THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF NUTRITIONAL SCIENCES

IMPACT OF BRAIN IRON LEVELS ON DOPAMINE METABOLISM

SABRINA RUSSO
Spring 2010

A thesis submitted
in partial fulfillment of
the requirements for a baccalaureate
degree in the Nutritional Sciences
with honors in Nutritional Sciences

Reviewed and approved* by the following:

Erica L. Unger
Research Associate of Nutritional Sciences
Thesis Supervisor

Rebecca Corwin
Associate Professor of Nutritional Neuroscience
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Iron (Fe) deficiency is one of the most common nutrient deficiencies in the world. Deficiency may occur due to decreased absorption rates that do not meet an individual's needs or by an excessive rate of excretion via blood loss. Iron deficiency causes a reduction in iron levels in the brain, which causally leads to numerous negative behavioral outcomes.

Fe functions in a number of bodily processes, including oxygen transport, facilitating electron transport in cytochromes, acting as monooxygenases, dioxygenases and peroxidases, and assisting the synthesis of niacin, hydroxylysine, myelin and neurotransmitters. One major neurotransmitter that is dependent upon Fe is dopamine (DA). DA is synthesized via a two-step process, and Fe is the cofactor of the initial step in DA production. Fe deficiency, therefore, may cause a secondary deficiency in DA. Fe and DA deficiencies are related to several disease states, including Restless Legs Syndrome.

To investigate the effects of iron status on dopamine metabolism in the brain, iron levels in the dopamine cell body region, the ventral midbrain, were manipulated and the effects on the dopamine transporter (DAT) in terminal regions (striatum and prefrontal cortex) were investigated. Several experiments were conducted on three groups of rats (Fe sulfate-, saline- and DFO-infusion) to examine how differing levels of brain Fe may affect DA metabolism. Infusion of the iron chelator, desferrioxamine (DFO), resulted in a reduction in prefrontal cortex (PFC) and striatal DAT on the cellular membrane without changing cytosolic DAT levels. Cytosolic levels of PFC DAT were increased in iron-infused rats compared to saline-treated animals. Overall, these data support a role for VMB iron levels in regulating DAT trafficking in dopamine terminal regions.

TABLE OF CONTENTS

AKNOWLEDGEMENTS

First, I'd like to thank my thesis advisor, Erica L. Unger. She has guided me throughout the year, provided advice regarding procedures, assisted in problem-solving in any difficult situations, taught me how to use several computer programs related to my experiments, and revised my thesis a countless number of times. Erica has taught me a great deal, and helped me to write a professional, eloquent thesis, which I am very proud of today. I truly appreciate all her time, guidance and support.

Next, I'd like to thank Laura E. Bianco. She has trained me to do all the necessary laboratory procedures in my thesis research. I came in with very limited experience and knowledge of the workings in a lab-setting, but with Laura's patience, and clear instructions, I learned a range procedures, from dissecting rat livers and spleens to performing gel electrophoresis. I thank her for the huge time-investment and effort she put into training me, and advising me throughout the process.

Lastly, I'd like to thank all other members of the lab. In addition to Erica and Laura, everyone in the lab was welcoming, friendly and supportive for the duration of the year. The group's company, insight, humor and light-heartedness was a true pleasure. I am so happy I have had an opportunity to work with such a wonderful group of individuals, and thank them all again.

Chapter 1: Specific Purpose and Concentrations

Iron (Fe) deficiency affects 2-5 billion people (WHO, 2009) at an estimated economic cost of tens of billions of dollars. This mineral deficiency is typically caused by absorption rates that do not meet an individual's needs or by an excessive rate of excretion via blood loss. Some groups at risk for Fe deficiency include menstruating females (blood loss), children (due to rapid growth and increased needs), vegans (decreased consumption of bioavailable Fe), the malnourished, and pregnant women (fetal requirements). In developing countries, it is estimated that between one-third to one-half of all women and children have iron deficiency anemia (WHO, 2009). The United States has much lower rates; however, it is estimated that 9% of children up to age 2, 12% of pregnant women and 11% of females between the ages of 16-49 y are Fe deficient (Trost, Bergfeld, & Calogeras, 2006).

Fe plays a vital role in a number of functions in the body including oxygen transport, assisting in electron transport in cytochromes, acting as monooxygenases, dioxygenases and peroxidases, and facilitating the synthesis of niacin, hydroxylysine, myelin and neurotransmitters (Patrick, 2007; Pinero & Connor, 2000). Thus, Fe absorption into the body and transport among vital organs are tightly regulated processes. Studies over the last three decades provide extensive evidence that Fe deficiency reduces brain Fe levels and alters dopamine (DA) system homeostasis. DA is synthesized via a two-step process – conversion of the amino acid tyrosine to L-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase and the conversion of LDOPA to DA by aromatic amino acid decarboxylase. The former reaction is the rate limiting step in DA synthesis for which Fe is a necessary cofactor.

In gestational, lactational and postweaning models of Fe deficiency, DA transporter (DAT) and receptor levels are reduced in several brain regions, causing disturbances in DA homeostasis. Rodent models appear to be a relatively good means to study Fe deficiency in humans given their similar impairments in behavioral and cognitive outcomes. In both humans and rodent models, symptoms of Fe deficiency include decreased cognitive ability, reduced attention, increased anxiety, hypoactivity, immune dysfunction, and an inability to regulate body temperature. Other symptoms described in human infants and adults include feelings of apathy, irritability, lethargy, lack of concentration, fatigue, lightheadedness, and breathing difficulties (Pinero & Connor, 2000).

Deficiencies in DA neurotransmission and Fe in the brain are related to Restless Legs Syndrome (RLS) in that the brain profile of Fe deficient rodents appears to have many similarities to the profile observed in RLS

patients. In particular, MRI and autopsy studies have revealed that Fe levels in substantia nigra and striatum are decreased and that DA receptor and transporter binding are reduced in RLS compared to healthy control subjects (Connor, et al., 2009). Thus, an increased understanding of the relationship between brain Fe status and DA metabolism may be beneficial in the treatment of this disorder.

Recent data from this laboratory shows that postweaning Fe deficiency reduces Fe levels in ventral midbrain and striatum, increases striatal extracellular dopamine concentrations, reduces striatal intracellular dopamine concentrations, alters total DAT protein levels in several brain regions, and changes the relative distributions of intracellular versus membrane DAT and DA 2 receptors (D2R) in striatum. Further, infusion of physiological amounts of iron into the Fe deficient ventral midbrain (VMB) restores extracellular dopamine levels to that of Fe sufficient rats. Thus, the overall objective of these experiments is to examine the extent to which VMB Fe status regulates key proteins of the dopamine (DA) system in striatum and prefrontal cortex.

Specific Aim #1: To determine the extent to which reduced VMB Fe levels affect DAT and D2 receptor distribution (membrane versus cytosol) in terminal regions. The ***hypothesis*** is that depletion of VMB Fe levels via desferrioxamine (DFO) infusion will alter the distribution of DAT and D2 receptors in striatum and prefrontal cortex.

Specific Aim #2: To determine the extent to which increased VMB Fe levels affect DAT and D2 receptor distribution (membrane versus cytosol) in terminal regions. The ***hypothesis*** is that the distribution of striatum and prefrontal cortex DAT and D2 receptors on the membrane and in the cytosol will be altered after infusion of Fe into the VMB.

Chapter 2: Background Literature

IRON (Fe)

Fe is a trace mineral usually found in amounts between 2-4 grams in the human body. Fe exists in food as heme Fe and non-heme Fe. Heme Fe is complexed to hemoglobin and myoglobin in animal products such as meat, poultry and fish, while non-heme Fe is found in both animal and plant products, including nuts, grains, vegetables, fruits and tofu.

Absorption of Fe

Fe absorption is the primary mechanism of homeostatic control, due mainly to the fact that there is no efficient way to eliminate excess Fe from the body. Heme Fe is the most readily absorbed form of Fe with absorption rates of 20-40%. After ingestion, heme Fe is first hydrolyzed from the globulin in hemoglobin or myoglobin by trypsin, pepsin and other proteases in the stomach. Absorption of heme Fe then occurs in the duodenum via receptors on the apical membrane. Within the enterocyte or intestinal cell, the porphyrin ring (four pyrrole [C_4H_5N] rings) of the heme group is hydrolyzed by heme oxygenase, which causes the release of inorganic Fe. Heme oxygenase is induced by Fe deficiency, thus increasing Fe absorption, suggesting that a feedback loop exists between Fe stores and heme oxygenase production (Fulton & Jeffery, 1993). This is just one means by which Fe absorption is regulated by the body's needs.

Non-heme Fe absorption is as low as 2% of intake but is absorbed at up to 80% in rare cases. Non-heme Fe digestion occurs in the stomach when hydrochloric acid, pepsin and other proteases free Fe from food. It is important to note that Fe in food is usually found in a Fe^{+3} oxidation state, and that absorption of non-heme Fe is typically less than that of heme Fe because Fe^{+3} (ferric Fe) can complex to form ferric hydroxide ($Fe(OH)_3$) in the intestines. Ferric hydroxide is not absorbed efficiently and must be reduced by duodenal cytochrome b (Dcytb) to Fe^{+2}. In the reduced state, divalent metal transporter 1 (DMT1) can then transport Fe^{+2} across the apical membrane.

There are a number of dietary factors that affect Fe absorption. Factors that increase non-heme Fe absorption include acids (absorbic, citric, lactic, tartaric acids), sugar, meats, poultry, fish and mucins. Factors that inhibit absorption include chelators, such as polyphenols, phytates, oxalates, EDTA (preservative), calcium, phosphorous, and antacids, and competitors, such as zinc, manganese and nickel. Once absorbed, Fe is used by the intestinal cell, stored in the intestinal cell for later use or excretion, or transported into the blood for use by other

cells. Fe is typically transported with proteins and/or amino acids due to its extreme reactivity.

Fe Transport, Storage and Functions

When the body needs Fe, Fe is secreted from the intestinal cell via the Fe exporter ferroportin, a process that is regulated by hepcidin activation. Two copper-containing proteins, hephaestin and ceruloplasmin, then oxidize Fe allowing it to bind to transferrin. Plasma transferrin transports Fe throughout the body, and iron uptake into organs is highly dependent on the number of transferrin receptors located on the membrane. The Fe requirement for bone marrow is high, and it is estimated that 80% of plasma Fe is delivered to bone marrow for red blood cell production. Most Fe that is not needed by the body is stored in the liver (60%), bone marrow and spleen bound to ferritin, the main storage protein for Fe. In response to Fe need, both Fe regulatory proteins (IRP1 and IRP2) and Fe response elements (IREs) work together to control the production of Fe-related proteins, including ferritin and transferrin. In Fe deficiency, low Fe levels increase transferrin production and reduce ferritin protein levels (Beard & Han, 2008).

Cellular Fe transport and distribution must be tightly regulated, because both deficiency and toxicity can cause many deleterious effects. These consequences result from the role of Fe in biological processes including transporting oxygen from the lungs to the tissues as a component of heme, facilitating electron transport in cytochromes, acting as monooxygenases, dioxygenases and peroxidases, and helping to synthesize niacin, hydroxylysine, myelin and neurotransmitters (Patrick, 2007; Pinero & Connor, 2000). As such, Fe deficiency has been associated with decreased cognitive ability, immune dysfunction, reduced work capacity, inability to regulate body temperature and in cases of anemia, fatigue, lightheadedness, breathing difficulties, pica, lighter skin, weak nails and an enlarged spleen have been reported (Pinero & Connor). Fe toxicity has been linked to dizziness, headaches, low blood pressure, coma and death (Frazin, 2003).

Fe and the Brain

Many studies have shown that Fe levels vary by brain region and that regions of the brain accumulate Fe at different rates (Morgan & Moss, 2002). In humans, the striatum, substantia nigra, and brain stem have among the highest Fe concentrations in older adults; while the cortical white matter and thalamus have lower levels (Morgan & Moss). Techniques including magnetic resonance imaging (MRI) can be used measure brain Fe *in vivo* by estimating

the Field-Dependent Relaxation Rate Increase (FDRI) (Morgan & Moss, 2002). Susceptibility-Weighted Imaging (SWI) is also used to measure Fe through MR phase effects (Morgan & Moss, 2002). These two methods of Fe detection have been used extensively to assess brain Fe in both young and elderly adults. Using these techniques, it has been shown that regardless of age, high Fe concentrations are present in the globus pallidus, while Fe levels in the caudate nucleus and putamen increase largely with age (Morgan & Moss, 2002; Ordidge, Gorell, Deniau, Knight & Helpern, 1994).

Fe distribution in the brain may be affected by hemopexin, an acute phase plasma glycoprotein produced primarily by the liver. The glycoprotein is transported into plasma where it binds heme with high affinity, and delivers it to the liver. This process protects against free heme-mediated oxidative stress, limits access by pathogens to heme and contributes to Fe homeostasis by recycling heme Fe. In cerebral spinal fluid (CSF), hemopexin is found in the sciatic nerve (a large nerve that runs from the lower back down towards the lower limb), skeletal muscle, retina, brain and CSF. Hemopexin in CSF is found in increased levels in Alzheimer's patients, which shows that it may be involved in heme detoxification in the brain. Researchers in Torina, Italy observed that the number of Fe-loaded oligodendrocytes in the basal ganglia and thalamus of hemopexin-null mice was double the levels found in the wild-type control group (Morello, et al., 2008). Hemopexin-null mice also showed a significant decrease in the number of ferritin-positive cells in the cerebral cortex and Fe deposits, and increased levels of malondialdehyde (an indicator of lipid peroxidation) and Cu-Zn superoxide dismutase-1 (an antioxidant). Overall, these results suggest that hemopexin may be involved in Fe delivery to and distribution within the brain (Morello, et al., 2008).

A number of studies have been conducted that show that transferrin is intricately involved in Fe transport into the brain. Although transferrin exists in large quantities in the liver, the presence of transferrin mRNA in oligodendrocytes and in the choroid plexus indicates that transferrin is produced de novo in the brain (Takeda, et al, 2002). A group of researchers from the University of Shizuoka investigated the effects of Fe-saturation on peripheral and brain Fe distribution (Takeda, et al., 2002). In these experiments, liver Fe levels in mice injected with Fe were four times that of control, while Fe levels in the brain were only 40% higher than control. Moreover, Fe levels in all regions of the brain in Fe injected mice were elevated 40-50%, except for the choroid plexus, where concentrations were equivalent between groups. These studies were the first to suggest that a controlled brain Fe uptake mechanism existed, and that transferrin bound Fe was likely the form that entered the brain. The high levels of Fe observed in the liver further suggested that non-transferrin bound Fe was primarily taken up by the liver, causing a reduction in

the amount of Fe available for the brain (Takeda et al., 2002).

In a related study in 2002, the mechanism of Fe uptake and developmental changes in Fe transport across the blood-brain barrier were investigated (Morgan & Moss). Importantly, these studies illustrated that BCECS (brain capillary endothelial cells) were the sight of transferrin bound Fe transport into the brain, and that Fe transport is enhanced when the rate of recycling transferrin receptors on BCECs is increased (Morgan & Moss, 2002). Subsequent studies have shown that ferro-transferrin binds to transferrin receptors on the outside of the cell, and that this complex is internalized into an endosome (Figure 1). The pH requirement around the Fe-transferrin molecule is 5.5, such that Fe can separate from transferrin and move into the cytoplasm. Fe transport molecules can move Fe around the cell to become incorporated into various structures and proteins, including mitochondria and ferritin. Ferritin stores excess Fe, and during autophagy, lysosomes engulf ferritin molecules. (Okam, 2001)

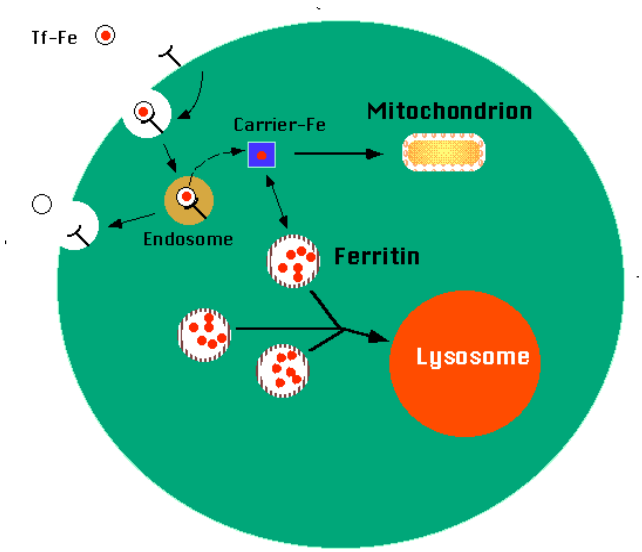

Figure 1: Illustration of Fe transport in a cell via transferrin/transferrin receptor mechanism.

Divalent metal transporter 1 (DMT1), the only known Fe importer, is also located on the blood brain barrier and plays a role in transporting Fe into the brain (Burido, et al., 1999). Much of the research involving the importance of DMT1 in brain Fe uptake comes from studies using Belgrade rats, which have a mutation in DMT1, resulting in a dramatic reduction in the functioning of this protein (Burido, et al., 1999). Immunohistochemical analyses have shown that Belgrade rats have a reduced level of Fe in oligodendrocytes, in myelin of white matter,

and in astrocytes of the cerebral cortex. Despite the dramatic loss of DMT1 function, a significant amount of iron is still present in the brain. These data suggest that while DMT1 is involved in Fe transport into the brain, there are other mechanisms that exist that allow for Fe import in the absence of this protein (Burido, et al., 1999).

Studies using hypotransferrinemic mice show that Fe can also enter the brain via a non-transferrin bound mechanism(s). Hypotransferrinemic mice have a mutation linked to the murine *Trf* locus that causes a deficiency in serum transferrin, resulting in <1% of normal values (Connor, et al., 2009). If mice are not treated with transferrin or red blood cell transfusions before weaning, death from anemia will occur. Treated hypotransferrinemic mice will survive after weaning without further treatment, but suffer from severe Fe deficiency anemia and acquire a severe tissue Fe overload in all nonhematopoietic tissues, including the liver where the Fe burden is 100 fold greater than wildtype mice (Trenor, et al., 2000). This animal model provides an excellent means to study Fe transport into the brain given that previous studies indicated that the transferrin/transferrin receptor Fe uptake mechanism is a major component of the uptake process. Surprisingly, hypotransferrinemic and wildtype mice have similar brain Fe uptake rates indicating that a significant, and possibly unidentified, brain Fe uptake mechanism exists that is independent of transferrin.

Fe Deficiency

Fe deficiency is the most common nutrient deficiency in the world, affecting 2-5 billion people (WHO, 2009). Most cases of Fe deficiency occur if absorption does not meet the body's needs because of low intake of bioavailable Fe or higher Fe requirements, or if too much is eliminated from the body due to excess blood loss, and pathological infections. Those at risk for Fe deficiency include menstruating females, children due to rapid growth and increased needs, vegetarians, the malnourished and pregnant women due to fetal development requirements. One third of all women and children in developing countries have Fe deficiency anemia (WHO, 2009). In the United States, it is reported that 9% of children up to age 2, 12% of pregnant women and 11% of females between the ages of 16-49 y are Fe deficient (Trost, Bergfeld & Calogeras, 2006).

Fe Assessment

Fe status is assessed using measures of plasma ferritin, transferrin saturation, total iron binding capacity or TIBC (how much transferrin is able to carry Fe), serum Fe, erythrocyte protoporphyrin or EPO (a hormone that increases with Fe deficiency), hemoglobin, and hematocrit (percentage of packed red blood cells, RBCs). The RBCs are also studied for their mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), which are both reduced in deficiency. Table 1 details current acceptable values for each Fe status marker.

Table 1: Acceptable Values of Fe Assessment Factors

Iron Status Marker	**Normal Range**
Plasma Ferritin	12-300 ng/mL (males), 12-150 ng/mL (females)
Transferrin Saturation	20-50%
TIBC	240-450 mcg/dL
Serum Iron	60-170 mcg/dL
EPO	0-19 mU/mL
Hemoglobin Concentration	14-18 g/dL (males), 12-16g/dL (females)
Hematocrit Concentration	41- 50% (males), 36-44% (females)

(NIH, 2009)

DOPAMINE (DA)

The DA Neurotransmitter System

Within the DA neurotransmitter system, neurons are densely organized into 4 main pathways, the nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular pathways. The nigrostriatal pathway, which extends DA projections from cell bodies in the substantia nigra pars compacta to striatum, is well known to be important in the initiation and control of movement (Koob & Volkow, 2009). High concentrations of DA also are present in the mesolimbic pathway, which has cell bodies located in the ventral tegmental area (VTA) that terminate throughout the basal forebrain in regions including nucleus accumbens, hippocampus and amygdala (Koob & Volkow). Considered the "reward center" of the brain, the neurochemical responses in these regions are hypothesized to be involved in the reinforcing effects of drugs of abuse. In the third pathway, mesocortical DA projections extend from the VTA-containing cell body region to the frontal, cingular, and entorrhinal cortices. Lastly, high levels of DA are present in the tuberoinfundibular pathway, which regulates the release of hormones from the anterior pituitary via projections from cell bodies in the hypothalamus.

DA Synthesis

DA is synthesized in the brain from the amino acid tyrosine, which is both produced in the periphery from phenylalanine via the enzyme phenylalanine hydroxylase and obtained directly from the diet. The large neutral amino acid carrier transports tyrosine into the brain where it is converted to L-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase, the rate limiting step in DA synthesis. L-DOPA is then metabolized to DA by L-aromatic amino acid decarboxylase (Figure 2). DA is the precursor of the other catecholamine neurotransmitters, norepinephrine and epinephrine.

DA is transported into vesicles by VMAT-2 and released into the synapse upon stimulation of the presynaptic neuron by an action potential (Figure 3). DA can be metabolized into dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) or 3-methoxytyramine (3-MT). Conversion of DA to DOPAC occurs after reuptake of DA into the neuron via MAO. In the extracellular space, DA can be metabolized to 3-MT by catechol-O-methyltransferase (COMT), which can also be sequestered by dopaminergic neurons and then converted to HVA by MAO.

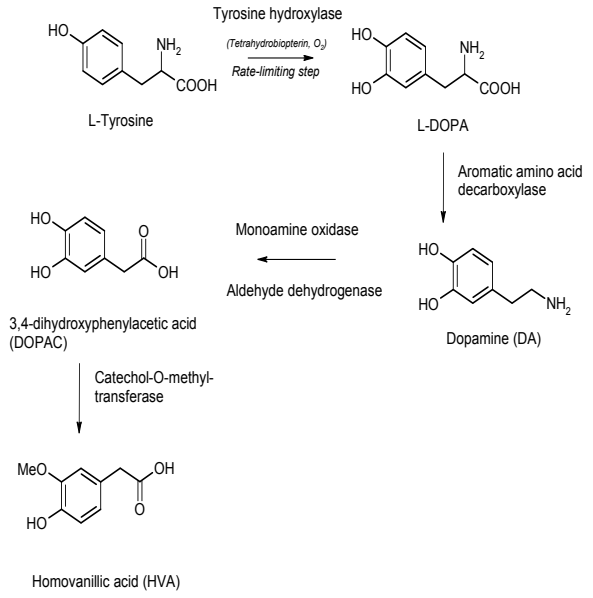

Figure 2: Synthesis and metabolism of DA

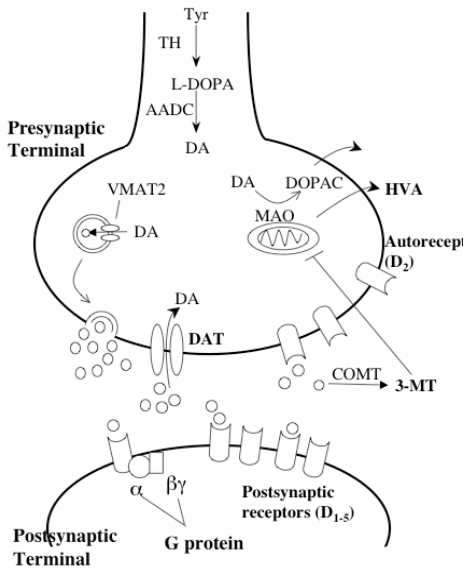

Figure 3: Simplified illustration of presynaptic dopamine neuron and a postsynaptic terminal. Tyr, tyrosine; TH, tyrosine hydroxylase; L-DOPA, L-dihydroxyphenyl-alanine; AADC, L-aromatic amino acid decarboxylase; DA, dopamine; MAO, monoamine oxidase; DOPAC, dihydroxyphenylacetic acid; COMT, catechol-O-methyltransferase; 3-MT, 3-methoxytyramine; HVA, homovanillic acid; DAT, dopamine transporter.

DA Regulation

The major regulator of extracellular DA levels in the brain is the DA transporter (DAT). DAT is a 12 transmembrane domain plasma membrane transporter that carries DA into presynaptic neurons to terminate its signal (Giros et al., 1992). Multiple amino acid residues are responsible for the binding of DA to DAT. Phosphorylation of DAT by PKC regulates the movement of this transporter protein off of the neuronal membrane and helps to mediate the ubiquitylation (the post-translational modification of a protein by the covalent attachment of ubiquitin monomers) and the subsequent degradation of DAT (Miranda, et al., 2005), thus regulating DA uptake.

The action of DA is also controlled by the dopamine receptors, D_1-D_5, which are classified as either D_1-like or D_2-like. (Civelli, 2000). These receptors interact with G protein complexes that are excitatory (Gs) or inhibitory (Gi). The D_1 and D_5 receptors interact with the Gs complex, activating adenylyl cyclase, while the D_2, D_3 and D_4 receptors interact with Gi, inhibiting cAMP production (Civelli, 2000). Only the D_2 receptor is located both presynaptically as an autoreceptor and postsynaptically. All of the other DA receptor subtypes are present on the postsynaptic membrane.

Iron deficiency, DA and behavior

Altered monoamine metabolism in the brain is a consistent finding in Fe deficiency animal models. Fe deficient rats consistently show reduced levels of DAT, D_1R, and D_2R, reduced concentrations of intracellular DA and increased concentrations of extracellular DA (Erikson, Jones, Hess, Zhang & Beard, 2001; Beard, Erikson & Jones, 2003; Unger, Wiesinger, Hao & Beard, 2008; Wiesinger, Buwen, Cifelli Unger, Jones & Beard, 2006.) In the study by Beard, et al (2003), both latational and postweaning Fe deficient rats showed reduced levels of brain Fe in striatum and nucleus accumbens that were correlated with decreases in DAT. Prefrontal cortex (PFC) iron was also reduced, but unlike other regions, DAT and D2R densities were increased compared to control levels. These data suggest that the mechanisms surrounding DA system regulation in response to Fe deficiency differ among brain regions. All of the studies within the last 15 years also indicate that Fe supplementation during lactation (preweaning) is within the critical period to correct brain neurotransmitter deficits, while supplementation post-weaning does not completely ameliorate the effects.

Changes in brain Fe status and dopaminergic functioning have been correlated with a number of behavioral changes in Fe deficient animals. Fe deficient rats are less active, are more hesitant to explore a novel environment, show decreased stereotypic behavior, are more fearful, and have reduced cognitive function compared to control rats (Beard et al., 2003; Youdim & Yehuda, 1989; Felt, et al; Pinero, Li, Connor & Beard, 2000; Beard, Erikson & Jones, 2002). In particular, reductions in VMB Fe and D_1R levels are related to reduced exploration and repeated movements in a novel environment, respectively. Additionally, changes in PFC DAT and D_1R densities are associated with observed increases in anxiety-like behaviors (Beard, Erikson & Jones, 2002). Other evidence suggests that Fe deficient mice have altered circadian rhythms of motor activity and body temperature regulation that are related to changes in circadian DA rhythms in striatum (Unger, 2009).

Individuals with Fe deficiency report symptoms that are similar to the behavioral changes observed in Fe deficient animal models. They present with feelings of apathy, lethargy, lack of concentration, increased anxiety, hypoactivity, altered perception and motivation, and decreased cognition, memory and attention (Beard, Erikson, & Jones, 2003; Beard & Connor, 2003; Piñero, Jones, & Beard, 2001; Youdim & Yehuda, 2000). In children, Fe deficiency is also associated with growth retardation and developmental delays, which are also observed in early ID rats (Pinero et al., 2001; Unger, Paul, Murray-Kolb, Felt, Jones, & Beard, 2007). Overall, these changes in behavior likely result from hypomyelination, impaired dopaminer function, and delayed neuromaturation (Beard et al., 2003).

Fe and DA-Related Diseases

Both excess and loss of DA and Fe have been linked to disease processes (Siegenthaler, 2003). DA deficiency in the striatum or substantia nigra and excess Fe in the substantia nigra likely contribute to Parkinson's disease-like symptoms (Siegenthaler; Bauminger, Barcikowska, Friedman, Galazka-Friedman, Hechel, & Nowik, 2005). Parkinson's disease is a neuronal disorder that is pathologically characterized by loss of neurons in the nigrostriatal DA pathway. This degenerative disorder progresses slowly and causes slowness in movement, rigidity, postural instability and tremors. In contrast low Fe levels in the CNS and reductions in dopamine indices in substantia nigra have been linked to Restless Leg Syndrome (RLS), a neurological movement disorder, which causes uncomfortable sensations in the legs typically during early evening hours (Patrick, 2007). Symptoms of RLS are alleviated by Fe treatments, and the worsening of symptoms at night may be related to altered circadian variations in Fe and/or DA levels ("Glossary…Q-Z"). In light of these disease processes, the connection between Fe homeostasis and DA system functioning is relevant, and an understanding of these mechanisms is an important contribution to Fe biology.

Chapter 3: Methods

Animals

Fifteen male Sprague Dawley rats were purchased from Harlan (Indianapolis IN) and housed 2 animals/cage. Upon arrival, animals were fed LabDiet 5001 rodent diet (Purina Mills LLC, Gray Summit, MO) containing 270 ppm Fe and had access to deionized distilled water *ad libitum*. All animals were maintained in a temperature (23 ± 2°C) and humidity (40%) controlled room on a 12:12 hr light/dark cycle (lights on at 0600). All protocols were approved by the Pennsylvania State University Animal Care and Use Committee.

Rat Surgery

At 120 d of age, rats were anesthetized with 3-5% isoflurane. The head of each animal was then shaved, opthamalic ointment was placed over their eyes, and the skin was then cleaned with providone/iodine solution and 70% ethanol in preparation for surgery. Animals were placed on a stereotaxic frame using the ear bars to stabilize the head, and sterile scissors were used to cut a small hole in the skin to expose the skull. A sterile surgical swab with 10% H_2O_2 was used to dissolve any tissue from the bone and to identify bregma. A small hole (<0.5 mm) was drilled into the skull using the coordinates determined in a rat brain atlas (Swanson 1998). The defined coordinates were: A-2.0, V -5.7, L -6.5. A sterile guide was then lowered into the hole and secured by dental cement. Animals were allowed to recover from surgery for 3 d before infusions.

Ventral Midbrain Infusion Cannula

Infusion and guide cannulas were made in house from stainless steel hypodermic tubing (Small Parts inc, Miramar, FL). The guide cannulas were assembled from 1.5 – 2.5 cm lengths of 26 gauge stainless steel tubing (O.D 0.018 in and I.D. 0.012 in) and 3.0 cm lengths of stainless steel wire (O.D. 0.0095 in). Infusion cannulas were assembled from 3.0 cm lengths of 32 gauge stainless steel tubing (O.D. 0.009 in, I.D. 0.004 in) and pieces of PE 10 and 50 polyethylene tubing (Becton and Dickinson and company, Parsippany, NJ). After assembly, the infusion cannulas were cut so that each cannula was 2 mm longer than its guide.

Iron Infusion

After 3 days of recovery from surgery, rats were anesthetized with 3-5% isoflurane, and then an infusion cannula (2mm) was inserted into the guide cannula. Rats were perfused with either 1 µM $FeSO_4$ (n=5 rats), 1 µM desferroxamine (DFO; n=5 rats) or artificial cerebral spinal fluid (aCSF; n=5 rats) for 20 minutes (1.3 µL/min). Rats were euthanized 16 hr post infusion.

Rat euthanasia and dissection

Animals were euthanized at the termination of the experiment by using CO_2 asphyxiation. Brains were rapidly removed and dissected for striatum (ST), nucleus accumbens (NA), prefrontal cortex (PFC) and ventral midbrain (VMB). Blood was collected at sacrifice into heparinized microcapillary tubes for hemoglobin and hematocrit determination (Pinero et al., 2001). Hemoglobin levels were determined photometrically using cyanomethemoglobin standard solution (Sigma Aldrich), and hematocrit was calculated after centrifugation (5 min at room temperature) of blood samples in heparinized microcapillary tubes. Additional blood was collected and centrifuged (5,000 X g, 15 min), and the remaining serum was collected and stored at -20°C until analysis for serum iron and total iron binding capacity. Livers were collected and frozen at -80°C until assessment of iron levels by colorimetric assay (Pinero et al., 2001).

Tissue Homogenization

To prepare samples for western blot analysis, brain tissues were separated into membrane and intracellular fractions using a Mem-PER kit and according to the manufacturer's instructions (Thermo Scientific, Rockford, IL). To each brain region, a total of 200 µ L of tris-buffered saline (TBS) was added for every 20 mg of tissue. Samples were vortexed briefly, and the wash was discarded. Tissues were then homogenized on ice in 200 µ L of TBS (RZR30 Heidolph teflon homogenizer, Heidolph, Polyscience, Niles, IL), passed 10 times through a 28G needle (Becton Dickinson, Franklin Lakes, New Jersey) to further homogenize the tissue, and \centrifuged at 1070 x g for 5 minutes at 4˚C. The supernatant was discarded and the pellet was resuspended in 150 µ L Reagent A (Eukaryotic Membrane Protein Extraction Reagent [Mem-PER] - Product #89826). The samples were then incubated for 10 min (room temperature) with occasional vortexing, and placed on ice. A total of 450 µ L of Reagent C (diluted as

indicated in the instructions) was added to each tube of lysed cells and vortexed. Tubes were incubated on ice for 30 min with vortexing every 5 min, and then centrifuged at 10,710 x g (3 min, 4˚ C). The supernatant was transferred to a new tube, incubated for 20 min (37˚C), and centrifuged (2 min, 12080 x g, room temperature) to isolate the hydrophobic fraction (i.e., the membrane protein portion) from the hydrophilic fraction. The hydrophilic phase (top layer) was removed from the hydrophobic protein phase (bottom) layer; separations were performed as quickly as possible, before the interface between the layers disappeared. Separated fractions were placed on ice or frozen at -80˚ C. The bicinchoninic acid (BCA) protein assay was used to determine protein content in each fraction with bovine serum albumin as the standard.

Protein Determination by Western Blot

The separating gel was made according to the following recipe:

9.4 mL	Acrylamide
16.4 mL	nanopure H_2O
8.8 mL	1.5 M Tris-HCl Buffer (pH 8.8)
100 µL	10% Sodium Dodecyl Sulfate (SDS)
150 µL	Ammonium Persulfide (APS)
15 µL	Tetramethylethylenediamine (TEMED)

The separating gel was cast, and isopropanol was added to produce a level surface. After the gel solidified (40-60 min), the isopropanol was removed, and the stacking gel was then prepared according to the following recipe:

1.3 mL	Acrylamide
6.1 mL	nanopure H_2O
2.5 mL	0.5 M Tris-HCl Buffer (pH 6.8)
100 µL	10% SDS
50 µL	APS
12.5 µL	Tetramethylethylenediamine (TEMED)

The stacking gel was poured over the separating gel, and a comb was placed between the glass plates in the stacking gel to form wells. After the stacking gel solidified (20-30 min), the comb was removed from the gel and the gel was placed into an electrophoresis running tank containing Tris-Glycine-SDS (TGS**)** buffer. Samples containing 25 µ g of protein (25 µ L total volume) were loaded into each well. A protein ladder (Santa Cruz) was loaded to identify molecular weights of separated proteins. Samples were then separated by electrophoresis at 40 V for 12 hr at room temperature.

Gel Transfer

Sponges, filter papers, nitrocellulose blotting paper, and the separating gel were equilibrated in transfer buffer (TGS, 20% MeOH). The transfer cassette was opened and laid flat on the table. On the clear side of cassette, a sponge, a piece of filter paper and the blot paper were layered, making sure that the blotting paper wasn't moved and that there were no air bubbles under the paper. The gel was carefully lifted, laid on top of blotting paper, and the second piece of filter paper and sponge were layered on top. The transfer cassette was then secured, and placed in transfer tank for transfer of proteins onto the nitrocellulose membrane (70 V, 2 hr). After transfer, the nitrocellulose membrane was removed from the transfer cassette, rinsed in PBS-Tween 20 (PBS-T), and then blocked in 5% dry milk in PBS-T for 1 hr (room temperature).

The following recipe was used to make TBS:

3.03g	25 mM Tris
14.4g	192 mM Glycine
1.0g	0.1% SDS
1.0L	nanopure H_2O

The following recipe was used to make PBS-T:

100 ml	10X PBS
900 ml	nanopure H_2O
0.5 ml	Tween 20

Protein determination

Membranes were washed 3 times (5 min each, room temperature) in PBS-T while shaking. A solution of 5% dry milk in PBS-T was made with the appropriate concentration of primary antibody (Table 2), and the membrane was incubated with the primary antibody for 1 hr while shaking (room temperature). After incubation with the primary antibody, membranes were again washed 3 times (5 min each, room temperature) in PBS-T. Secondary antibodies were diluted in 5% dry milk in PBS-T (Table 2) and then incubated with the membrane for 1 hr (room temperature). Membranes were washed in TBS-T (4X, 10 min, room temperature) prior to film development.

Table 2: Concentrations of Antibodies and Corresponding Brain Region

Brain Region	Primary Antibody	Concentration	Secondary Antibody	Concentration
Prefrontal Cortex	DAT, Santa Cruz	1:1000	Anti Rat	1:2000
Prefrontal Cortex	Actin, Santa Cruz	1:4000	N/A	N/A
Striatum	DAT, Santa Cruz	1:1000	Anti Rat	1:2000
Striatum	Actin, Santa Cruz	1:4000	N/A	N/A

Film Development

Membranes were incubated for 1 min in Supersignal West Pico Chemiluminescent substrate (Thermo Scientific, Rockford, IL) for film development. The membrane was then placed between two plastic sheet protectors that were smoothed over to ensure there were no bubbles. In the dark room, CL-XPosure (Thermo Scientific) film was placed over the blot and secured in a cassette for up to 10 min. Time exposure was dependent on the antibody and brain region utilized. After the appropriate amount of development time, the film was removed and developed.

Table 3: Developing Times for Antibodies and Corresponding Brain Regions

Brain Region	Primary Antibody	Groups	Developing Time
Prefrontal Cortex	DAT	Fe, Saline	5 minutes
Prefrontal Cortex	DAT	DFO, Saline	5 minutes
Prefrontal Cortex	Actin	Fe, Saline	10 minutes
Striatum	DAT	Fe, Saline	2 minutes
Striatum	DAT	DFO, Saline	2 minutes
Striatum	Actin	Fe, Saline	5 minutes

Statistics

Data are shown as means ± standard error of the mean (SEM). A student's T-test was performed to determine differences in integrated densities between DFO and vehicle treated and iron and vehicle treated groups. Significance was set at $P<0.05$.

Chapter 4: Results

In this study, we investigated the effects of VMB iron status on dopamine transporter regulation in terminal regions of prefrontal cortex and striatum. Data are shown as integrated densities of western blots, and results were compared between saline and DFO groups and saline and Fe groups in each blot. In the membrane fraction of the PFC, DAT protein levels were significantly greater in iron-treated rats compared to saline-treated rats ($p<0.05$; Figure 1). DFO treatment had the opposite effect, with membrane DAT protein levels being lower in DFO-treated rats relative to saline-treated rats ($p<0.05$; Figure 1). Intracellular DAT levels in PFC were not significantly different between iron- and saline-treated rats ($p=0.2522$) or DFO- and iron-treated rats ($p=0.1459$). Actin levels were not different between treatment groups, confirming that loaded protein levels were similar.

Results are below in the form of integrated densities (top) of each blot and representative blot images (bottom):

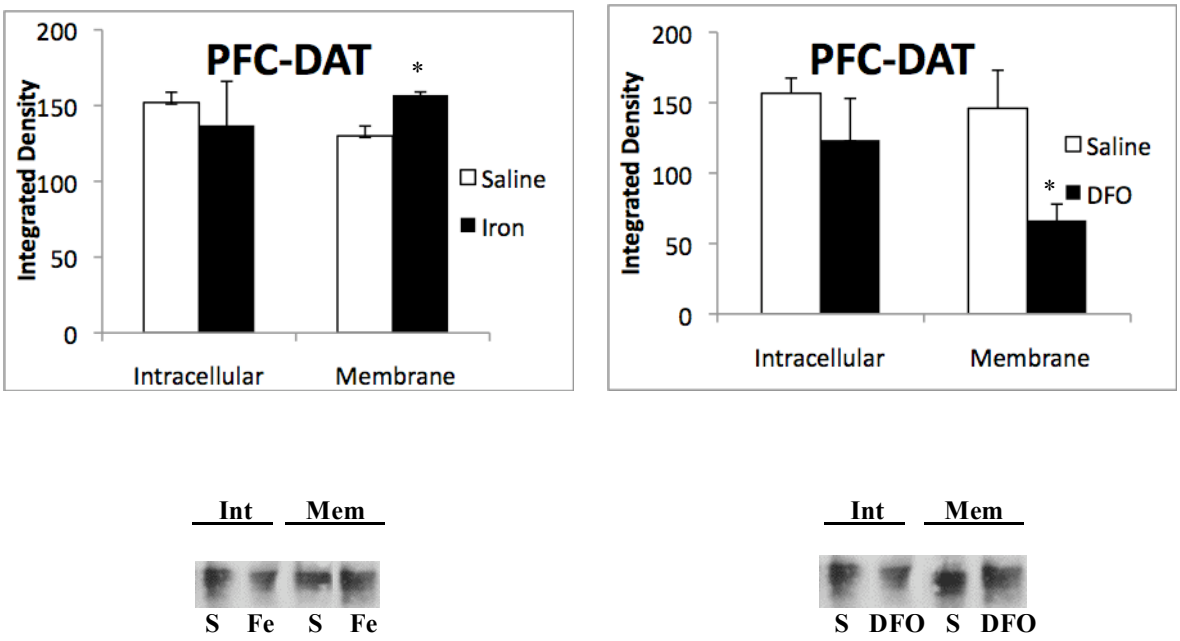

Figure 1. Membrane and cytosolic DAT levels in PFC. Rats were infused with either saline, iron or DFO and intracellular (Int) and membrane (Mem) fractions of DAT were collected and measured by western blot. Western blots were quantified, and the data are shown as integrated densities. *$p<0.05$ relative to saline-treated rats.

Membrane levels of DAT in ST were similar between saline- and Fe-treated rats, while the intracellular levels of DAT were reduced in iron-treated rats compared to controls ($p<0.05$; Figure 2). DFO-treated rats showed significant reductions in both membrane ($p<0.05$) and intracellular ($p<0.05$) levels of striatal DAT. Again, actin levels were not different between treatment groups.

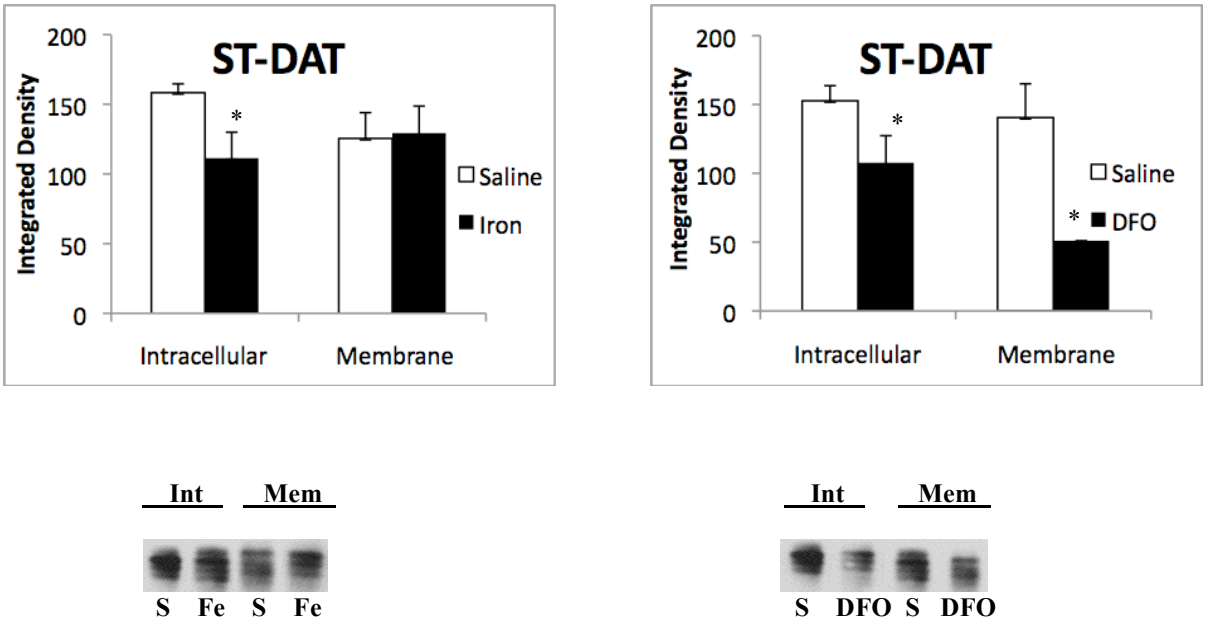

Figure 2. Membrane and cytosolic DAT levels in ST. Rats were infused with either saline, iron or DFO and intracellular (Int) and membrane (Mem) fractions of DAT were collected and measured by western blot. Western blots were quantified, and the data are shown as integrated densities. *$p<0.05$ relative to saline-treated rats.

Chapter 5: Discussion

The purpose of this study was to investigate the effect of VMB Fe status on DAT localization (membrane versus cytosol) in DA terminal regions. Previous data from this research group have shown that Fe deficiency reduces functional DAT levels and increases extracellular DA in the striatum (Bianco, 2008). Moreover, Fe infusion into the Fe deficient VMB corrects extracellular and intracellular DA levels to that of Fe sufficient rats. These experiments serve to investigate whether modulation of Fe status in the VMB can alter membrane and cytosol pools of DAT in ST and PFC, and thus change extracellular DA levels. The two original hypotheses stated that reduced VMB Fe levels (via DFO infusion) and increased VMB Fe levels (via Fe sulfate infusion) would alter membrane and cytosol levels of DAT in the ST and PFC regions of the brain.

Extensive studies have been conducted over the past thirty years, which conclude that Fe deficiency reduces brain Fe levels and affects DA metabolism and functioning (Erikson, Jones, Hess, Zhang & Beard, 2001; Beard, Erikson & Jones, 2003**;** Unger, Wiesinger, Hao & Beard, 2008; Wiesinger, Buwen, Cifelli Unger, Jones & Beard, 2006). DAT is the key regulator of DA uptake that is present in high levels in ST and nucleus accumbens (NA), and to lesser extent in substantia nigra, ventral tegmentum and PFC (Giros et al., 1992). The protein expression of this transporter protein is consistently reduced in ST by brain Fe deficiency, leading to increased striatal extracellular DA levels. The mechanisms involved in this regulation have yet to be described. Given that Fe is present in significant levels in substantia nigra and ventral tegmentum, it is reasonable to suggest that Fe levels in these cell body regions can alter not only transcription and translation of DAT, but also regulatory signaling events important for DAT function (Giros et al., 1992). We chose the PFC and ST for these studies because they are key regions involved in behaviors that are altered by Fe deficiency. The cell bodies of the mesolimbic pathway are present in the ventral tegmentum, and their axons terminate in several regions including PFC (Koob & Volkow). This DA pathway is involved in the regulation of cognition and emotion, which both show deficits in Fe deficiency (Beard, Erikson & Jones, 2002). The nigrostriatal pathway, in which DA projections extend from the substantia nigra of the VMB, plays a key role in the control of movement, which is also significantly altered by low Fe status (Koob & Volkow).

In the current study, membrane DAT levels were reduced in PFC and striatum after DFO infusion into VMB. DFO is a metal chelator that has a higher affinity for Fe than all other metals. Therefore, infusion of DFO into VMB can produce a regional Fe deficiency by binding available iron. Previous studies have shown a reduction in

total DAT protein levels in PFC and striatum in Fe deficient animals, thus the observations in this study agree with previous findings (Erikson, Jones, Hess, Zhang & Beard, 2001; Beard, Erikson & Jones, 2003**;** Unger, Wiesinger, Hao & Beard, 2008; Wiesinger, Buwen, Cifelli Unger, Jones & Beard, 2006). The cytosolic fractions of DAT were not different from saline-treated controls, suggesting that the change in regulation of DAT may occur via signaling at the cell membrane. DA uptake into the presynaptic membrane is dependent on membrane DAT expression; thus the reduction in membrane expression of DAT helps to explain the increased levels of extracellular dopamine observed in Fe deficient animals. The fact that reductions in VMB iron alone regulate DAT movement in ST is a novel observation. Previous research investigating this interaction has been limited by Fe deficiency in several brain regions in Fe deficient animals, and thus there is a lack of knowledge as to which brain regions are specifically involved in regulation of striatal DAT.

Infusion of Fe into the VMB increased membrane DAT in the PFC, which is the opposite effect that was observed in DFO-treated animals. In the ST, membrane DAT levels were unaffected while intracellular DAT levels were reduced. These data suggest that different mechanisms are involved in regional DAT regulation when excess Fe is available in the cell body region. The amount of Fe sulfate that was added to VMB has been shown by our lab to not produce oxidative stress in the brain. Further, Fe staining revealed that Fe sulfate did not diffuse out of the VMB region. Thus, changes in DAT in ST and PFC are unlikely the result of free radical damage or diffusion of infused Fe into other brain regions.

These data have implications for disease states including Fe deficiency and Fe deficiency anemia. Rodent models, such as rats, have been used in a number of studies concerning Fe deficiency due to the fact that they exhibit many similar behavioral and cognitive outcomes to Fe deficient humans (Pinero & Connor, 2000). In both humans and rodent models, symptoms of Fe deficiency include decreased cognitive ability, reduced attention, increased anxiety, hypoactivity, immune dysfunction, and an inability to regulate body temperature (Pinero & Connor, 2000). The DA neurotransmitter system is known to be involved in all of these behavioral processes at various levels. To understand why Fe deficiency leads to the described behavioral outcomes, we must understand the mechanisms involved. This series of experiments is one step in that process.

Fe deficiency and subsequent DA deficiency are also directly related to symptoms of RLS. RLS is a sensorimotor movement disorder, which causes uncomfortable sensations in the legs typically during early evening hours (Patrick, 2007). Symptoms worsen in the evening (prior to sleep) with a clear protective period in the early

morning hours. Studies have shown that DAT and D2 receptor levels are correlated with RLS severity scores; the worse the RLS symptoms, the lower the DAT and D2 receptor levels in striatum (Patrick, 2007). Intravenous Fe treatment has been shown to ameliorate RLS symptoms, but the mechanisms involved are unknown. These data, along with our microdialysis data (described above) may provide an indication of the underlying processes involved in this treatment strategy.

Summary

Fe is a key micronutrient needed for various bodily functions, including DA production. A number of disease states are correlated to Fe deficiency, including Fe deficiency anemia and RLS. RLS is associated with a secondary decline in DA production due to reduced Fe concentrations. Decreased levels of DA markers, such as DAT, in various brain regions, including the ST and PFC, are consistent findings in Fe deficient animal models, which display concurrent DA deficiencies. In the future, further research on additional DA and Fe-related proteins and brain regions may benefit in developing a deeper understanding of the underlying mechanisms seen in Fe deficient states.

Future Implications

In addition to measuring DAT levels in the PFC and ST, several other proteins can be measured, and additional brain regions may be assessed when comparing Fe status and DA metabolism. Proteins such as transferrin, D1R and D2R have been analyzed in previous studies as Fe and DA markers. Other brain regions have also been assessed in previous studies, including the nucleus accumbens (NA) and VMB.

Transferrin acts to transport Fe throughout the body and to assist with organ uptake of Fe, with receptors located on the membrane. Preliminary studies suggest that transferrin is produced de novo in the brain, and that transferrin bound Fe is the predominant form that entered the brain (Takeda, et al, 2002). In Fe deficiency, transferrin receptor expression increases in order to transport as much Fe as possible, partially compensating for decreased Fe levels (Beard & Han, 2008). Transferrin, therefore, could be used to decipher the extent to which Fe transport may differ between control, DFO-treated, and Fe-treated groups.

D1R and D2R are two subtypes of DA receptors. D1 receptors are located on the postsynaptic membrane, and D2R is found both presynaptically as an autoreceptor and postsynaptically (Civelli, 2000). Numerous studies

have shown that D_1R, and D_2R are reduced in Fe deficiency in ST, NA and VMB (Erikson, Jones, Hess, Zhang & Beard, 2001; Beard, Erikson & Jones, 2003; Unger, Wiesinger, Hao & Beard, 2008; Wiesinger, Buwen, Cifelli Unger, Jones & Beard, 2006). To date, no studies have investigated the influence of Fe status on membrane and cytosolic levels or these DA receptor subtypes. Previous studies though have indicated that D2R regulates DAT movement on and off the cellular membrane via a mechanism involving protein kinase C. Therefore, future investigations as to the mechanisms involved in these interactions may prove insightful into understanding the relationship between Fe deficiency and specific behavioral outcomes

Continuing research on Fe and DA levels in the brain can help create a deeper understanding of the underlying mechanisms and consequences of Fe and DA deficiencies. By analyzing the PFC, ST, VMB and NA regions of the brain for markers such as DAT, transferrin, D1R, and D2R, additional data can be collected regarding the effects of altered brain Fe levels on DA metabolism. This research may be an important scientific contribution in regard to the neurological outcomes of brain Fe deficiency.

References

Actin (2009). Retrieved April 14, 2010, from the Medical Dictionary website: http://medical-dictionary.thefreedictionary.com/actin.

Bauminger, E. R., M. Barcikowska, A. Friedman, J. Galazka-Friedman, D. Hechel, & Nowik, I (2005, August 15). Does Iron Play a Role in Parkinson's Disease? *Hyperfine Interactions Journal,* 91(1), 853-857.

Beard, J. & Connor, J. R. (2003, April 10). Iron Status and Neural Functioning. *Annual Reviews Journals* 23, 41-58.

Beard, J. & Han, O. (2008, September 30). Systemic Iron Status. *Biochimica et Biophysica Acta,* 1790 (7), 584-588.

Beard, J., Erikson, K. M. & Jones, B. C. (2002, August 21). Neurobehavioral analysis of developmental iron deficiency in rats. *Behavioral Brain Research Journal*, 134(1-2), 517-24.

Beard, J., Erikson, K. M. & Jones, B. C. (2003, April). Neonatal Iron Deficiency Results in Irreversible Changes in Dopamine Function in Rats. *The Journal of Nutrition*, 133, 1174-1179.

Burido, J. R., Martin, J., Menzies, S. L., Dolan, K. G., Romano, M. A., Fletcher, R. J., Garrick, L. M. & Connor, J. R. (1999) Cellular distribution of iron in the brain of the Belgrade rat. *Neuroscience Journal*, 93 (3), 1189-1196.

Cameron, T. C., Campagna, D. R., Sellers, V. M., Andrews, N. C. & Fleming, M. D. (2000, August). The Molecular Defect in Hypotransferrinemic Mice. *Blood Journal,* 96, 1113-1118.

Civelli, O. (2000). *Molecular Biology of the Dopamine Receptor Subtypes*. Retrieved July 2009, from ACNP website: http://www.acnp.org/G4/GN401000014/CH014.html.

Connor, J. R., Wang, X, Allen, R.P., Beard, J. L., Wiesinger, J.A., Felt, B. T. & Earley, C. J. (2009, May 25). Altered Dopaminergic Profile In Putamen and Substantia Nigra in Restless Leg Syndrome. *Brain: A Journal of Neurology*, 132 (9), 2403-2412.

Encyclopedia (2009, February 26). Retrieved September 13, 2009, from National Institute of Health website: http://www.nlm.nih.gov/medlineplus/encyclopedia.html.

Erikson, K. M., Jones, B. C. & Beard, J. L. (2000, August 2) Iron Deficiency Alters Dopamine Transporter Functioning in Rat Striatum. *The Journal of Nutrition*, 130, 2831-2837.

Erikson, K. M., Jones, B. C., Hess, E.J., Zhang, Q. & Beard J.L. (2001). Iron Deficiency Decreases Dopamine D1 and D2 Receptors in Rat Brain. *Pharmacology, Biochemistry, and Behavoir*, 69(3-4), 409-18.

Erikson, K. M., Pinero, D. J., Connor, J. R., & Beard, J. L. (1997, October 10). Regional Brain Iron, Ferritin and Transferrin Concentrations during Iron Deficiency and Iron Repletion in Developing Rats. *The Journal of Nutrition*, 127 (10), 2030-2038.

Felt, B. T., Beard, J. L., Schallert, T., Shao, J., Aldridge, J. W., Connor, J. R., Georgieff, M. K. & Lozoff, B. (2006, August 10). Persistent neurochemical and behavioral abnormalities in adulthood despite early iron supplementation for perinatal iron deficiency anemia in rats. *Behavioral Brain Research Journal,* 171(2), 261-70.

Frazin, N. (2003, August 11). *Study Links Restless Leg Syndrome to Poor Iron Uptake in Brain.* Retrieved August 2009, from National Institute of Neurological Disorders and Stroke website: http://www.ninds.nih.gov/news_and_events/news_articles/news_article_rls_ iron.htm.

Fulton, B. & Jeffery, E. H. (1993, March 28) *Heme oxygenase induction*.

Giros, B., Mestikawy, S., Godinot, N., Zheng, K., Han, H., Yang-Feng, T. & Caron, M. G. (1992, September). Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Molecular Pharmacology*, 42(3), 383-390.

Hill, J. M., Ruff, M. R., Weber, R. J. & Pert, C. B. (1985, July 1). *Transferrin receptors in rat brain: neuropeptide-like pattern and relationship to iron distribution*. Retrieved July 2009, from Proceedings of the National Academy of Sciences of the United States of America website: http://www.pnas.org/content/82/13/4553.abstract.

Koob, G. F. & Volkow, N. D. (2009, August 26). Neurocircuitry of Addiction. *Neuropsychopharmacology,* 35, 217–238.

Li, M., Bermak, J.C., Wang, Z.W., & Zhou, Q.Y. (2000, March 1). *Modulation of Dopamine D_2 Receptor Signaling by Actin-Binding Protein (ABP-280).*

Micronutrient Deficiencies. Retrieved September 26, 2009, from the World Health Organization website: http://www.who.int/nutrition/topics/ida/en/.

Marso, S. P., & Rutherford, B. D. (2002). Is a High Hematocrit Level Good For Patients With Heart Failure? *Journal of the American College of Cardiology*, 39, 1703-1704.

McGourthy (2000, March). *Chelation*. Retrieved July 2009, from NBIA Disorders Association website: http://www.nbiadisorders.org/chelation.htm.

Miranda, M., Wu, C. C., Sorkina, T., Korstjens, D. & Sorkin, A. (2005, August 18). *Enhanced Ubiquitylation and Degradation of the Dopamine Transporter Mediated by Protein KInase C.*

Morello, N., Tanoli, E., Logrand, F, Fiorito, V., Fagoonee, S., Turco, E., Silengo, L., Vercelli, A., Altruda, F. & Tolosano, E. (2008, December 16). *Hemopexin affects iron distribution and ferritin expression in mouse brain.*

Morgan, E. H. & Moss, T. (2002). Mechanism and Developmental Changes in Iron Transport Across the Blood-Brain Barrier. *Developmental Neuroscience Journal*, 11, 595-603.

Moss, T., Skjoerringe, T., Gosk, S., & Morgan, E.H. (2006, January 2). Brain capillary endothelial cells mediate iron transport into the brain by segregating iron from transferrin without the involvement of divalent metal transporter 1. *Journal of Neurochemistry,* 98(6), 1946-58.

Okam, M. *Transferrin and Iron Transport Physiology* (2001, January 29). Retrieved July 2009, from Information Center for Sickle Cell and Thalassemic Disorders website: http://sickle. bwh.harvard.edu/iron_transport.html.

Ordidge, R. J., Gorell, G. M., Deniau, J. C., Knight, R. A. & Helpern, J. A. (1994). *Relative Assessment of Brain Iron Levels Using MRI at 3 Telsa.*

Patrick, L. (2007, June). Restless Legs Syndrome: pathophysiology and the role of iron and folate. *Alternative Medicine Review: A Journal of Clinical Therapeutic,* 12(2), 101-12.

Pfefferbaum, A., Adalsteinsson, E., Rohlfing, T & Sullivan, E. V. (2009). MRI estimates of brain iron concentration in normal aging: Comparison of field-dependent (FDRI) and phase (SWI) methods. *Neuroimage Journal*, 47(2), 493-500.

Pinero, D. J., & Connor, J. R. (2000). *Iron in the Brain: An Important Contributor in Normal and Diseased States.*

Pinero, D. J., Li, N., Connor, J. R. & Beard, J.L. (2000). Variations in Dietary Iron Alter Brain Iron Metabolism in Developing Rats. *Journal of Nutrition*, 85(4), 931 - 945.

Piñero, D. J., Jones, B. C. & Beard, J. L. (2001). Variations in Dietary Iron Alter Behavior in Developing Rats. *Journal of Nutrition,* 131, 311-318.

Siegenthaler, M. (2003). *Dopamine*. Retrieved July 2009, from Davidson College website: http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/Siegenthaler/Dopaminesite.htm.

Substantia Nigra. Retrieved June 2009, from *Merrium-Webster Dictionary* website: http://www. merriam-webster.com/dictionary/substantia%20nigr.

Takeda, A., Takatsuka, K., Sotogaku, N., & Oku, N. (2002, March 18). Influence of iron-saturation of plasma transferrin in iron distribution in the brain. *Neurochemistry International Journal*, 14(4), 223-228.

Takeda, A. (2001). Significance of Transferrin in Iron Delivery to the Brain. *Journal of Health Science*, 47(6), 520–524.

Trenor**,** C.C., Campagna, D. R., Sellers**,** V. M., Andrews, N. C., & Fleming, M. D. (2000, August 1). The molecular defect in hypotransferrinemic mice. *Blood Journal,* 96(3), 1113-1118.

Trost, L.B., Bergfeld, W.F., & Calogeras, E. (2006, May). The diagnosis and treatment of iron deficiency and its potential relationship to hair loss. American Acadamy of Dermatology. 54(5): 824-844.

Unger, E. L., Bianco, L. E., Burhans, M. S., Jones, B. C. & Beard, J. L. (2006, June 1). Acoustic startle response is disrupted in iron-deficient rats. *Pharmacology Biochemistry and Behavior Journal*, 84 (2), 378-384).

Unger, E. L., Paul, E. L., Murray-Kolb, L. E., Felt, B., Jones, B. C. & Beard, J. L. (2007, January). Early Iron Deficiency Alters Sensorimotor Development and Brain Monoamines in Rats. *Journal of Nutrition,* 137, 118-124.

Unger, E. L., Wiesinger, J. A., Hao, L. & Beard, J. L. (2008, December). Dopamine D_2 Receptor Expression Is Altered by Changes in Cellular Iron Levels in PC12 Cells and Rat Brain Tissue. *Journal of Nutrition,* 138(12), 2487-2494.

Wiesinger, J. A., Buwen, J. P., Cifelli, C. J., Unger, E. L., Jones, B. C. & Beard, J. L. (2006, August 17). Down-regulation of dopamine transporter by iron chelation *in vitro* is mediated by altered trafficking, not synthesis. *Journal of Neurochemistry*, 100(1), 167 – 179.

Youdium, M. B.H., Ben-Shachar, D. & Yehuda, S. (1989). Putative biological mechanisms of the effect of iron deficiency on brain biochemistry and behavior. *American Journal of Clinical Nutrition*, 85(4): 931-945.

Youdim, M.B.H., & Yehuda, S. (1989) Brain iron: a lesson from animal models. *American Journal of Clinical Nutrition,* 85(4), 931-945. Youdim, M.B.H., & Yehuda, S. (2000, May). The neurochemical basis of cognitive deficits induced by brain iron deficiency: involvement of dopamine-opiate system. *Cellular and Molecular Biology Journal,* 46(3): 491-500.

Academic Vita

Sabrina Russo

Education

8/2007-Present: The Pennsylvania State University, University Park, PA
Major: Nutritional Sciences
Honors: Nutritional Sciences
Current GPA: 3.93

8/2006-12/2006: Johnson and Wales University, Providence, RI
Major: Baking and Pastry Arts
Honors: Baking and Pastry Arts
Overall GPA: 3.75

Work Experience and Professional Membership

10/2009-Present: Member of the American Dietetic Association

8/2009-Present: Thesis Laboratory Researcher
The Pennsylvania State University, State College, PA

- Use a variety of laboratory techniques to separate samples of mice's brains and test how brain iron levels affect dopamine functioning
- Compose a thesis paper on the background information and results of the study

8/2009-12/2009: Teacher's Assistant for a food science course (Nutrition 120)
The Pennsylvania State University, State College, PA

- Conducted review sessions prior to each exam
- Distributed and collected exams
- Graded exams

5/2009-11/2009: Job shadowing of a dietitian/volunteering
Lehigh Valley Hospital, Allentown, PA

- Assisted in planning menu and activities, and shopping for children's diabetes camp, Camp Red Jacket
- Created take-home recipe booklet and calculated calories for each dish for Camp Red Jacket participants
- Prepared food and work with children during activities in Camp Red Jacket
- Audited charts of diabetic patients

3/2007-Present: Cashier
Wegmans Food Markets, Allentown, PA

- Provided excellent customer service to a wide range of customers
- Assisted in checking-out and helping customers locate items in the store
- Completed multiple tasks throughout store

Campus Involvement:

Student Nutrition Association 2008-2010
- Raised money for dance marathon for children with cancer (THON), donated blood

Health and Human Development Honors Club 2008-2010
- Wrapped presents for needy children and their families

Blue and White Society 2007
- Raised money for THON

Community Service Club 2007
- Played bingo and sung Christmas carols at local nursing home

Keystone Honor Society 2007
- Raised money for THON

Awards/Honors:

2008-2010: Schreyer's Honors College Student
2007-2010: Dean's List
2007: Barnes & Noble Award
2007: President's Freshman Award
2007: Certificate of Achievement in Keystone Honor Society