked differences in the personality of the affected individual, but often do not have a noticeable effect on intelligence quotient (IQ) testing results (Stuss *et al.,* 1985). Unfortunately, the frontal cortex is commonly damaged due to its relatively large surface area and placement at the front of the cranium (Levin *et al.,* 1987). The reason for the use of this brain region to study dopamine uptake is because the frontal lobe contains most of the dopaminergic projections that are contained within the cerebral cortex. Thus, synaptosomes prepared

from this region of the brain will contain an adequate number of dopamine transporters to ensure uptake.

The striatum (striate nucleus), on the other hand, is a subcortical part of the cerebrum that develops from the embryonic telencephalon (Jain *et al.,* 2001). It is the largest structure of and major input center for the basal ganglia, which are a clustered group of neuronal cell bodies that are present in vertebrate brains (Hoshi *et al.,* 2005). In primates, the striatum is divided by an area of white matter in the brain called the internal capsule into two functionally distinct regions: the caudate nucleus and putamen (Vorn *et al.,* 2004). The caudate nucleus is primarily involved with the control of voluntary motor actions, learning, and memory (Packard and Knowlton, 2002). The putamen is also implicated with motor skills. Specifically, the putamen is important in the learning of motor movements (Delong, Alexander, *et al.* 1984), preparation of movement (Delong, Georgopoulos, *et al.* 1984), and determining the force and sequences of movements (Packard and Knowlton, 2002). The caudate nucleus is highly innervated by dopaminergic neurons from the ventral tegmental area (VTA) and substantia nigra regions of the brain (Hu *et al.,* 2004; Nicola *et al.,* 2000). The putamen also requires intact dopaminergic innervation to function properly (Nicola *et al.,* 2000). The high proportion of dopaminergic neurons in the substructures of the striatum makes homogenized tissue from this region amenable to studying dopamine uptake.

Genetically modified mice, which are common model organisms used in biomedical research, have been engineered to mimic organisms with reduced amounts of SERT (Holmes, Murphy, *et al.* 2003). Mice can be produced with SERT +/+, SERT +/-, and SERT -/- genotypes. SERT +/- variants have SERT expression reduced by approximately 50%

(Lesch *et al.,* 1996; Bengel *et al.*, 1998). SERT -/- mice, produced by breeding SERT +/- mice, on the other hand, do not express SERT (Bengel *et al.,* 1998). Despite the fact that excess serotonin in the extracellular fluid during development has been shown to lead to cranial, facial, and heart defects, these mice have a normal phenotypic appearance (Bengel *et al.,* 1998). SERT null mutant mice show an increase in anxiety-like behaviors, as shown in light/dark exploration and elevated plus-maze tests, when compared to SERT +/+ mice (Holmes *et al.,* 2003; Popa *et al.*, 2008).

Both SERT and DAT are known to have high affinity for their respective substrates. However, these neurotransmitter systems also have overlapping control of many physiological functions (Murphy *et al.,* 1998; Torres *et al.,* 2003). In addition, some promiscuity of transport has been shown in each transporter, since experimental evidence has illustrated that the transporters are not completely selective for their corresponding neurotransmitters (Faraj *et al.,* 1994; Pan *et al.,* 2001; Schmidt and Lovenberg, 1985). A clear linkage between these transporters appears in the control of reward responses of drugs of abuse, such as cocaine (Bubar *et al.,* 2003; Uhl *et al.,* 2002; Sora *et al.*, 2001) and MDMA (Bankson and Cunningham, 2001; Bankson and Yamamoto, 2004; Bengel *et al.,* 1998). Although it was previously believed that inhibition of DAT was the sole of action in cocaine reward, the actions of this drug are more broadly reaching (Uhl *et al.,* 2002). Specifically, cocaine exerts its rewarding and aversive actions through multiple alternate transporters, including SERT and other monoamine transporters (Uhl *et al.,* 2002). Other research confirms the association of these transporters as they pertain to the abuse of cocaine. Rats treated with the selective serotonin reuptake inhibitors fluoxetine and fluvoxamine exhibited enhanced cocaine-induced dopamine release (Bubar *et al.,* 2003).

Similarly, MDMA, the popular recreational amphetamine analog, has also helped to elucidate the potential relationship between these transporters (Bankson and Cunningham, 2001; Bankson and Yamamoto, 2004; Bengel *et al.,* 1998). As is seen with cocaine, MDMA also causes serotonin-modulated dopamine release (Bankson and Cunningham, 2001; Bankson and Yamamoto, 2004). Also, in SERT knockout mice, the locomotor enhancing and hyperactivity effects of MDMA are lost (Bengel *et al.,* 1998). Contrarily, the calming effect of psychostimulant drugs in DAT knockout mice is eliminated by the SERT blocker fluoxetine (Gainetdinov *et al.,* 1999). These data suggest that this primary calming effect of psychostimulants in DAT knockout mice is mediated by the serotonin neurotransmission system (Gainetdinov *et al.,* 1999). Since the existence of crosstalk between the dopamine and serotonin neurotransmission systems is clearly illustrated by these drugs of abuse, further experiments to investigate the molecular underpinnings of the associations between these systems is the underlying hypothesis to be tested in this thesis.

Conditioned place preference (environmental place conditioning) is a common technique used in animal studies to determine the potentially rewarding properties of drugs (Tzschentke, 1998). In this procedure, animal subjects are injected with drugs of interest in a chamber with specific environmental cues. This process is then repeated in the same fashion for several days. During this period of conditioning, animals begin to associate the rewards of the drug with the environmental cues that are continually present in the training process. Subsequently, when the animal is tested in an apparatus that contains one compartment with the same drug-related environmental cue used in the conditioning phase, and another compartment with neutral cues, it will gravitate towards the one with the drug-related cues. In comparison to other mice strains, SERT knockout

mice display enhanced cocaine place preference, while DAT knockout mice show no distinct changes in place preference to cocaine (Sora *et al.,* 1998). Only mice with dual loss of both SERT and DAT expression show complete abolition of cocaine place preference (Sora *et al.,* 2001). Again, these data suggest a complex relationship between the serotonin and dopamine neurotransmission systems, and, specifically, the serotonin and dopamine transporters, and justifies further study of the linkage between these systems.

In sum, research shows that drug reward pathways and locomotor behavioral pathways depend on a synergy between the serotonin and dopamine neurotransmission systems, as opposed to being solely controlled by the dopamine system. The present study was performed to gather preliminary data concerning additional interactions between these systems. Here, homogenized brain tissue from SERT +/- mice was used to investigate dopamine uptake. All uptake was monitored via the technique of chronoamperometry. In the future, similar dopamine uptake experiments will also be performed with SERT +/+ and SERT -/- mice. The ultimate goal of this project is to investigate compensatory changes in dopamine transporter function in response to reduced or absent expression of SERT. More specifically, this study seeks to determine whether decreases in SERT expression have a compensatory effect to modify dopamine uptake by either promiscuous uptake of dopamine by SERT or by compensatory alterations in DAT expression. Since DAT has been shown to have the ability to take up serotonin, it was hypothesized that in mice with reduced or absent SERT, that DAT might be upregulated to compensate for the lack of SERT. Thus, the rate of dopamine uptake may be increased indirectly due to the effects caused by DAT upregulation and compensation. In addition, this study also seeks to determine whether dopamine that has been cleared by reuptake is lost during the process

of vacuum filtration. This loss would dictate that radiochemical methods that use vacuum filtration are underestimating the amount of dopamine taken up during experimentation due to the rupturing of synaptosomal membranes.

## Experimental Methods

### Animals

SERT +/- mice were housed in the Penn State University animal facility (University Park, PA) and received water and food *ad libitum*. All animal procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee and were in accordance with the National Institutes of Health Animal Care Guidelines.

### Electrode Preparation and Calibration

Borosilicate glass capillaries (1.2 mm x 0.68 mm) were used for microelectrode fabrication by pulling with an automatic puller. Capillaries were threaded with 30 μm carbon fibers and pulled to create two electrodes whose carbon fiber tips were cut to 60-100 μm in length (Textron Specialty Materials; Lowell, MA). The microelectrodes tips were then dipped in or injected with an epoxy solution (8 parts epoxy solution A, 2 parts epoxy solution B) and briefly dipped in a solution of acetone, all of which were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Subsequently, the microelectrodes were heated in an oven for at least five hours at 100°C.

After heating, microelectrodes were calibrated against known concentrations of dopamine (0.25 μM – 1.25 μM). Only electrodes showing a high degree of linearity in response to the dopamine ($r^2 > 0.90$) were kept and used for further experimentation with synaptosomes. Those not showing a high degree of linearity were discarded.

**Chronoamperometry**

To measure dopamine, a working carbon fiber electrode was placed in a solution of synaptosomes at a 0 V resting potential as compared to a Ag/AgCl reference electrode (Gerhardt *et al.,* 1984). Changes in current as a result of dopamine oxidation were recorded in response to a 1-Hz square wave potential step generated by an EI-400 bipotentiostat (ESA Biosciences; Chelmsford, MA). A drift current was recorded for 100 ms. The potential was then stepped to +0.7 V, which exceeds the dopamine oxidation potential, and was held there for 100 ms (Daws and Toney, 2007). The potential was then held at 0 V for 100 ms, which is below the dopamine oxidation potential, causing dopamine to be reduced. Finally, the electrode was allowed to rest for 700 ms to decrease the amount of products that will adsorb to electrode surfaces (Daws and Toney, 2007; Perez and Andrews, 2005).

**Synaptosome Preparation**

Bilateral frontal cortex and striatum of SERT +/- mice were quickly removed by dissection and placed immediately on ice. These structures were then homogenized separately with 10 volumes of Tris-sucrose buffer (0.5 mM Tris-HCl, 0.32 mM sucrose, pH 7.4) in a glass homogenizer with a Teflon pestle. Homogenates were centrifuged at 2000 g (4,500 rpm) for 10 min The supernatant from this initial spin was then centrifuged again at 16,000 g (13,000 rpm) for 10 min The resulting pellet was resuspended in 40 volumes of synaptosomal assay buffer (124 mM NaCl, 1.80 mM KCl, 1.24 mM $KH_2PO_4$, 1.40 mM $MgSO_4$, 2.50 mM $CaCl_2$, 26.0 mM $NaHCO_3$, 10.0 mM glucose, saturated with 95% $O_2$/5% $CO_2$, pH 7.4 with phosphoric acid).

**Uptake Experiments**

Microelectrodes that were precalibrated and showed a high degree of linearity in response to dopamine ($r^2 > 0.90$) were placed in synaptosomal solutions in a final volume of 2 mL assay buffer. A +0.7 V pulsed potential was applied until a stable baseline current was attained. Dopamine was added to each synaptosomal solution to yield a final concentration of 0.25 – 1.50 μM. Measuring dopamine uptake at these different concentrations was performed to determine a maximum rate of uptake ($V_{max}$) at which filtration experiments could be carried out. The synaptosomal solution was subsequently stirred, and the change in current with respect to time was recorded for 20 minutes. Prior to the addition of dopamine, synaptosomes were centrifuged at 16,000 g (13,000 rpm) for 5 min and placed in fresh, oxygenated assay buffer. After addition, a postcalibration was performed in fresh assay buffer to ensure electrodes were still functional, and not damaged or fouled.

**Chemicals**

Dopamine and all of the chemicals used in both the Tris-sucrose and synaptosomal assay buffers were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO).

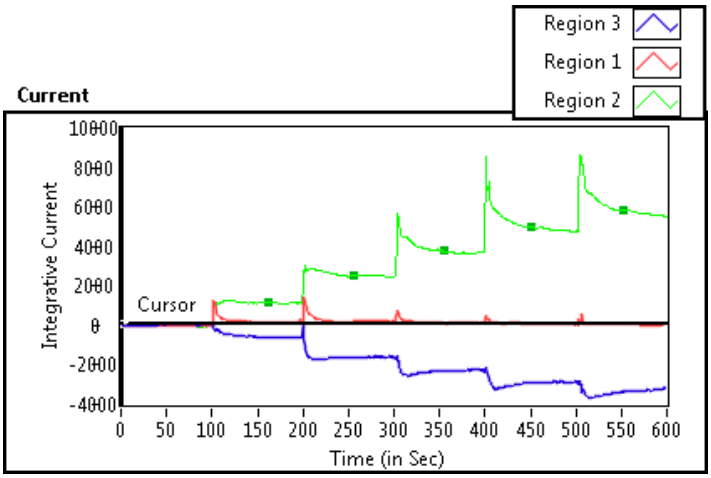
# Results

## Dopamine Uptake in SERT +/– Synaptosomes Showed a Low Success Rate

Carbon fiber microelectrode responses to various concentrations of dopamine were assessed between 0.25 – 1.25 μM to test the efficacy of the electrode. A sample of calibrations is depicted in Figure 1-1. As shown in Table 1-1, there was a large amount of difficulty performing the dopamine uptake experiments in both the frontal cortex and striatum synaptosomes. Only 6 of the 31 total uptake experiments showed valid and measureable uptake. Summary tables and figures of the successful uptake experiments are presented.

**Figure 1-1 – Sample Microelectrode Calibrations.** Linear microelectrode responses were obtained for concentrations of dopamine between 0.25 and 1.25 μM for the oxidative (diamond shape) and reductive (square shape) currents. Calibrations with $r^2$ values of 0.99 or greater linearity in response to dopamine were preferred in the uptake experiments involving synaptosomes. Those electrodes with $r^2$ values of less than 0.90 were discarded and not used in experimentation. Region 2 (green lines) represent the oxidative current, while region 3 (blue lines) represent the reductive current. All figures presented show microelectrodes with oxidative current $r^2$ values of 0.99 or higher. The spikes indicate times at which dopamine was added and subsequently stirred, producing a rapid jump in current.

**A.**


Region 3
Region 1
Region 2
Current
Integrative Current
10000
8000
6000
4000
2000
0
-2000
-4000
Cursor
0 50 100 150 200 250 300 350 400 450 500 550 600
Time (in Sec)

**B.**

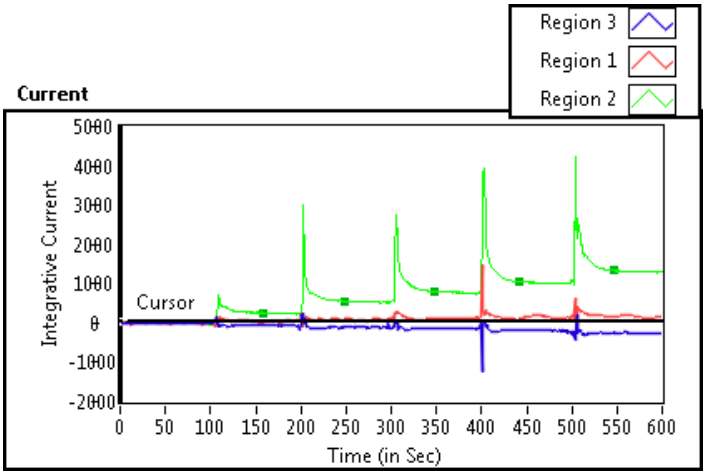

Region 3
Region 1
Region 2
Current
Integrative Current
5000
4000
3000
2000
1000
0
-1000
-2000
Cursor
0 50 100 150 200 250 300 350 400 450 500 550 600
Time (in Sec)

**Table 1-1 – Totality of SERT +/– Frontal Cortex (FC) and Striatal (S) Synaptosome Uptake Experiments.** Experiments 1, 3, 8, 9, 16, 28 were deemed to show successful uptake. In experiment 13, although a value for uptake was measured, the current dropped below baseline during the course of the experiment. In experiments 17 and 29, the current rose throughout the experiment. In experiment 20, an adequate baseline was not obtained. In experiment 30, the electrode was found to be fouled after postcalibration. In all other experiments, no measurable uptake was detected, thus no postcalibration was performed. One commonly observed problem in this set of experiments was that microelectrodes recorded increases in current. However, current should have been decreasing, as dopamine was cleared from the extrasynaptosomal solution during the uptake process. Another commonly encountered problem was spiking current levels. It was hypothesized that these were due to either difficulties with the potentiostat or fouling of the microelectrodes. Finally, some microelectrodes showed excessive background noise, or could not be properly baselined prior to the injection of dopamine.

| Experiment # | Brain Region Studied | Tissue Wt. (g) | [Dopamine] (μM) | Uptake Rate (nmol/g tissue-min) | Postcalibration $r^2$ Value |
|---|---|---|---|---|---|
| 1 | FC | 0.03663 | 1 | 2.7 | 0.9259 |
| 2 | S | 0.01975 | 1 | N/A | N/A |
| 3 | FC | 0.01620 | 1 | 2.7 | 0.9868 |
| 4 | S | 0.01850 | 1 | N/A | N/A |
| 5 | S | 0.01470 | 1 | N/A | N/A |
| 6 | S | 0.02010 | 1 | N/A | N/A |
| 7 | FC | 0.02460 | 1 | N/A | N/A |
| 8 | S | 0.01920 | 1 | 5.0 | 0.9864 |
| 9 | FC | 0.02090 | 1 | 5.4 | 0.9468 |
| 10 | FC | 0.02350 | 1 | N/A | N/A |
| 11 | S | 0.02070 | 1 | N/A | N/A |
| 12 | S | 0.03480 | 1 | N/A | N/A |
| 13 | FC | 0.01200 | 1 | 19 | 0.8499 |
| 14 | S | 0.02120 | 0.25 | N/A | N/A |
| 15 | FC | 0.02910 | 1 | N/A | N/A |
| 16 | FC | 0.01820 | 1 | 3.7 | 0.9797 |
| 17 | S | 0.02180 | 1 | 6.9 | 0.9517 |
| 18 | FC | 0.04090 | 0.5 | N/A | N/A |
| 19 | S | 0.03260 | 0.5 | N/A | N/A |
| 20 | FC | 0.01810 | 0.5 | 2.3 | 0.6178 |
| 21 | S | 0.03230 | 0.5 | N/A | N/A |
| 22 | FC | 0.03660 | 1.5 | N/A | N/A |
| 23 | S | 0.03870 | 1.5 | N/A | N/A |
| 24 | FC | 0.02240 | 1.5 | N/A | N/A |
| 25 | S | 0.02940 | 0.5 | N/A | N/A |
| 26 | FC | 0.03630 | 1.5 | N/A | N/A |
| 27 | S | 0.06730 | 1.5 | N/A | N/A |
| 28 | S | 0.03810 | 1.5 | 1.9 | 0.9783 |
| 29 | FC | 0.01860 | 1.5 | 4.3 | 0.9788 |
| 30 | FC | 0.03870 | 1 | 0.74 | -0.0021 |
| 31 | S | 0.05900 | 1 | N/A | N/A |

| Experiment # | Brain Region Used | Tissue Wt. (g) | [Dopamine] (μM) | Uptake Rate (nmol/g tissue-min) | Postcalibration $r^2$ Value |
|---|---|---|---|---|---|
| 1 | FC | 0.03660 | 1 | 2.7 | 0.9259 |
| 3 | FC | 0.01620 | 1 | 2.7 | 0.9868 |
| 9 | FC | 0.02090 | 1 | 5.3 | 0.9468 |
| 16 | FC | 0.01820 | 1 | 3.7 | 0.9797 |

**Table 1-2 – Summary of Successful SERT +/– Mice Frontal Cortex Synaptosome Uptake Experiments**. Experiments characterized as successful showed measurable uptake and were performed with electrodes with high linearity in response to dopamine during postcalibration. Only four experiments out of the 15 total experiments run in frontal cortex synaptosomes met this criteria.

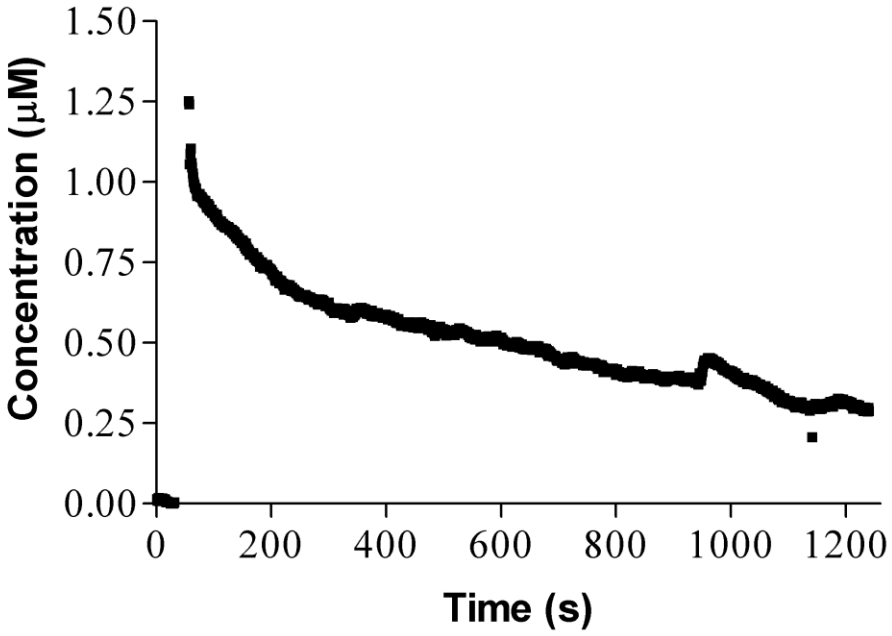



**Figure 1-2 – Frontal Cortex Uptake Experiment #1.** This figure shows changes in extrasynaptosomal dopamine concentration as a function of time. In this experiment, 1 μM of dopamine was added to the extrasynaptosomal solution. Dopamine was cleared at a rate of 2.7 nmol/g tissue-min. Each point in the graph is one complete scan, which takes approximately one second in chronoamperometry in the delayed pulse mode.

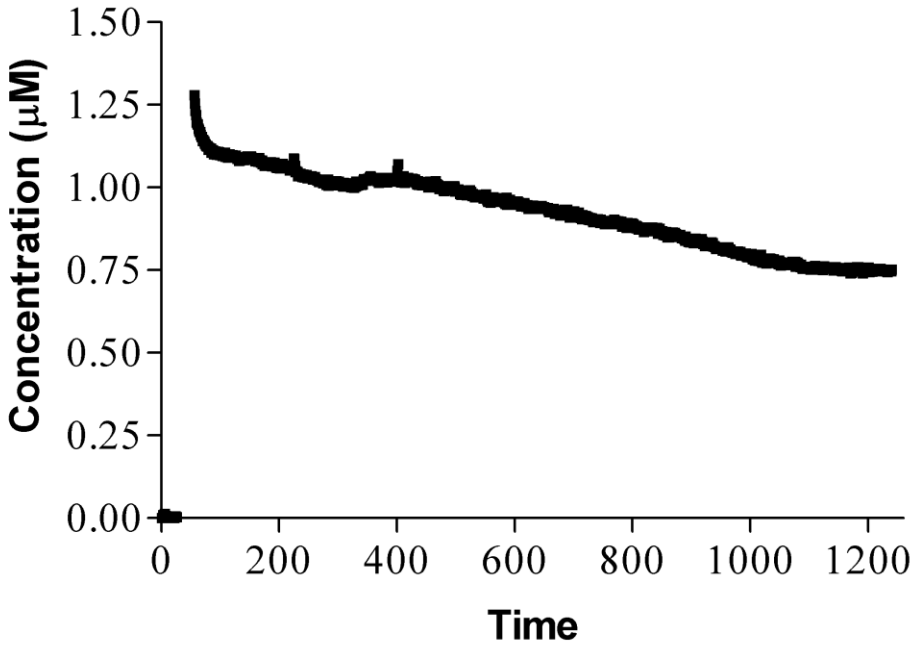



**Figure 1-3 – Frontal Cortex Uptake Experiment #3.** This figure shows changes in extrasynaptosomal dopamine concentration as a function of time. In this experiment, 1 μM dopamine was added to the synaptosome assay buffer. Dopamine was cleared at a rate of 2.7 nmol/g tissue-min.

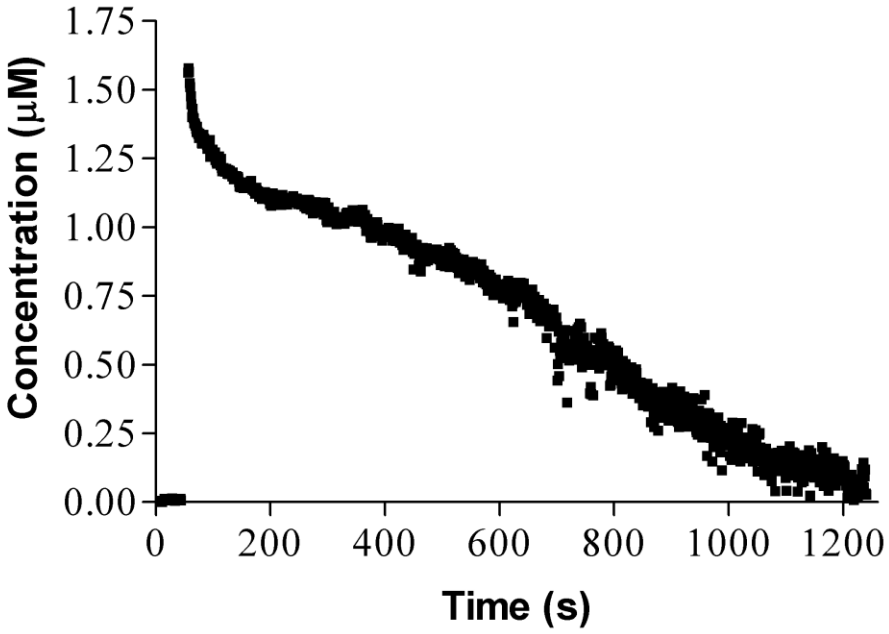



**Figure 1-4 – Frontal Cortex Uptake Experiment #9.** This figure shows changes in extrasynaptosomal dopamine concentration as a function of time. In this experiment, 1 μM dopamine was added to the extrasynaptosomal solution. Dopamine was cleared at a rate of 5.4 nmol/g tissue-min.

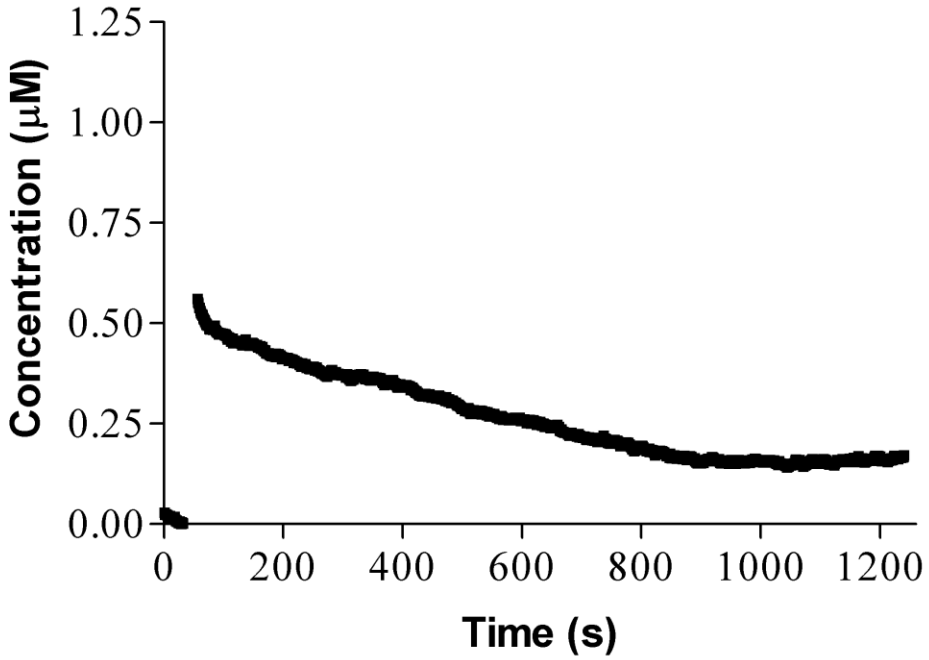



**Figure 1-5 – Frontal Cortex Uptake Experiment #16.** This figure shows changes in extrasynaptosomal dopamine concentration as a function of time. In this experiment, 1 μM dopamine was added to synaptosomes. Dopamine was cleared at a rate of 3.7 nmol/g tissue-min. In this experiment, although 1 μM of dopamine was added, the electrode did not record 1 μM of dopamine at the outset of the uptake curve.

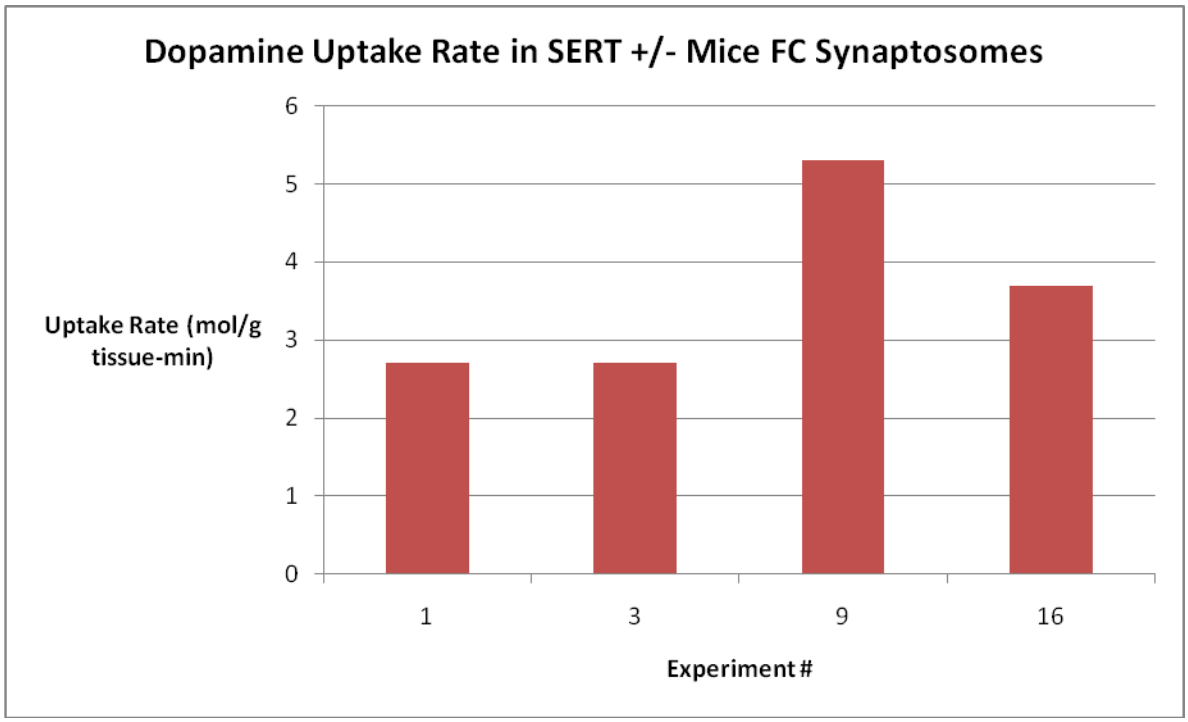



**Figure 1-6 – Dopamine Uptake Rates in SERT +/– Mice Frontal Cortex Synaptosomes**. Dopamine uptake in frontal cortex synaptosomes incubated with 1 μM of dopamine. The mean dopamine uptake rate was 3.6 ± 0.6 nmol/g tissue-min.

| Experiment # | Brain Region Studied | Tissue Wt. (g) | [Dopamine] (μM) | Uptake Rate (nmol/g tissue-min) | Postcalibration $r^2$ Value |
|---|---|---|---|---|---|
| 8 | S | 0.01920 | 1 | 5.0 | 0.9864 |
| 28 | S | 0.03810 | 1.5 | 1.9 | 0.9783 |

**Table 1-3 – Summary of Successful SERT +/– Mice Striatal Synaptosome Uptake Experiments.** Only two experiments showed measureable uptake out of the 16 total experiments run in striatal synaptosomes.

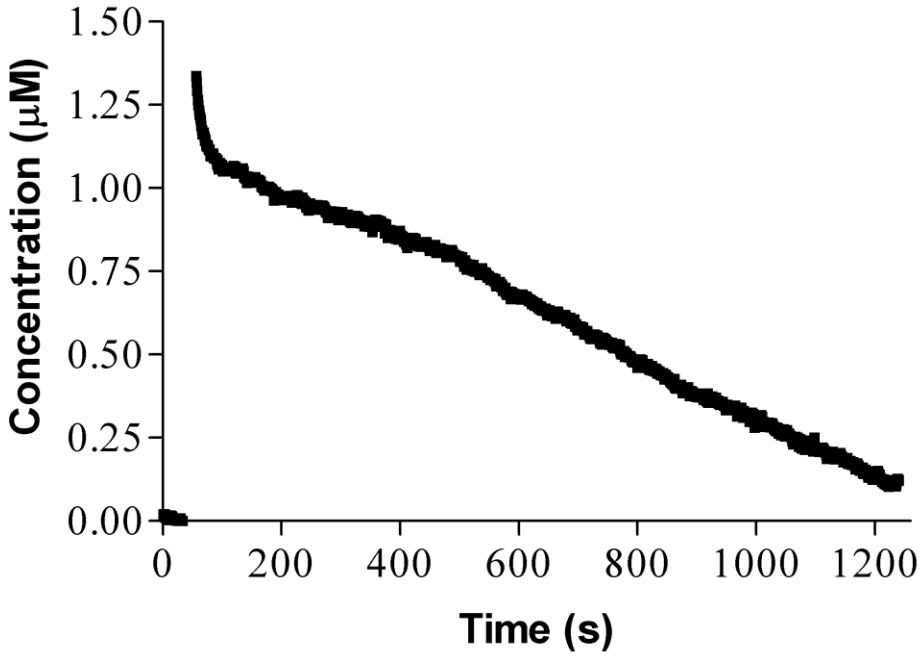



**Figure 1-7 – Striatal Uptake Experiment #8.** In this experiment, 1 μM dopamine was added to striatal synaptosomes. It was cleared at a rate of 5.0 nmol/g tissue-min.

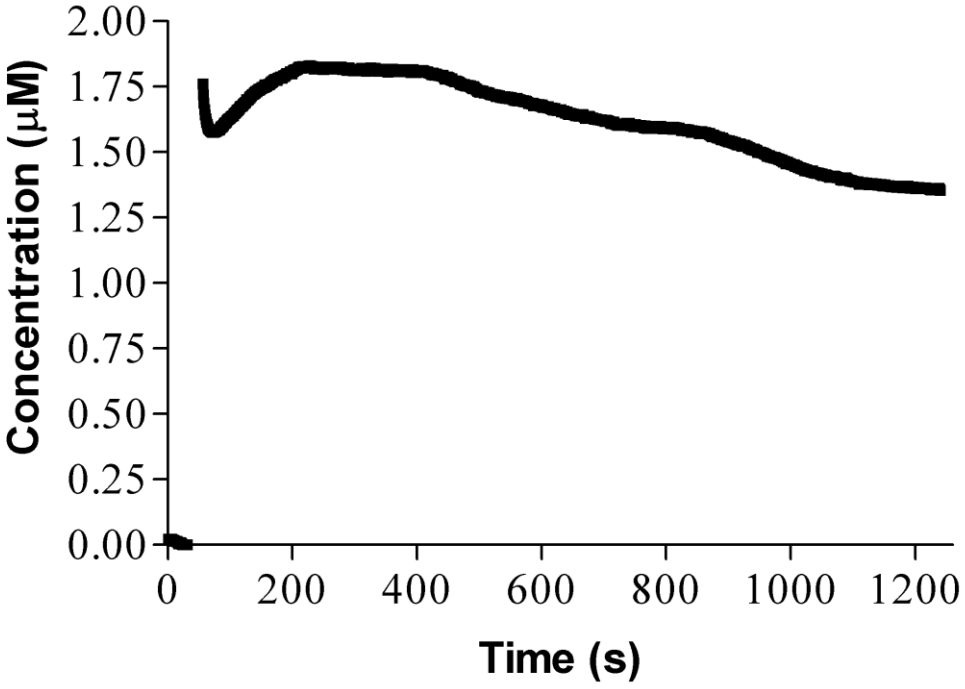



**Figure 1-8 – Striatal Uptake Experiment #28.** In this experiment, 1.5 μM of dopamine was added to the extrasynaptosomal solution. Dopamine was cleared at a rate of 1.9 nmol/g tissue-min.

## Discussion

In this study, only a fraction of the synaptosomal preparations showed measureable uptake of dopamine. In the frontal cortex synaptosomes, four experiments were successful. All of these synaptosomal solutions were incubated with 1 μM dopamine, which was allowed to clear (uptake) for 20 minutes. The average dopamine uptake rate was 3.6 nmol/g tissue-min, with a standard error of 0.6 nmol/g tissue-min The striatal synaptosome experiments also had a low overall success rate. In the striatal synaptosomes, two preparations showed successful uptake. However, these two solutions were treated with two different concentrations of dopamine, so no average rate of uptake was calculated. The solution treated with 1 μM dopamine showed an uptake rate of 5.0 nmol/g tissue-min, while the solution treated with 1.5 μM only showed an uptake rate of 1.9 nmol/g tissue-min.

Similar research coming from the same laboratory showed that the mean dopamine uptake rate was 446 nmol/g tissue-min in striatal synaptosomes from SERT +/+ mice when incubated with 1 μM dopamine. On the other hand, synaptosomes from SERT +/- showed a mean uptake rate of 318 nmol/g tissue-min, and synaptosomes from SERT -/- mice showed a mean uptake rate of 305 nmol/g tissue-min. All of these values were also obtained by chronoamperometry (Perez *et al.*, 2001). The same author obtained lower values when the experiment was performed another time, with striatal synaptosomes showing a mean uptake rate of 285 nmol/g tissue-min in tissue from SERT +/+ mice, 235 nmol/g tissue-min in tissue from SERT +/- mice, and 242 nmol/g tissue-min in tissue from SERT -/- mice (Perez, 2004). In radiochemical studies of dopamine uptake in striatal synaptosomes, mean

uptake rates appear to be significantly different. One study investigating uptake in CD-1 mouse striatal synaptosomes using [$^3$H]-dopamine reported a mean uptake rate of 10 nmol/g tissue-min (Ramassamy *et al.*, 1994). Another study showed a mean uptake rate of [$^3$H]-dopamine ranging from 0-25 nmol/g tissue-min depending on the concentration of sodium in the preparation (Zimanyi *et al.*, 1989). Other values reported in the literature for [$^3$H]-dopamine uptake into striatal synaptosomes include 29 nmol/g tissue-min (Sershen *et al.*, 1987), 40 nmol/g tissue-min (Wheeler *et al.,* 1993), 10-30 nmol/g tissue-min depending on the concentration of potassium chloride in the preparation (Woodward *et al.,* 1986), 35 nmol/g tissue-min (Yi and Johnson, 1990), 19.4 nmol/g tissue-min (Izenwasser *et al.*, 1992), and 16 nmol/g tissue-min (Copeland *et al.*, 2005). Overall, the values for dopamine uptake into striatal synaptosomes as measured by chronoamperometry were approximately 10-fold greater than those measured by radiochemical uptake. This trend is reminiscent of similar studies using serotonin (Perez *et al.*, 2006). The values obtained in this study for dopamine uptake (5 nmol/g tissue-min and 1.9 nmol/g tissue-min) in striatal synaptosomes were low, especially in comparison to the studies that also used chronoamperometry to monitor uptake.

Due to the difficulties that I encountered in carrying out these experiments, a discussion of the problems and potential improvements is justified. First, I hypothesize that the problems with the synpatosomal solutions might have arisen as a result of the makeshift apparatus used to stir the solutions upon addition of the varying concentrations of dopamine. It is known that synaptosomal plasma membranes are weaker and less resistant to stress when compared with cellular plasma membranes (Ishioka *et al.,* 1990; Perez and Andrews, 2005). In addition, several studies have recognized the detriment of

filtering and stirring synaptosomal solutions. Recognizing the relative weakness of synaptosomal membranes is what led to the discovery that radiochemical analysis studies that used subsequent vacuum filtration of synaptosomal solutions underestimated values of dopamine uptake due to membrane rupture (Perez *et al.*, 2006). Another study determined that the use of a magnetic stirring bar at the lowest possible stirring plate speed completely abolished all uptake in the synaptosomal solutions (Perez and Andrews, 2005). Thereby, a reasonable proposal for future experiments would be to ensure the use of a delicate stirring apparatus with a low speed setting.

Initially microelectrodes were prepared by dipping them in an epoxy solution (8 parts epoxy solution A, 2 parts epoxy solution B) and then briefly dipping them in a solution of acetone. This method was used in other similar experiments with success (Perez *et al.*, 2006; Unger *et al.,* 2006; Singh *et al.,* 2010). However, when I prepared electrodes using this method, they frequently lost their carbon fiber tips, presumably due to a lack of epoxy localized at the tips of the glass capillaries. Once this was discovered, the microelectrodes were then prepared by injecting the epoxy solution directly into the tips using a syringe. This method was also effectively employed in the past (Perez and Andrews, 2005). Microelectrodes with epoxy directly injected into the tips did not show the problem of carbon fiber removal, and this method is recommended for future experiments.

Similarly, electrodes were initially made by heating in an oven for 2 hours at 80 °C (Perez and Andrews, 2005), or by heating in an oven overnight at 80 °C (Perez *et al.*, 2006). However, electrodes that were prepared in this fashion often showed incomplete sealing by the epoxy, causing the carbon fiber tips to be easily pulled out of the glass capillaries, or current values that would spike throughout the calibrations and synaptosomal uptake

experiments. After encountering these problems, microelectrodes were then heated overnight at 100 °C. Microelectrodes heated in this manner were not as susceptible to losses of carbon fiber tips, or irregular current readings. This method is also recommended for future experimental attempts.

Finally, after heating, all microelectrodes were calibrated against known concentrations of dopamine ranging from 0.25 μM – 1.25 μM. Only electrodes showing linearity in response to dopamine of $r^2 > 0.90$ were used in these synaptosomal uptake experiments. Those showing a lower degree of linearity were not used in experimentation. Similar experiments limited the electrodes used in synaptosomal uptake to those that had $r^2$ values of greater than 0.99 (Perez and Andrews, 2005; Perez *et al.*, 2006). Many of the problems with the uptake experiments could potentially be explained by improperly functioning microelectrodes including failure to achieve stable baselines, rising current levels, and spiking current readings. Thereby, a potential revision to the methods of this experiment would be to hold electrodes to this high standard of linearity in response to dopamine.

In the future, several additional experiments will need to be carried out. First, additional dopamine uptake experiments should be conducted in SERT +/- mice in synaptosomes from both homogenized frontal cortex and striatum. Since only four frontal cortex uptake experiments showed measureable uptake, as did only two striatum uptake experiments, more data will need to be gathered to add to the preliminary data presented here. Using the above suggestions should improve the reproducibility and robustness of uptake experiments. In addition, uptake experiments should be extended to the other genotypes of mice, including SERT +/+ and SERT -/- mice to compare the uptake rates. An

important future addition will be vacuum filtering the synapatosomal solutions after the period of uptake to determine the integrity of synaptosomal membranes. In previous experiments, after the uptake period, synaptosomal solutions were put on ice immediately to stop the uptake process, and then vacuum filtered. It was determined that a pressure of 300 mm Hg was sufficient to rupture synaptosomal membranes (Perez *et al.*, 2006). In future experiments, a pressure control gauge could be used to employ varying pressures when filtering synaptosomes. In this way, an optimal pressure in which synaptosomal solutions can be filtered without rupturing their membranes could be determined. Integrity of the membranes could be confirmed by using high performance liquid chromatography (HPLC) to measure dopamine concentrations remaining in the filtrates after filtering. If membranes have remained intact, the amount of dopamine measured in the filtrates by HPLC should be negligible.

Recent research that has shown that both SERT and DAT allow some promiscuity of transport (Faraj *et al.,* 1994; Pan *et al.,* 2001; Schmidt and Lovenberg, 1985; Baganz *et al.,* 2008), and that these transporters are both involved in reward responses to cocaine (Bubar *et al.,* 2003; Uhl *et al.,* 2002) and MDMA (Bankson and Cunningham, 2001; Bankson and Yamamoto, 2004; Bengel *et al.,* 1998). Additionally, overlapping roles are played by the serotonin and dopamine neurotransmitter systems in many physiological functions (Murphy *et al.,* 1998; Torres *et al.,* 2003). All of these data illustrate that the dopamine and serotonin neurotransmitter systems work synergistically. Accordingly, the ultimate goal of this project is to further investigate the relationship between these neurotransmission systems by determining if there are compensatory effects in the dopamine neurotransmission system in response to reduced or absent expression of SERT.

Specifically, this study will seek to determine the ability of SERT to modify dopamine uptake in homogenized brain tissue by either promiscuous uptake of dopamine or by altering DAT expression. Recent data indicate that there were no alterations in DAT expression in the striatum across the three SERT genotypes (Perez, 2004). Also, dopamine uptake in striatal synaptosomes was unchanged as a result of reduced or absent SERT expression (Perez, 2004). Furthermore, these same authors found no component of dopamine uptake as a result of SERT activity. Promiscuous uptake of dopamine by SERT was hypothesized to cause reduced dopamine uptake rates in synaptosomes prepared from SERT +/- and SERT -/- mice when compared to those from SERT +/+ mice. In addition, the dopamine uptake inhibitor WIN 35428 caused complete inhibition of dopamine uptake when incubated with striatal synaptosomes, showing that DAT is the primary transporter clearing dopamine in striatum (Perez, 2004). These data illustrate that dopamine neurochemistry is unchanged in response to changes in SERT expression (Perez, 2004). Further work in this study will seek to verify the results obtained by Perez *et al*. Additionally, these studies could be extended to investigate compensatory changes in the frontal cortex, which is also known to express DAT. An examination of changes in the frontal cortex may be particularly useful, as dopamine uptake by the norepinephrine transporter (NET) has been observed in genetically engineered mice that express low levels of DAT (Moron *et al.*, 2002). Thus, a reasonable hypothesis would be that promiscuous dopamine uptake might also occur via SERT in brain regions with low DAT expression.

This study will also seek to determine whether dopamine that has been taken up from solution is lost during the process of vacuum filtration. This loss would confirm that

radiochemical methods that use vacuum filtration are underestimating the amount of dopamine uptake during experimentation due to the rupturing of synaptosomal membranes, as presented in recent research (Perez *et al.*, 2006). Further experimentation into what vacuum pressures are acceptable for filtering synaptosomes would also prove valuable for other methods of research. Overall, despite the low success rate in uptake experiments, the furthering of the goals and data for this project are warranted.

## References

Agu GO, Rajkumar K, Murphy BD (1986) Evidence for dopaminergic regulation of prolactin and a luteotropic complex in the ferret. Biol Reprod 35: 508-515.

Alenina N, Kikic D, Todiras M, Mosienko V, Qadri F, Phlem R, Boye P, Vilianovitch L, Sohr R, Tenner K, Hortnagl H, Bader M (2009) Growth retardation and altered autonomic control in mice lacking brain serotonin. Proc Natl Acad Sci 106: 10332-10337.

Arias-Carrion O, Poppel E (2007) Dopamine, learning, and reward-seeking behavior. Acta Neurobiol 67: 481-488.

Axelrod BN, Goldman RS, Henry RR (1992) Sensitivity of the mini-mental state examination to frontal lobe dysfunction in normal aging. J Clin Psychol 48: 68-71.

Baganz NL, Horton RE, Calderon AS, Owens WA, Munn JL, Watts LT, Koldzic-Zivanovic N, Jeske NA, Koek W, Toney GM, Daws LC (2008) Organic cation transporter 3: keeping the brake on extracellular serotonin in serotonin-transporter-deficient mice. Proc Natl Acad Sci 105: 18976-18981.

Bankson MG, Cunningham KA (2001) 3,4-methylenedioxymethamphetamine (MDMA) as a unique model of serotonin receptor function and serotonin-dopamine interactions. J Pharmacol Exp Ther 297: 846-852.

Bankson MG, Yamamoto BK (2004) Serotonin-GABA interactions modulate MDMA-induced mesolimbic dopamine release. J Neurochem 91: 852-859.

Ben-Jonathan N, Hnasko R (2001) Dopamine as a prolactin (PRL) inhibitor. Endo Reviews 22: 724-763.

Bengel D, Murphy DL, Andrews AM, Wichems CH, Feltner D, Heils A, Mossner R, Westphal H, Lesch KP (1998) Altered brain serotonin homeostasis and locomotor insensitivity to 3,4-methylenedioxymethamphetamine ("ecstasy") in serotonin transporter-deficient mice. Mol Pharmacol 53: 649-655.

Benmansour S, Cecchi M, Morilak DA, Gerhardt GA, Javors MA, Gould GG, Frazer A (1999) Effects of chronic antidepressant treatments on serotonin transporter function, density, and mRNA level. J Neurosci 19: 10494-10501.

Benton, AL (1968) Differential behavioral effects in frontal lobe disease. Neuropsychologia 6:53-60.

Berger M, Gray JA, Roth BL (2009) The expanded biology of serotonin. Annu. Rev. Med. 60: 355–366.

Berardelli A, Rothwell JC, Thompson PD, Hallett M (2002) Pathophysiology of bradykinesia in Parkinson's disease. Brain 124: 2131-2146.

Booth RF, Clark JB (1978) A rapid method for the preparation of relatively pure metabolically competent synaptosomes from rat brain. J Biochem 176: 365-370.

Bradford HF, Thomas AJ (2006) Metabolism of glucose and glutamate by synaptosomes from mammalian cerebral cortex. J Neurochem 16: 1495-1504.

Brazell MP, Kasser RJ, Renner KJ, Feng J, Moghaddam B, Adams RN (1987) Electrocoating carbon fiber microelectrodes with Nafion improves selectivity for electroactive neurotransmitters. J Neurosci Meth 22: 167-177.

Brooks DJ, Salmon EP, Mathias CJ, Quinn N, Leenders KL, Bannister R, Marsden CD, Frackowiak RS (1990) The relationship between locomotor disability, autonomic dysfunction, and the integrity of the striatal dopaminergic system in patients with

multiple system atrophy, pure autonomic failure, and Parkinson's disease, studied with PET. Brain 113: 1539-1552.

Brownman KE, Curzon P, Pan JB, Molesky AL, Komater VA, Decker MW, Brioni JD, Moreland RB, Fox GB (2005) A-412997, a selective dopamine D4 agonist, improves cognitive performance in rats. Pharmacol Biochem Behav 82: 148-155.

Bubar MJ, McMahon LR, De Deurwaerdere P, Spampinato U, Cunningham KA (2003) Selective serotonin reuptake inhibitors enhance cocaine-induced locomotor activity and dopamine release in the nucleus accumbens. Neuropharmacol. 44: 342-353.

Cantello R, Gianelli M, Bettucci D, Civardi C, De Angelis MS, Mutani R (1991) Parkinson's disease rigidity. Neurol 41: 1449.

Carlsson I, Wendt PE, Risberg J (2000) On the neurobiology of creativity. Differences in frontal activity between high and low creative subjects. Neuropsychologia 38: 873-885.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301: 386.

Casseron W, Genton P (2005). DOPA-sensitive dystonia-plus syndrome. Devel. Med. Child Neurol. 47: 200-203.

Channon S (2004) Frontal lobe dysfunction and everyday problem-solving: social and non-social contributions. Acta Psychologica 115: 235-254.

Chen NH, Reith ME, Quick MW (2004) Synaptic uptake and beyond: the sodium- and chloride-dependent neurotransmitter family SLC6. Euro J Physiol 447:519-531.

Cheng HY, McCreery RL (1977) Potential dependent chronoamperometry: experimental verification. J Electroanalytic Chem 85: 361-369.

Coleman M (1973) Serotonin and central nervous system syndromes of childhood. J Autism Devel Disorders 3: 27-35.

Copeland BJ, Heff NH, Hadjiconstantinou M (2005) Enhanced dopamine uptake in the striatum following repeated restraint stress. Synapse 57: 167-174.

Collu R, Jequier JC, Letarte J, Leboeuf G, Ducharme JR (1974) Endocrine effects of brain serotonin depletion by 5,6-dihydroxytryptamine in prepuberal male rats. Neuroendocrinol 14: 139-150.

Cote F, Thevenot E, Fligny C, Fromes Y, Darmon M, Ripoche MA, Bayard E, Hanoun N, Saurini F, Lechat P, Dandolo L, Hamon M, Mallet J, Vodjdani G (2003) Disruption of the nonneuronal *tph1* gene demonstrates the importance of peripheral serotonin in cardiac function. Proc Natl Acad Sci 100: 13525-13530.

Crabb DW, Matsumoto M, Chang D, You M (2004) Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology. Proc Natl Nutr Soc 63: 49–63.

Crinion JT, Lambon-Ralph MA, Warburton EA, Howard D, Wise RJ (2003) Temporal lobe regions engaged during normal speech comprehension. Brain 126: 1193-1201.

Daws LC, Toney GM (2007) High-speed chronoamperometry to study kinetics and mechanisms for serotonin clearance *in vivo*. Electrochem Meth Neurosci 7: 1-11.

Daws LC, Montanez S, Owens WA, Gould GG, Frazer A, Toney GM, Gerhardt GA (2005) Transport mechanisms governing serotonin clearance *in vivo* revealed by high-speed chronoamperometry. J Neurosci Meth 143: 49-62.

De Jong EB, Horsten BP, Goldschmidt HM (1983) Determination of nine catecholamine metabolites and 5-hydroxyindolacetic acid in urine by capillary gas chromatography. J Chromatogr 279: 564-572.

De Jong TR, Veening JG, Waldinger MD, Cools AR, Olivier B (2006) Serotonin and the neurobiology of the ejaculatory threshold. Neurosci Biobehav 30:893–907.

Delong MR, Alexander GE, Georgopoulos AP, Crutcher MD, Mitchell SJ, Richardson RT (1984) Role of basal ganglia in limb movements. Hum Neurobiol 2: 235-244.

Delong MR, Georgopoulos AP, Crutcher MD, Mitchell SJ, Richardson RT, Alexander GE (1984) Functional organization of the basal ganglia: contributions of single-cell recording studies. Ciba Found Symp 107: 64-82.

Depue RA, Collins PF (1999) Neurobiology of the structure of personality: dopamine, facilitation of incentive motivation, and extraversion. Behav Brain Sci 22: 491-517.

Dorofeev VM, Khnychev SS (1970) Effect of exogenous serotonin of blood platelets and some blood clotting indices in acute experimental radiation sickness. Bull Exp Biol Med 69: 21-23.

El-Ghundi M, Fletcher PJ, Drago J, Sibley DR, O'Dowd BF, George SR (1999) Spatial learning deficit in dopamine D1 receptor knockout mice. Eur J Pharmacol 27: 95-106.

Elsworth JD, Roth RH (1997) Dopamine synthesis, uptake, metabolism, and receptors: relevance to gene therapy of Parkinson's disease. Exp Neurol 144: 4-9.

Enerback L, Jarlstedt J (1975) A cytofluorometric and radiochemical analysis of the uptake and turnover of 5-hydroxytryptamine in mast cells. J Histochem Cytochem 23: 128-135.

Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Cohen RM (1997) Low medial prefrontal dopaminergic activity in autistic children. Lancet 350: 638-634.

Eslinger PJ, Grattan LM, Damasio H, Damasio AR (1992) Developmental consequences of childhood frontal lobe damage. Arch Neurol 49: 764-769.

Faraj BA, Olkowski ZL, Jackson RT (1994) Active [3H]-dopamine uptake by human lymphocytes: correlates with serotonin transporter activity. Pharmacol 48: 320-327.

Faulkner LR (1983) Understanding electrochemistry: some distinctive concepts. J Chem Educ 60:262

Gainetdinov RR, Wetsel RC, Jones SR, Levin ED, Jaber M, Caron MG (1999) Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science 283: 397-401.

Gobbi M, Crespi D, Foddi MC, Fracasso C, Mancini L, Parotti L, Mennini T (1997) Effects of chronic treatment with fluoxetine and citalopram on 5-HT uptake, 5-HT1B autoreceptors, 5-HT3 and 5-HT4 receptors in rats. Naunyn Schmiedebergs Arch Pharmacol 356: 22-28.

Golimbet VE, Alfimova MV, Shcherbatykh, Abramova LI, Kaleda VG, Rogaev EI (2001) Insertion/deletion polymorphism of the serotonin transporter gene and neuroticism as a temperament trait in affective patients and healthy individuals. Mol Biol 35: 336-338.

Gelernter J, Pakstis AJ, Kidd KK (1995) Linkage mapping of serotonin transporter protein gene slc6a4 on chromosome 17. Hum Genet 95: 677-680.

Gelernter J, Kranzler H, Cubells JF (1997) Serotonin transporter protein (SLC6A4) allele and haplotype frequencies and linkage disequilibria in African- and European-

American and Japanese populations and in alcohol-dependent subjects. Hum Genet 101: 243-246.

Gerhardt GA, Oke AF, Nagy G, Moghaddam B, Adams RN (1984) Nafion-coated electrodes with high selectivity for CNS electrochemistry. Brain Res 290: 390–394.

Gillman PK (1999) The serotonin syndrome and its treatment. J Psychopharmacol 13: 100-109.

Giuliano F, Allard J (2001) Dopamine and sexual function. Int J Impot Res 13: S18-S28.

Goetz CG, Tanner CM, Levy M, Wilson RS, Garron DC (1985) Pain in Parkinson's disease. Mov Disorders 1: 45-49.

Goodkin P, Howard BD (1973) Studies on acetylcholinesterase of rat brain synaptosomal plasma membranes. J Neurochem 22: 129-136.

Gordon J, Barnes NM (2003) Lymphocytes transport serotonin and dopamine: agony or ecstacy? Trends Immunol 24: 438-443.

Greenwood TA, Alexander M, Keck PE, McElroy S, Sadovnick AD, Remick RA, Kelsoe JR (2001) Evidence for linkage disequilibrium between the dopamine transporter and bipolar disorder. Am J Med Gen 105: 145-151.

Greenwood TA, Schork NJ, Eskin E, Kelsoe JR (2006) Identification of additional variants within the human dopamine transporter gene provides further evidence for an association with bipolar disorder in two independent samples. Mol Psychiatry 11: 125-133.

Hegerl U, Bottlender R, Gallinat J, Kuss HJ, Ackenheil M, Moller HJ (1998) The serotonin syndrome scale: first results on validity. Eur Arch Psychiatry Clin Neurosci 248: 96-103.

Heijtz RD, Kolb B, Forssberg H (2007) Motor inhibitory role of dopamine D1 receptors: implications for ADHD. Physiol Behav 92: 155-160.

Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, Lesch KP (1996) Allelic variation of human serotonin transporter gene expression. J Neurochem 66: 2621-2624.

Hernandez MT, Sauerwein HC, Jambaque I, De Guise E, Lussier F, Lortie A, Dulac O, Lassonde M (2002) Deficits in executive functions and motor coordination in children with frontal lobe epilepsy. Neuropsychologia 40: 384-400.

Holmes A, Murphy DL, Crawley JN (2003) Abnormal behavioral phenotypes of serotonin transporter knockout mice: parallels with human anxiety and depression. Biol Psychiatry 54: 953-959.

Holmes A, Lit Q, Murphy DL, Gold E, Crawley JN (2003) Abnormal anxiety-related behavior in serotonin transporter null mutant mice: the influence of genetic background. Genes Brain Behav 2: 365-380.

Holroyd CB, Coles MG (2002) The neural basis of human error processing: reinforcement learning dopamine, and the error-related negativity. Psych Rev 109: 679-709.

Hoshi E, Tremblay L, Feger J, Carras PL, Strick PL (2005) The cerebellum communicates with the basal ganglia. Nat Neurosci 8: 1491-1493.

Hu ZL, Cooper M, Crockett DP, Zhou R (2004) Differentiation of the midbrain dopaminergic pathways during mouse development. J Comp Neurol 476: 301-311.

Hyland K (2008) Clinical utility of neurotransmitter metabolite analysis in cerebrospinal fluid. Clin Chem 54: 633-641.

Kilty JE, Lorang D, Amara SG (1991) Cloning and expression of a cocaine-sensitive rat dopamine transporter. Science 254: 578-579.

Insel TR, Mueller EA, Alterman I, Linnoila M, Murphy DL (1985) Obsessive-compulsive disorder and serotonin: is there a connection? Biol Psychiatry 20: 1174-1188.

Isbister GK, Buckley NA, Whyte IM (2007) Serotonin toxicity: a practical approach to diagnosis and treatment. Med J of Aus 187: 361-365.

Isbister GK, Bowe SJ, Dawson A, Whyte IM (2004) Relative toxicity of selective serotonin reuptake inhibitors (SSRIs) in overdose. J Toxicol Clin Toxicol 42: 277-285.

Ishioka N, Oda T, Natake Y, Kurioka S (1990) Analysis and separation of synaptosomal membrane proteins. Neurochem Res 15: 475-481.

Izenwasser S, Rosenberger JG, Cox BM (1992) Inhibition of [3H]dopamine and [3H]serotonin uptake by cocaine: comparison between chopped tissue slices and synaptosomes. Life Sci 50: 541-547.

Jain M, Armstrong RJE, Barker RA, Rosser AE (2001) Cellular and molecular aspects of striatal development. Brain Res Bulletin 55: 533-540.

Jankovic J (2008) Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 79: 368-376.

Jeffrey JJ, Ehlich LS, Roswit WT (1991) Serotonin: an inducer of collagenase in myometrial smooth muscle cells. J Cell Physiol 146: 399–406.

Jonakait GM, Gintzler AR, Gershon MD (1979) Isolation of axonal varicosities (autonomic synaptosomes) from the enteric nervous system. J Neurochem 32: 1387-1400.

Kahlig KM, Javitch JA, Galli A (2004) Amphetamine regulation of dopamine transport: combined measurements of transporter currents and transporter imaging support endocytosis of an active carrier. J Biol Chem 279: 8966-8975.

Kaupinnen RA, Nicholls DG (1986) Synaptosomal bioenergetics. Eur J Biochem 158: 159-156.

Kish SJ, Shannak K, Hornykiewicz (1988) Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. New England J Med 318: 876-880.

Kizer DE, Chan SK (1963) The Effect of Hepatocarcinogenesis upon 5-hydroxytryptophan decarboxylase and serotonin deaminase. Cancer Res 21: 489-495.

Konishi S, Jimura K, Asari T, Miyashita Y (2003) Transient activation of superior prefrontal cortex during inhibition of cognitive set. J Neurosci 23: 7776-7782.

Korpi ER (1982) Kinetics of tryptophan influx into brain slices depleted of sodium and loaded with L-histidine. J Neurochem 40: 428-433.

Kuhar MJ, Ritz MC (1991) The dopamine hypothesis of the reinforcing properties of cocaine Trends Neurosci 14: 299-302.

Krack P, Benazzouz A, Pollak P, Limousin P, Piallat B, Hoffmann D, Xie J, Benabid AL (2004). Treatment of tremor in Parkinson's disease by subthalamic nucleus stimulation. Mov Disorders 13: 907-914.

Lai JC, Clark JB (1976) Preparation and properties of mitochondria derived from synaptosomes. Biochem J 154: 423-432.

Lange KM, Mecklinger L, Walitza S, Becker G, Gerlach M, Naumann M, Tucha O (2006) Brain dopamine and kinematics of graphomotor functions. Hum Mov Sci 25: 492-509.

Leathwood PD (1987) Tryptophan availability and serotonin synthesis. Proc Nutr Soc 46: 143-156.

Leblanc AC. Apoptosis: Techniques and Protocols. 2nd. Totowa: Humana, 2002.

Lebrec D (1990) Portal hypertension: serotonin and pathogenesis. Cardio Drugs Therapy 4: 33-35.

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274: 1527-1531.

Levin HS, Amparo E, Eisenberg HM, Williams DH, High WM, McArdle CB, Weiner RL (1987) Magnetic resonance imaging and computerized tomography in relation to the neurobehavioral sequelae of mild and moderate head injuries. J Neurosurg 66:706-713.

Liu T, Borjigin L (2006) Relationship between nocturnal serotonin surge and melatonin onset in rodent pineal gland. J Circad Rhythms 4:12-13.

Lovinger DM (1999) The role of serotonin in alcohol's effects on the brain. Current Separations 18: 23-29.

McEntee WJ, Crook TH (1990) Serotonin, memory, and the aging brain. Psychopharmacol 103: 143-149.

Michelhaugh SK, Fiskerstrand C, Lovejoy E, Bannon MJ, Quinn JP (2001) The dopamine transporter gene (SLC6A3) variable number of tandem repeats domain enhances transcription in dopamine neurons. J Neurochem 79: 1033-1038.

Miller EK, Cohen JD (2001) An integrative theory of prefrontal cortex function. Annu Rev Neurosci 24: 167-202.

Miller GM, Madras BK (2002) Polymorphisms in the 3'-untranslated region of human and monkey dopamine transporter genes affect reporter gene expression. Mol Psychiatry 7: 44-55.

Miller GW, Yang Y, Gainetdinov RR, Caron MG (2001) Dopamine transporter and vesicular monoamine transporter knockout mice. Meth Mol Med 6: 179.

Minami M, Endo T, Hirafuji M, Hamaue N, Liu Y, Hiroshige T, Nemoto M, Saito H, Yoshioka M (2003) Pharmacological aspects of anticancer drug–induced emesis with emphasis on serotonin release and vagal nerve activity. Pharmacol Ther 99:149–165.

Morgan DG, May PC, Finch CE (1987) Dopamine and serotonin systems in human and rodent brain: effects on age and neurodegenerative diseases. J Am Geriatr Soc 35: 334-345.

Moron JA, Brockington A, Wise RA, Rocha BA, Hope BT (2002) Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. J Neurosci 22: 389-395.

Morris M (2000) Postural instability in Parkinson's disease: a comparison with and without a concurrent task. Gait Posture 12: 205-216.

Mukhin EI, Adrianov OS (1982) Role of the brain dopaminergic system in generalization. Bull Exp Biol Med 94: 1053-1055.

Murphy DL, Andrews AM, Wichems CH, Li Q, Tohda M, Greenberg B (1998) Brain serotonin neurotransmission: an overview and update with an emphasis on serotonin subsystem heterogeneity, multiple receptors, interactions with other neurotransmitter systems, and consequent implications for understanding the actions of serotonergic drugs. J Clin Psychiatry 59: 4-12.

Murphy DL, Lerner A, Rudnick G, Lesch KP (2004) Serotonin transporter: gene, genetic disorders, and pharmacogenetics. Mol Interv 4: 109-123.

Neville M, Morton J, Umemura S (2001) Lactogenesis: the transition from pregnancy to lactation. Ped Clinics North Am 48: 35-52.

Nichols CD, Sanders-Bush E (2001) Serotonin Receptor Signaling and Hallucenogenic Drug Action. Heffter Rev Psych Res 2: 73-80.

Nicola SM, Surmeier J, Malenka RC (2000) Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Annu Rev Neurosci 23: 185-215.

Ormsbee HS, Fondacaro JD (1985) Action of serotonin on the gastrointestinal tract. Proc Soc Exp Biol Med 178: 333-338.

Owens MJ, Nemeroff CB (1994) Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. Clin Chem 40: 288-295.

Packard MG, Knowlton BJ (2002) Learning and memory functions of the basal ganglia. Annu Rev Neurosci 25: 563-593.

Pan Y, Gembom E, Peng W, Lesch KP, Mossner R, Simantov R (2001) Plasticity in serotonin uptake in primary neuronal cultures of serotonin transporter knockout mice. Brain Res Dev Brain Res 126: 125-129.

Parsons JA, Nicoll CA (1971) Mechanism of action of prolactin-inhibiting factor. Neuroendocrinol 8: 213-227.

Patterson DS, Trachtenberg FL, Thompson EG, Belliveau RA, Beggs AH, Darnall R, Chadwick AE, Krous HF, Kinney HC (2006) Multiple serotonergic brainstem abnormalities in suddent infant death syndrome. J Am Med Assoc 296: 2124-2132.

Perez XA (2004) Using chronoamperometry to investigate serotonin and dopamine transporter function. Graduate Thesis, Pennsylvania State University. 161 p.

Perez XA, Bressler AJ, Andrews AM (2007) Determining serotonin and dopamine uptake rates in synaptosomes using high-speed chronoamperometry. Electrochem Meth Neurosci 7: 1-8.

Perez XA, Andrews AM (2005) Chronoamperometry to determine differential reductions in uptake in brain synaptosomes from serotonin transporter knockout mice. Anal Chem 77: 818-826.

Perez XA, Unger E, Andrews AM. Dopamine uptake in SERT knockout mice by chronoamperometry." Poster session presented at: Society for Neuroscience 31st annual meeting; 2001 Nov. 10-15; San Diego, CA.

Perez XA, Bianco LE, Andrews AM (2006) Filtration disrupts synaptosomes during radiochemical analysis of serotonin uptake: comparison with chronoamperometry. J Neurosci Meth 154: 245-255.

Poewe WH, Lees AJ (1988) Dystonia in Parkinson's disease: clinical and pharmacological features. Annals Neurol 23: 73-78.

Popa D, Lena C, Alexandre C, Adrien J (2008) Lasting syndrome of depression produced by reduction of serotonin uptake during postnatal development: evidence from sleep, stress, and behavior. J Neurosci 28: 3546-3554.

Ralph RJ, Paulus MP, Fumagalli F, Caron MG, Geyer MA (2001) Prepulse inhibition deficits and perseverative motor patterns in dopamine transporter knockout mice: differential effects of D1 and D2 receptor antagonists. J Neurosci 21: 305-313.

Ramage AG (2006) The role of central 5-hydroxytryptamine (5-HT, serotonin) receptors in the control of micturition. Br J Pharmacol 147:S120–131.

Ramassamy C, Girbe F, Pincemail J, Christen Y, Costentin J (1994) Modifications of the synaptosomal dopamine uptake and release by two systems generating free radicals: ascorbic acid/Fe2+ and L-arginine/NADPH. Ann N Y Acad Sci 17: 141-152.

Roca M, Parr A, Thompson R, Woolgar A, Torralva T, Antoun N, Manes F, Duncan J (2009) Executive function and fluid intelligence after frontal lobe lesions. Brain 133: 234-247.

Sabol S, Hu S, Hamer D (1998) A functional polymorphism in the monoamine oxidase a gene promoter. Hum Genet 103: 273–9.

Sander T, Harms H, Podschus Jm Finckh U, Nickel B, Rolfs A, Rommelspacher H, Schmidt LG (1997) Allelic association of a dopamine transporter gene polymorphism in alcohol dependence with withdrawal seizures or delirium. Biol Psychiatry 41: 299-304.

Schmidt CJ, Lovenberg W (1985) *In vitro* demonstrations of dopamine uptake by neostriatal serotonergic neurons of the rat. Neurosci Lett 59: 9-14.

Schofield GC, Ho AKS, Southwell JM (1967) Enterochromaffin cells and the 5-hydroxytryptamine content of the colon of mice. J Anat 101:711-721.

Scott ID, Nicholls JD (1980) Energy transduction in intact synaptosomes. Influence of plasma-membrane depolarization on the respiration and membrane potential of internal mitochondria determined *in situ*. Biochem J 186: 21-33.

Scott S, Caird FS, Williams BO (1984) Evidence for an apparent sensory speech disorder in Parkinson's disease. J Neurol Neurosurg Psychiatry 47: 840-843.

Seitz RJ, Stephan KM, Binofski F (2000) Control of action as mediated by the human frontal lobe. Exp Brain Res 133: 71-80.

Sershen H, Debler EA (1987) Effect of ascorbic acid on the synaptosomal uptake of [$^{3}$H]MPP+, [$^{3}$H]dopamine, and [$^{14}$C]GABA. J Neurosci Res 17: 298-301.

Shepherd JJ (1963) Serotonin and Gut Motility. Br Med J 2: 1589.

Singh YS, Sawarynski LE, Michael HM, Ferrell RE, Murphey-Corb MA, Swain GM, Patel BA, Andrews AM (2010) Boron-doped diamond microelectrodes reveal reduced serotonin uptake rates in lymphocytes from adult rhesus monkeys carrying the short allele of the 5-HTTLPR. ACS Chem Neurosci 1: 49-64.

Sommer C (2004) Serotonin in pain and analgesia: actions in the periphery. Mol Neurobiol 30: 117–25.

Sora I, Hall FS, Andrews AM, Itokawa M, Li XF, Wei HB, Wichems C, Lesch KP, Murphy DL, Uhl GR (2001) Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. Proc Natl Acad Sci 98: 5300-5305.

Sora I, Wichems C, Takahaski N, Li XF, Zeng Z, Revay R, Lesch KP, Murphy DL, Uhl GR (1998) Cocaine reward models: conditioned place preference can be established in dopamine- and serotonin-transporter knockout mice. Proc Natl Acad Sci 95: 7699-7704.

Spanagel R, Weiss F (1999) The dopamine hypothesis of reward. Trends Neurosci 22: 521-527.

Star MA, Page ME (2008). "MDMA (3,4-Methylenedioxymethamphetamine)-Mediated Distortion of Somatosensory Signal Transmission and Neurotransmitter Efflux in the Ventral Posterior Medial Thalamus. J Pharmacol Exper Therapeutics 327: 20-31.

Steinhardt RA, Bi G (1994) Cell membrane resealing by a vesicular mechanism similar to neurotransmitter release. Science 263: 390-393.

Sternbach H (1991) The serotonin syndrome. Am J Psychiatry 148: 705-713.

Stunkard AJ, Allison KC (2009) A biobehavioural model of the night eating syndrome. Obes Rev 10: 69-77.

Stuss DT, Ely P, Hugenholtz H, Richard MT, LaRochelle S, Poirier CA, Bell I (1985) Subtle neuropsychological deficits in patients with good recovery after closed head injury. Neurosurg 17: 41-47.

Swanson JM (2000) Dopamine-transporter density in patients with ADHD. Lancet 355: 1461-1462.

Swerdlow NR and Koob GF (1987) Dopamine, schizophrenia, mania, and depression: toward a unified hypothesis of cortico-striato-pallido-thalamic function. Behav Brain Sci 10: 197-245.

Sze JY, Victor M (2000) Food and metabolic signaling defects in a Caenorhabditis elegans serotonin-synthesis mutant. Nature 403 (6769): 560–564.

Tecott LH, Abdallah L (2003) Mouse genetic approaches to feeding regulation: serotonin 5-HT2C receptor mutant mice. CNS Spectr 8: 584–88.

Torres GE, Gainetdinov RR, Caron MG (2003) Plasma membrane monoamine transporters: structure, regulation, and function. Nat Rev Neurosci 4: 13-25.

Turner TJ, Adams ME, Dunlap K (1993) Multiple Ca2+ channels types coexist to regulate synaptosomal neurotransmitter release. Proc Natl Acad Sci 90: 9518-9522.

Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Progress Neurobiol. 56: 613-672.

Ueno S, Nakaumure K, Mikami M, Kondoh K, Ishiguro H, Arinami T, Korniyama T, Mitsushio T, Sano M, Tanabe H (1999) Identification of a novel polymorphism of the human dopamine transporter (DAT1) gene and the significant association with alcoholism Mol Psychiatry 4: 552-557.

Uhl GR, Hall FS, Sora I (2002) Cocaine, reward, movement and monoamine transporters. Mol Psychiatry 7: 21-26.

Unger EL, Eve DJ, Perez XA, Reichenbach DK, Xu Y, Lee MK, Andrews AM (2006) Locomotor hyperactivity and alterations in dopamine neurotransmission are associated with overexpression of A53T mutant human –synuclein in mice. Neurobiol Disease 21: 431-443.

Van Der Wee NJ, Van Heen NJ, Stevens H, Van Vliet IM, Van Rijk PP, Westenberg HG (2008) Increased serotonin and dopamine transporter binding in psychotropic medication – naïve patients with generalized social anxiety disorder shown by $^{123}$I-β-(4-Iodophenyl)-Tropane SPECT. J Nucl Med 59: 757-763.

Vatassery GT, Sheridan MA, Krezowski AM (1981) Spectrophotofluorometry of serotonin in blood platelets. Clin Chem 27: 328-330.

Vorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM (2004) Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci 27: 468-474.

Wheeler DD, Edwards AM, Ondo JG (1993) Dopamine uptake in five structures of the brain: comparison of rate, sodium, dependence, and sensitivity to cocaine. Neuropharmacol 32: 501-508.

Whittaker VP (1962) Pharmacological studies with isolated cell components. Biochem. Pharmacol 9: 61-69.

Whittaker VP (1993) Thirty years of synaptosome research. J Neurocytol 22: 735–742.

Wise RA (1996) Neurobiology of Addiction. Curr Opin Neurobiol 6: 243-251.

Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5: 1-13.

Woodward JJ, Wilcox RE, Leslie SW, Riffee WH (1986) Dopamine uptake during fast-phase endogenous dopamine release from mouse striatal synaptosomes. Neurosci Letters 71: 106-112.

Yang B, Chan RC (2007) A meta-analysis of association studies between the 10-repeat allele of a VNTR polymorphism in the 3'-UTR of dopamine transporter gene and attention deficit hyperactivity disorder. Am J Med Genet B Neuropsychiatry Genet 144B: 541-550.

Yi SJ, Johnson KM (1990) Effects of acute and chronic administration of cocaine on striatal uptake, compartmentalization and release of [3H] dopamine. Neuropharmacol 29: 475-486.

Zimanyi I, Lajtha A, Reith ME (1989) Comparison of characteristics of dopamine uptake and mazindol binding in mouse striatum. Naunyn-Schmiedeberg's Arch Pharmacol 340: 626-632.

Zubieta JK, Alessi NE (1993) Is there a role of serotonin in the disruptive behavior disorders? Child Adolesc Psychopharmacol 3: 11-36.

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**EDUCATION**:

High School Diploma, Conestoga High School (Berwyn, PA), 2006
Bachelor of Science Degree in Biology (Vertebrate Physiology), Pennsylvania State University, 2010
Honors in Veterinary and Biomedical Science
Thesis Title: *Investigating Dopamine Uptake in Mouse Brain Synaptosomes via Chronoamperometry*
Thesis Supervisor: Anne Milasincic Andrews, Ph.D.
Doctor of Medicine Candidate, University of Virginia School of Medicine, 2014

**RELATED EXPERIENCE**:

Internship, Fox Medical Oncology Center (Paoli, PA)
Supervisor: Stephen C. Fox, M.D., F.A.C.P.
*May 2006 – Present*

Honors Undergraduate Research, Huck Institutes of the Life Sciences (University Park, PA)
Supervisor: Anne Milasincic Andrews, Ph.D.
*September 2008 – Present*

Internship, Drexel University College of Medicine (Philadelphia, PA)
Supervisor: Gianluca Gallo, Ph.D.
*Summers 2008, 2009*

Internship, Lankenau Institute for Medical Research (Wynnewood, PA)
Supervisor: Iramoudi Ayene, Ph.D.
*Summer 2007*

**COLLEGIATE AWARDS:**

Class Valedictorian and Student Marshal, Pennsylvania State University Department of Biology, 2010
Dean's List (all semesters)
Evan Pugh Scholar Award (Senior), 2009-2010
Schreyer Ambassador Travel Grant, 2009-2010
Claire L. and Charles L. Miller Alumni Memorial Scholarship, 2009-2010

American Reinvestment and Recovery Act National Institute of Mental Health Summer Research Award, 2009
Schreyer Summer Research Grant, 2009
Evan Pugh Scholar Award (Junior), 2008-2009
Edward C. Hammond, Jr. Memorial Scholarship, 2008-2009
Alpha Epsilon Delta (Pennsylvania Beta Chapter) Inductee, 2008
President Sparks Award, 2007-2008
President's Freshman Award, 2006-2007
Academic Excellence Scholarship (Schreyer Honors College), 2006-2010

**HIGH SCHOOL AWARDS:**

Conestoga All-Scholar Award
AP Scholar with Distinction
PHEAA Certificate of Merit (for the CollegeBoard SAT)
National Honor Society
Service and Leadership Award
Distinguished Honor Roll (all semesters)

**ACTIVITIES:**

Teaching Assistant for Biol472 (Mammalian Physiology)
Amateur Triathlete (USA Triathlon)
Alpha Epsilon Delta (Pennsylvania Beta Chapter)
Research in Costa Rica
Springfield THON
Tutor in Biology and Organic Chemistry
Philosophy Club
Schreyer Honors College Student Council