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The effects of neonatal manganese exposure on impulsivity, unlearned motoric function, and reward

Carmela Marie Reichel

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THE EFFECTS OF NEONATAL MANGANESE EXPOSURE ON IMPULSIVITY, UNLEARNED MOTORIC FUNCTION, AND REWARD

A Thesis
Presented to the Faculty of California State University, San Bernardino

In Partial Fulfillment of the Requirements for the Degree Master of Arts in Psychology: General Experimental

By
Carmela Marie Reichel
June 2005
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Approved by:

Dr. Sanders McDougall, Chair, Psychology

Dr. Cynthia Crawford

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ABSTRACT

Manganese is an essential trace mineral that, at high doses, damages the dopamine neurotransmitter system. Evidence suggests that children may be particularly prone to manganese neurotoxicity and may have an amplified risk of neuronal damage. Infant formulas contain substantially more manganese than the recommended daily allowance, so this sub-population of infants may be at particular risk for developing long-term behavioral deficits and neuronal alterations. The purpose of this thesis, therefore, was to examine the effects of low to moderate doses of manganese (0, 250, or 750 μg per day from PD 1-21) on a comprehensive battery of behaviors during the neonatal period, preweanling period, and in adulthood. Additionally, this thesis evaluated manganese-induced changes in dopamine transporter binding sites. Manganese exposure did not affect the appearance of developmental landmarks, nor did it alter neonatal motor activity or olfactory orientation. Manganese did impair neonatal motor coordination and depressed body weight gain on PD 13-21. Manganese exposure did not affect basal motor activity, balance, or coordination. When tested at PD 90, however, manganese-exposed rats exhibited both reduced responding on a
differential reinforcement of low rates of response (DRL) task and a reduction in cocaine-induced motor activity. Moreover, early manganese exposure caused a persistent reduction of striatal dopamine transporters. Manganese did not alter the rewarding properties of cocaine, as measured by the acquisition, extinction, and reinstatement of conditioned place preference. When considered together, the most parsimonious conclusion is that early manganese exposure causes neurotoxicity, which is evidenced by short- and long-term changes in behavior and reductions in striatal dopamine transporters.
ACKNOWLEDGMENTS

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DEDICATION

To my husband, Gary Reichel, for his unwavering support. Also, to my mother, Mary Ann Montagne, just because I miss her.
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CHAPTER ONE
MANGANESE AND HUMAN EXPOSURE

Introduction

Manganese is a complex trace mineral that has opposing roles as an essential nutrient and a potential neurotoxicant. The roles oppose because although manganese is potentially harmful to the central nervous system, it is an important trace mineral required to maintain enzyme levels and cellular transmission throughout the body (Aschner, Vrana, & Zheng, 1999). Most people get an appropriate amount of manganese through dietary intake of natural foods such as whole grains, legumes, avocados, blueberries, and green leafy vegetables (Aschner et al., 1999). Failure to maintain basal levels of manganese can cause seizure activity and skeletal abnormalities (Critchfield, Carl, & Keen, 1993; Zidenberg-Cherr, Keen, Lonnerdal, & Hurley, 1983). However, since sufficient quantities of manganese are typically obtained through diet, dietary deficiencies are secondary to the threat of overexposure (Aschner et al., 1999).

The toxic effects of manganese were first identified in 1837 by a doctor who treated five patients that inhaled
excessive amounts of manganese in a pyrolusite mill (Cawte, 1985). Since then manganese poisoning has been reported world-wide in factory alloy workers and miners (Beuter, Edwards, deGeoffrou, Mergler, & Hudnell, 1999; Cawte, 1985; Lucchini, Bergamaschi, Smargiassi, Festa & Apostoli, 1997; Mergler et al., 1994), as well as in individuals accidentally exposed to manganese in drinking water (He, Liu, & Zhang, 1994; Woolf, Wright, Amarasiriwardena, & Bellinger, 2002).

Behavioral and neurological manifestations of manganese poisoning differ depending upon the length of exposure. Early in the illness, behavioral symptoms are similar to schizophrenia, including compulsiveness, violent behavior, emotional instability, and hallucinations (Aschner, 2000; Beuter et al., 1999; Mergler, 1999; Mergler et al., 1994). These initial symptoms are later replaced by extrapyramidal motor system impairments that result in bradykinesia and dystonia (Aschner, 2000). Additionally, manganese-intoxicated individuals gradually lose speech capabilities: speech becomes slurred, slow and irregular, while voices become monotone and low (Aschner, 2000). Although the final stages of chronic manganese poisoning closely resemble Parkinson’s disease (i.e., manganese
poisoning and Parkinson’s disease are both characterized by bradykinesia and widespread muscular rigidity), the differences between the two disorders are distinct. Specifically, manganese poisoning is characterized by less intense resting tremor, more frequent dystonia, the propensity to fall backwards, and inconsistent responding to levodopa (Calne, Chu, Huang, Lu, & Olanow, 1994). Importantly, these clinical symptoms progressively worsen even after exposure has stopped and tissue mineral concentrations have returned to normal levels (Hochberg et al., 1996; Huang, Chu, Lu, Chen, & Calne, 1998; Huang, Lu, Chu, Hochberg, Lilienfeld, Olanow, & Calne, 1993).

Manganese and Parenteral Nutrition Supplementation

There are no reports that normal dietary intake causes manganese poisoning; however, children and adults receiving parenteral nutrition supplementation occasionally suffer from manganese intoxication (Dickerson, 2001; Fell et al., 1996; Komaki, Maisawa, Sugai, Kobayashi, & Hashimoto, 1999; Nagatomo et al., 1999). In separate case studies, two adult patients were admitted into hospital care following several months of total parenteral nutrition that increased manganese levels to 20 µmol daily (Nagatomo et al., 1999).
Both patients exhibited classic symptoms of manganese poisoning that included psychiatric and Parkinsonian symptoms (Nagatomo et al., 1999).

Similar to adults, children exposed to high levels of manganese during parenteral nutrition supplementation have both higher levels of whole blood manganese and an excess accumulation of manganese in certain brain regions (e.g., the basal ganglia) (Fell et al., 1996; Komaki et al., 1999). Children with the highest whole blood manganese concentrations demonstrated dystonic limb movements, abnormal posturing, seizure, tremor, and abnormal MRI (Fell et al., 1996; Komaki et al., 1999). For children and adults, psychiatric and behavioral dysfunctions subsided after manganese supplementation was discontinued (Komaki et al., 1999).

Manganese and Environmentally Exposed Children

Very little is known about the consequences of manganese exposure during infancy or childhood; however, it is generally believed that children may be more susceptible to manganese neurotoxicity than adults (Cawte, 1985; Kostial, Kello, Jugo, Rabar, & Maljkovic, 1978; Mergler, 1999; Weiss, 1999). In China, children exposed to elevated
manganese in drinking water performed worse than controls on short-term memory tasks, manual dexterity tasks, and visuo-perceptual tasks (He et al., 1994). A case study in the United States provided similar results, because a boy inadvertently exposed to manganese through drinking water had difficulty performing memory tasks and coordinating rapid alternating motor movements (Woolf et al., 2002).

Manganese and Infant Formula

It has been established that exposure to high doses of manganese induces toxicity. Consequently, there is increasing concern that chronic exposure to lower amounts of manganese may also have toxic effects (Lucchini et al., 1997; Mergler, 1999). Children could be at particular risk, because a child’s diet in the United States typically exceeds the daily requirement for maintaining basal manganese levels (Hunt & Meacham, 2001; Milner, 1990). For example, the recommended daily manganese requirement for infants ranges from 0.3 mg to 0.6 mg (Milner, 1990). In the United States, the average dietary intake of manganese for infants is 1.0 mg/kg per day, with 49% of the daily intake being provided through infant foods (Hunt & Meacham, 2001). Importantly, commercially available infant formulas contain
substantially more manganese than human milk (Lonnerdal, 1994). Specifically, human milk contains approximately 3-8 μg/l manganese, while cow milk-based formulas contain 50-100 μg/l manganese, and soy-based products contain as much as 200-300 μg/l manganese (Lonnerdal, 1994). The high manganese levels in soy-based formulas are of particular concern, because these types of formulas are commonly recommended for infants with protein allergies, lactose intolerance, or general gastrointestinal discomfort (Lonnerdal, 1994).

Manganese and Attention Deficit/Hyperactivity Disorder

Manganese levels in infant formulas are of particular interest because these levels may be sufficient to cause lasting learning disabilities and behavioral dysfunctions associated with Attention Deficit/Hyperactivity Disorder (ADHD) (Cawte, 1985; Lonnerdal, 1994; Weiss, 1999). Several lines of evidence support manganese involvement in ADHD. Before discussing this evidence in detail, however, the next section will provide an overview of ADHD classifications, behavioral manifestations, and neuroimaging studies.
Attention Deficit/Hyperactivity Disorder

The prevalence of ADHD is reported to be between 3%-7% of school age children (American Psychiatric Association, 1994), making this disorder one of the most commonly diagnosed neurological disorders among children and adolescents in the United States (Solanto, 1998). ADHD is characterized by persistent inattention, hyperactivity, and/or impulsivity. These characteristics are exaggerated and disabling in individuals with the disorder and are inconsistent with typical behavior within the individual’s developmental period (American Psychiatric Association, 1994).

The Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV divides behavioral symptoms into two basic categories: inattention and hyperactivity/impulsivity (American Psychiatric Association, 1994). These two categories are further classified into three different diagnostic subtypes: ADHD Predominantly Inattentive Type, ADHD Predominantly Hyperactive-Impulsive Type, or ADHD Combined Type. Additionally, ADHD can co-occur with other psychiatric conditions including oppositional defiance disorder, conduct disorder, mood disorders and
Tourette’s/tic disorders (Goldman, Genel, Bezman, & Slanetz, 1998).

Attention Deficit/Hyperactivity Disorder and Behavioral Dysfunction

In laboratory assessments, ADHD patients display a wide variety of behavioral dysfunctions. For example, ADHD children exhibit impairments on short-term memory tasks (Paule et al., 2000), complex visual memory tasks (Berman, Douglas, & Bar, 1999), and on nonverbal learning and memory tasks (O’Toole, Abramowitz, Morris, & Dulcan, 1997). Inattentiveness and impulsivity interfere with the ability of ADHD children and adults to exhibit sustained attention (Oades, 2000; Solanto, Wender, & Bartell, 1997; Swaab-Barneveld et al., 2000; van der Meere, Shalev, Borger, & Gross-Tsur, 1995). Tasks that require participants to withhold a previously conditioned or reinforced motor response are difficult for children, adolescents, and adults with ADHD (Dinn, Robbins, & Harris, 2001; Nigg, 1999; Schachar, Moto, Logan, Tannock, & Klim, 2000). Additionally, ADHD children show an inability to perform tasks that require temporal processing in order to correctly execute a response (Barkley, Koplowitz, Anderson, & McMurray, 1997; Gordon, 1979; McClure & Gordon, 1984).
Attention Deficit/Hyperactivity Disorder and Neuroimaging

Neuroimaging techniques consistently reveal differences in the frontal cortex, caudate nucleus, and globus pallidus of ADHD and nonADHD patients. Specifically, ADHD boys, when compared to controls, have 5% less total brain volume (Castellanos et al., 1994), 4.7% less total cerebral volume (Castellanos et al., 1996), and less frontal cortex volume (Filipek et al., 1997). In normal children, the caudate nucleus exhibits a size asymmetry; however, ADHD children do not show typical right > left asymmetry (Castellanos et al., 1994, 1996; Mataro, Garcia-Sanchez, Junque, Estevez-Gonzalez, & Pujol, 1997). The degree to which caudate asymmetry is abnormal is correlated with the inattentiveness exhibited by ADHD individuals (Schrimsher, Billingsley, Jackson, & Moore, 2002). Additionally, ADHD is characterized by atypical caudate maturation. Specifically, caudate volume normally decreases substantially across maturation; however, the caudate volume of children with ADHD does not show the normal decline (Castellanos et al., 1994). Conversely, the globus pallidus, but not the putamen, is smaller in people
diagnosed with ADHD (Aylward, 1996; Castellanos et al., 1996; Giedd, Blumenthal, Molloy, & Castellanos, 2001). These volumetric differences are consistent with positron emission tomography (PET) studies and single photon emission computerized tomography (SPECT) studies that demonstrate decreased metabolism in the frontal lobes and striatum (i.e., caudate-putamen) of ADHD patients (Ernst, Zametkin, Mattochik, Jons, & Cohen, 1998; Lou, Henrikson, Bruhn, Berner, & Nielsen, 1989; Zametkin et al., 1990). Additionally, functional magnetic resonance imaging (fMRI) studies have shown decreased activation of the frontal lobes and striatum during performance of cognitive tasks that require inhibition of a previously reinforced response (Durston et al., 2003; Rubia et al., 1999, 2000; Teicher et al., 2000; Vaidya & Gabrieli, 1999).

Taken together, these various neuroimaging techniques consistently demonstrate that corticostriatal circuitry is dysfunctional in ADHD (Casey et al., 1997; Johansen, Aase, Meyer, & Sagvolden, 2002; Solanto, Wender, & Bartell, 1997). In addition, dysfunction of the nigrostriatal dopamine system has been implicated in motor abnormalities associated with ADHD, while dysfunction of the mesolimbocortical system has been implicated in altered
reward function (Johansen et al., 2002; Sagvolden et al., 1998).

**Relationship Between Manganese Toxicity and Attention Deficit/Hyperactivity Disorder**

As mentioned previously, there is evidence suggesting a causal relationship between manganese toxicity and ADHD. Manganese levels in hair samples provide the first line of evidence. Trace minerals, including manganese, found in hair samples accumulate at a differential rate depending on age and sex of the child (Collipp, Chen, & Maitinsky, 1983; Sakai, Wariishi, & Nishiyama, 2000). Therefore, it is not surprising that children directly exposed to manganese show increased concentrations of the mineral in hair samples (He et al., 1994; Fell et al., 1996; Komaki et al., 1999; Woolf et al., 2002). Interestingly, learning disabled children also have elevated levels of manganese in their hair samples, even though they have not been previously exposed to excessive levels of the mineral (Collipp, Chen, & Maitinsky, 1983; Pihl & Parkes, 1977). Consequently, these data suggest that absorption and retention of environmental metals may be directly related to the development of learning disabilities as well as disruptive and hyperactive behaviors, and emotional disturbances (Marlowe & Bliss,
manganese concentrations in hair are higher in formula-fed infants than in breast-fed infants (Collipp, Chen, & Maitinsky, 1983).

Neurobehavioral tasks also suggest that a causal relationship may exist between manganese toxicity and ADHD (Barkley, Koplowitz, Anderson, & McMurray, 1997; Durston et al., 2003; Rubia et al., 1999, 2000; Teicher et al., 2000; Vaidya & Gabrieli, 1999). Although manganese neurotoxicity has not been directly associated with function of the frontal cortex (Calabrassi et al., 2001; Kontur & Fletcher, 1985), manganese impairs performance on neurobehavioral tasks mediated by corticostriatal circuitry. For example, manganese-exposed children show memory impairment, poor manual dexterity skills, and poor motor coordination, which are consistent with behavioral alterations observed in ADHD children (i.e., memory, motor, attention, and response inhibition difficulty) (He et al., 1994; Woolf et al., 2002). Combined, these results suggest that both manganese neurotoxicity and ADHD may involve alterations of corticostriatal functioning, which is indicated by disinhibition of performance on neurobehavioral tasks.
(Barkley, 1997, 2003; Calabrassi et al., 2001; Casey et al., 1997).

Another line of evidence, provided by neuroimaging studies, suggests that the same neural substrates are involved in both manganese toxicity and ADHD. More precisely, manganese toxicity and ADHD involve dopamine system impairment of the nigrostriatal and mesolimbic pathways. Manganese accumulates in the same basal ganglia nuclei (i.e., striatum and globus pallidus) that are consistently found by neuroimaging studies to function abnormally in ADHD (Durston et al., 2003; Erickson et al., 2002; Lou et al., 1989; Teicher et al., 2000; Vaidya & Gabrieli, 1999). Abnormal functioning of the nigrostriatal dopamine pathway is responsible for the motoric dysfunction observed in both manganese intoxication and ADHD (Aschner, 2000; Aylward, 1996; Castellanos et al., 1996; Erickson et al., 2002; Giedd, Blumenthal, Molloy, & Castellanos, 2001; Johansen et al., 2002). Motoric dysfunction is most typically expressed as impaired timing assessments, poor motor control, hyperactivity, and impulsivity (Johansen et al., 2002; Kadesjjo & Gillberg, 1999; Sagvolden et al., 1998).
Another neural substrate involved in both manganese neurotoxicity and ADHD is the globus pallidus. Neuroimaging studies suggest that the globus pallidus functions abnormally in ADHD, perhaps preventing normal responding to reinforcing or rewarding events (Barkley, 1997, 2003; Douglass & Parry, 1994; Johansen et al., 2002; Sagvolden et al., 1998). Manganese accumulation in the globus pallidus may interrupt reward processes, because stimulation of dopamine terminals in this brain region increases responding for drug and food reward (Fletcher, Korth, Sabijan, & DeSousa, 1998; Olive & Maidment, 1998; Ono, Nishijo, & Nishino, 2000). Taken together, the brain areas affected by manganese accumulation are the same areas implicated in ADHD; therefore, it is reasonable to suggest that manganese accumulation in the basal ganglia may cause motoric dysfunction and reward deficiencies consistent with symptoms observed in ADHD.

Summary

This chapter provides evidence that manganese is neurotoxic to adults and children. Humans are primarily exposed to manganese via environmental contamination (Beuter et al., 1999; Cawte, 1985; He et al., 1994; Mergler
et al., 1994; Woolf et al., 2002) or orally through parenteral nutrition (Dickerson, 2001; Fell et al., 1996; Komaki et al., 1999; Nagatomo et al., 1999). At high doses, manganese causes motoric and cognitive dysfunction (Aschner, 2000; Beuter et al., 1999; Cawte, 1985; Mergler et al., 1994); however, it is unclear if low doses cause similar behavioral and cognitive symptoms. There is increasing evidence that children may be particularly prone to manganese neurotoxicity, because infant formulas contain about 50% more manganese than the recommended daily allowance (Hunt & Meacham, 2001). Children fed these formulas may be at risk for lasting brain changes that result in long-term behavioral dysfunction (Cawte, 1985; Lonnerdal, 1994; Mergler, 1999; Weiss, 1999). Specifically, a causal relationship may exist between manganese exposure and ADHD (Cawte, 1985; Lonnerdal, 1994; Weiss, 1999).
CHAPTER TWO

BASAL GANGLIA CIRCUITRY

Introduction

Based on data presented in Chapter 1, it is apparent that manganese has toxic effects in both children and adults. These neurotoxic effects are manifested as changes in cognition, personality, and gross motor movement. The accumulated evidence indicates that these changes are a result of manganese-induced alterations of central nervous system functioning. Manganese also affects a variety of neurotransmitter systems, with most of the research implicating dopamine, glutamate, and γ-aminobutyric acid (GABA). Manganese-induced changes in neurotransmission are predominantly found in the basal ganglia; therefore, the purpose of Chapter 2 will be to provide background information about neurotransmitter system functioning within the basal ganglia.

Basal Ganglia Circuitry: Structure

The basal ganglia consists of four primary nuclei: the striatum (caudate nucleus and putamen), the globus pallidus (internal and external segments), the substantia nigra (pars reticulata and pars compacta), and the subthalamic

Pathways between the cortex, basal ganglia, thalamus, and brain stem make up the basal ganglia-thalamocortical circuits (see Figure 1) (Alexander et al., 1990).

Specifically, the basal ganglia receives input from the cortex and sends information back to the cortex via connections with the thalamus (Alexander et al., 1990). The primary input nucleus of the basal ganglia is the striatum (Albin et al., 1989; De Long, 2000), although the subthalamic nucleus also receives excitatory glutamatergic input from the cortex (Afsharpour, 1985; Alexander & Crutcher, 1990; Carlson & Carlson, 1990; De Long, 2000; Greenamyre, 2001; Young, Bromberg, & Penny, 1981).

The primary output structures of the basal ganglia are the globus pallidus internal segment and substantia nigra pars reticulata (SNr), which are functionally similar nuclei (Albin et al., 1989; De Long, 2000). Although the
Figure 1. Schematic representation of basal ganglia-thalamocortical circuitry.
Abbreviations: GPe, globus pallidus external segment; GPi, globus pallidus internal segment; SNr, substantia nigra pars compacta; SNr, substantia nigra pars reticulate.
thalamus is not considered part of the basal ganglia, it has an important role in basal ganglia-thalamocortical circuitry. Specifically, the thalamus receives inhibitory GABAergic input from the SNr and GPi and, in turn, sends excitatory glutamatergic output to the cortex (Albin et al., 1989; Alexander & Crutcher, 1990; Alexander et al., 1990; Carlson & Carlson, 1990; De Long, 2000; Greenamyre, 2001).

The cortex provides glutamatergic input to the striatum which, in turn, inhibits two parallel pathways: the direct pathway and the indirect pathway (Albin et al., 1989; Alexander & Crutcher, 1990; Alexander et al., 1990; De Long, 2000). The direct pathway consists of striatal cells projecting to the SNr and Globus pallidus internal segment (Albin et al., 1989; Alexander & Crutcher, 1990; Alexander et al., 1990; De Long, 2000). The indirect pathway begins with striatal efferents projecting from the striatum to the GPe, then from the GPe to the subthalamic nucleus, and finally from the subthalamic nucleus to the SNr and globus pallidus internal segment (Albin et al., 1989; Alexander & Crutcher, 1990; Alexander et al., 1990; De Long, 2000; Greenamyre, 2001).
In terms of neurotransmitters, 90% of striatal neurons are GABAergic (Alexander et al., 1990; De Long, 2000; O’Conner, 1998). The remaining 10% of striatal neurons, consisting of substance P, dynorphin, enkephalin, neurotensin, and acetylcholine interneurons, are responsible for regulating tonic control of the striatum (Alexander et al., 1990; De Long, 2000; O’Conner, 1998).

The neurons of the direct pathway co-release GABA, dynorphin, and substance P in the SNr and Globus pallidus internal segment (Albin et al., 1989; De Long, 2000), whereas neurons of the indirect pathway co-release GABA, enkephalin, and neurotensin in the globus pallidus external segment (GPe) (Albin et al., 1989; De Long, 2000). In addition, GABA neurons project from the GPe to the subthalamic nucleus (Albin et al., 1989; De Long, 2000). Glutamate axons project from the subthalamic nucleus to the SNr and Globus pallidus internal segment, which is the only excitatory connection involving basal ganglia circuitry (Afsharpour, 1985; Alexander & Crutcher, 1990; Carlson & Carlson, 1990; De Long, 2000; Greenamyre, 2001; Young, Bromberg, & Penny, 1981).

Dopaminergic cell bodies in the SNc project to the striatum, where they synapse on cells of the direct and
indirect pathways (Albin et al., 1989; Alexander & Crutcher, 1990; Alexander et al., 1990; De Long, 2000). Specifically, excitatory D1-like dopamine receptors are located on neurons composing the direct pathway; whereas, inhibitory D2-like dopamine receptors are located on neurons of the indirect pathway (De Long, 2000; Gerfen, 1992; Gerfen, Engber, Mahan, Susel, Chase, Monsma, & Sibley, 1990; O'Conner, 1998).

Basal Ganglia Circuitry: Function

As just mentioned, basal ganglia-thalamocortical circuits are involved in a variety of behavioral and motor functions via parallel pathways that begin and end in specific areas of the cortex and engage different areas of the basal ganglia and thalamus (Alexander et al., 1990; Alexander & Crutcher, 1990; De Long, 2000; Hoover & Strick, 1993, 1999). Activation of the direct pathway disinhibits thalamocortical transmission. More specifically, the inhibitory drive of striatal GABAergic projections to the SNr and Globus pallidus internal segment, in turn, inhibits GABA transmission to the thalamus (Alexander et al., 1990; Alexander & Crutcher, 1990). In contrast, activation of the indirect pathway ultimately results in increased inhibition
of the thalamus via glutamatergic projections from the subthalamic nucleus. In this case, increased activation of striatal GABAergic projections to the GPe suppresses neuronal firing, which decreases inhibitory control over the subthalamic nucleus (Alexander et al., 1990; Alexander & Crutcher, 1990). Disinhibition of the subthalamic nucleus increases excitatory glutamatergic transmission to the SNr and Globus pallidus internal segment, which ultimately increases the inhibitory drive to the thalamus (Alexander et al., 1990; Alexander & Crutcher, 1990). To summarize, activation of the direct pathway disinhibits the thalamus increasing thalamocortical transmission, while activation of the indirect pathway causes increased inhibition of thalamocortical neurons (Alexander & Crutcher, 1990; Alexander et al., 1990; De Long, 2000).

Dopamine modulates this system. In the striatum, D1-like and D2-like receptors are located on different sets of neurons and modulate both the direct and indirect pathway. Specifically, stimulation of striatal D1-like receptors increases the firing rate of the striatonigral GABAergic pathway (i.e., the direct pathway), which results in disinhibition of the thalamus (Gerfen, 1992; Gerfen et al., 1990; O'Conner, 1998). Stimulation of striatal D2-like
receptors reduces the firing rate of the striatopallidal GABA pathway (i.e., the indirect pathway), which decreases inhibition of the thalamus (Gerfen, 1992; Gerfen et al., 1990; O’Conner, 1998). Although striatal D₁-like and D₂-like receptors modulate different pathways, ultimately both receptor types facilitate motor movement by reducing inhibition of thalamocortical neurons (De Long, 2000; Gerfen, 1992; Gerfen et al., 1990; O’Conner, 1998).

Since the striatum is the main input nucleus of the basal ganglia and since the SNr and Globus pallidus internal segment are the main output nuclei, it is understandable that manganese accumulation in these areas may cause basal ganglia dysfunction. Chapter 3 will provide information about the dopamine system, because manganese intoxication often impairs dopamine neurotransmission within the basal ganglia. Chapter 4 will then discuss manganese-induced alterations in basal ganglia functioning.
CHAPTER THREE

THE DOPAMINE SYSTEM

Introduction

As previously mentioned, dopaminergic neurotransmission is important for motor movement and reward processes. Since the dopamine system is implicated in both manganese toxicity and ADHD, the next section will include a more thorough discussion of the dopamine system. Specifically, the section will provide information about the effects of psychostimulant drugs on the two main dopaminergic pathways as well as dopamine-mediated behaviors (e.g., locomotor activity, reward seeking behaviors, and impulsivity). The last section will describe maturation of the dopamine system.

Dopamine Receptor Classification

Originally dopamine receptors were classified by anatomical location and function (Clark & White, 1987). Subsequent work has categorized dopamine receptors into families depending upon the ability of the receptor subtype to activate the enzyme adenylyl cyclase (Clark & White, 1987; Kebabian & Calne, 1979). $D_1$-like receptors stimulate adenylyl cyclase activity, while $D_2$-like receptors either
inhibit or are unlinked to adenylyl cyclase activity (Clark & White, 1987; Kebabian & Calne, 1979). On this basis, two basic dopamine receptor families have been classified: the D<sub>1</sub>-like family, which consists of the D<sub>1</sub> and D<sub>5</sub> subtypes; and the D<sub>2</sub>-like family, which consists of the D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> receptor subtypes (Clark & White, 1987). D<sub>1</sub>-like receptors are located on postsynaptic terminals, whereas D<sub>2</sub>-like receptors are present on presynaptic and postsynaptic terminals (Clark & White, 1987).

**Dopaminergic Drugs**

Psychotropic drugs that enhance the function of a neurotransmitter system are considered agonists; whereas, antagonists attenuate the effects of endogenous neurotransmitters. Dopamine agonist drugs work by either directly stimulating receptors or by indirectly altering cellular processes in a way that increases the amount of endogenous neurotransmitter in the synaptic cleft. Amphetamine, cocaine, and methylphenidate are indirect dopamine agonists, although their mechanisms of action differ. Amphetamine increases extracellular dopamine by reversing the direction of the dopamine transporter, thus increasing the release of free-floating dopamine from the...
presynaptic terminal (Heikkila, Orlansky, Mytilineou, & Cohen, 1975; Jones, Gainetdinov, Wightman, & Caron, 1998; Sonders, Zhu, Zahniser, Kavanaugh, & Amara, 1997). Cocaine and methylphenidate increase extracellular dopamine levels by blocking the dopamine transporter, thus preventing the removal of dopamine from the synaptic cleft (Kimko, Cross, & Abernethy, 1999; Kuhar, Ritz, & Boja, 1991; Ritz, Lamb, Goldberg, & Kuhar, 1987; Volkow et al., 1995).

Direct dopamine receptor agonists alter dopaminergic functioning by directly stimulating pre- and postsynaptic receptors. For example, SKF 38393 and quinpirole selectively stimulate D₁-like and D₂-like receptors, respectively. In contrast, direct dopamine receptor antagonists block the receptor, which prevents endogenous or exogenous ligands from stimulating the receptor complex. Selective receptor antagonists exist for both the D₁-like (e.g., SCH 23390) and D₂-like (e.g., sulpiride) receptor.

Dopamine Pathways

Behavioral responses to dopaminergic drugs are mediated by the nigrostriatal pathway and the mesocorticolimbic pathway. Drugs that increase dopamine levels in the synapse (i.e., indirect agonists) initiate
both locomotor activity and reward seeking behaviors (Cho et al., 1999; Costall et al., 1975; Wise & Bozarth, 1987; Wise & Hoffman, 1992). Conversely, drugs that block dopaminergic functioning decrease motoric functioning and inhibit reward processes (Arnt, 1987; Josselyn, Miller, & Beninger, 1997; Miller, Wickens, & Beninger, 1990; Wise & Rompre, 1989). The nigrostriatal pathway is most commonly associated with intense and perseverative motoric effects of dopaminergic drugs, whereas the mesocorticolimbic pathway is most commonly associated with the rewarding properties of dopaminergic drugs (Arnt, 1987; Wise & Bozarth, 1987).

**Nigrostriatal Dopamine Pathway**

The nigrostriatal dopamine pathway is part of the basal ganglia and consists of cell bodies located in the substantia nigra and projecting to the striatum. This pathway is primarily associated with motor movement (Arnt, 1987), because deterioration of the nigrostriatal pathway impairs motor function and results in the behavioral abnormalities associated with Parkinson’s disease (e.g., tremor, rigidity, and bradykinesia) (Calne, Chu, Huang, Lu, & Olanow, 1994; Carey, 1986; Goldberg et al., 2003; Olanow et al., 1996). Not surprisingly, blocking dopamine
transmission or reducing dopamine levels in the striatum decreases motor activity. For example, depleting vesicular stores of dopamine with reserpine reduces spontaneous locomotor activity and stereotypy (Florin, Kuczenski, & Segal, 1995). Additionally, dopamine receptor antagonists block hyperactivity induced by systemic or intrastriatal injections of direct and indirect dopamine agonists (Costall & Naylor, 1976; Fontana, Post, Weiss, & Pert, 1993; Levin, See, & South, 1989).

Indirect dopamine agonists differentially induce locomotor activity and stereotypical responding depending on drug dose (Cho et al., 1999; Costall et al., 1975; Segal & Kuczenski, 1997). At low doses, cocaine and amphetamine increase locomotor activity due to increased dopamine release in the nucleus accumbens (Dickson, Lang, Hinton, & Kelly, 1994; Mele, Thomas, & Pert, 1998). On the other hand, acute treatment with a high dose of cocaine or amphetamine increases dopamine release in the striatum and produces stereotypy (Dickson et al., 1994; Waszczak, Martin, Boucher, Zahr, Sikes, & Stellar, 2001). Taken together, these findings indicate that low doses of an indirect agonist initially increases dopaminergic transmission in the mesolimbic pathway; whereas, high doses
preferentially activate the nigrostriatal dopamine system (Dickson et al., 1994; Mele et al., 1998; Stahl, Ferger, & Kuschinsky, 1997; Waszczak et al., 2001).

Direct dopamine agonists are also capable of increasing locomotor activity and inducing stereotypy (Defts & Kelly, 1990; Einat & Szechtman, 1993; Molloy & Waddington, 1987; Starr, 1988; Van Hartesveldt, Cottrell, Potter, & Meyer, 1992). For example, the D₁-like receptor agonist SKF 38393 increases sniffing, rearing, and locomotion of rats habituated to the testing environment (Molloy & Waddington, 1987); whereas, stimulation of D₂-like receptors with quinpirole increases locomotor activity (Einat & Szechtman, 1993; Van Hartesveldt et al., 1992). Combined treatment with both SKF 38393 and quinpirole induces intense stereotypies (Defts & Kelly, 1990; Molloy & Waddington, 1987; Starr, 1988). Not surprisingly, D₂-like receptor antagonists block quinpirole-induced behaviors and D₁-like receptor antagonists block SKF 38393-induced behaviors (Defts & Kelly, 1990; Levin, See, & South, 1989; Starr, 1988; Storey, Middlemiss, & Reavill, 1995; White, Bednarz, Wachtel, Hjorth, & Brooderson, 1988). The D₁-like receptor antagonist SCH 23390 also attenuates both D₂-like agonist-induced behaviors, suggesting that the D₁-like
receptor has a "permissive" or "enabling" role in dopamine transmission (Dall'Olio, Roncada, Vaccher, Gandolfi, & Montanaro, 1989; Defts & Kelly, 1990; Molloy & Waddington, 1987; Starr, 1988; White et al., 1988).

Mesocorticolimbic Dopamine Pathway

The mesocorticolimbic dopamine pathway is composed of dopaminergic cell bodies located in the ventral tegmental area and projecting to the nucleus accumbens and prefrontal cortex (see Figure 2) (Ikemoto & Panksepp, 1999; Oades & Halliday, 1987; Ungerstedt, 1971). Accumbal output neurons project from the nucleus accumbens to the Globus pallidus internal segment, and then from the Globus pallidus internal segment to the ventral tegmental area (McBride, Murphy, & Ikemoto, 1999; Pierce & Kalivas, 1997); whereas, cortical output neurons project from the frontal cortex to the nucleus accumbens and ventral tegmental area. Thus, the ventral tegmental area, nucleus accumbens, Globus pallidus internal segment, and frontal cortex make up the primary sites mediating the reinforcing properties of dopaminergic drugs (Ikemoto & Panksepp, 1999; McBride et al., 1999; Pierce & Kalivas, 1997). Drug-induced activation of this neural system is typically associated with subjective
Figure 2. Schematic representation of mesocorticolimbic system. Abbreviations: GPi, globus pallidus internal segment.

Several different behavioral paradigms demonstrate that the mesocorticolimbic system mediates the rewarding properties of direct and indirect dopamine agonists. These paradigms include drug self-administration, locomotor activity, conditioned place preference (CPP), and schedule-controlled operant tasks (Bardo, 1998; Ikemoto & Panksepp, 1999; Mayorga, Popke, Fogle, & Paule, 2000; Paule et al., 1999). For example, both direct and indirect dopamine agonists can induce CPP (Carr & White, 1983; 1996; White, Packard, & Hiroi, 1991), and are readily self-administered by both rodents and primates (Johanson, Balster, Bonese, 1976; Neilsen, Duda, Mokler, & Moore, 1984; Woolverton, Goldberg, & Ginos, 1984). Conversely, destroying dopamine terminals and cell bodies of the mesocorticolimbic pathway attenuates self-administration of dopamine agonists, blocks CPP, and decreases both apomorphine (a direct dopamine agonist) and amphetamine-induced locomotor activity (Gerrits & Van Ree, 1996; Gold, Swerdlow, & Koob, 1988; Joyce, Stinus, & Iversen, 1983; Kelly & Iversen, 1976; Kelly, Seviour, & Iversen, 1975; Lyness, Friedle, & Moore, 1979; Pettit, Ettenberg, Bloom, & Koob, 1984; Roberts &
Koob, 1982; Spyraki, Fibiger, & Phillips, 1982). The latter finding is relevant, because locomotor hyperactivity is an indirect measure of reward (Wise & Bozarth, 1987).

Development of the Dopamine System

The dopamine system is not adult-like at birth, but continues to mature during the postnatal period. In rodents, dopamine transporters, D₁-like and D₂-like receptor densities increase daily throughout early postnatal development (Galineau, Kodas, Guilloteau, Vilar, & Chalon, 2004; Murrin & Zeng, 1986; 1990; Rao, Molinoff, & Joyce, 1991; Tarazi & Baldessarini, 2000; Tarazi, Tomasini, & Baldessarini, 1998, Zeng, Hyttel, & Murrin, 1988). The dopamine receptor subtypes develop independently of each other, because D₁-like receptors are expressed prior to D₂-like receptors in the striatum, substantia nigra, and globus pallidus (Rao, Molinoff, & Joyce, 1991). During the periadolescent period dopamine receptors are overproduced in the striatum and prefrontal cortex, peaking by PD 40, and are subsequently pruned back to adult levels by PD 120 (Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000; Teicher, Andersen, & Hostetter, 1995). Dopamine transporters, on the other hand, reach peak levels between
PD 28 - 35 and remain constant until adulthood (Galineau, Kodas, Guilloteau, Vilar, & Chalon, 2004; Tarazi, Tomasini, & Baldessarini, 1998).

Dopaminergic drugs have generally similar behavioral profiles in postnatal and adult animals. For instance, indirect dopamine agonists activate reward processes, stimulate motor activity, and induce stereotyped behaviors by as early as PD 2 (Barr & Lithgow, 1986; Lal & Sourkes, 1973; Lanier & Isaacson, 1977; Laviola, Dell’Omo, Alleva, & Bignami, 1992). Direct dopamine agonists (e.g., SKF 38393 and quinpirole) stimulate unlearned motor movements in preweanling rats, while direct dopamine antagonists (e.g., SCH 23390 and sulpiride) reduce motor function (Camp & Rudy, 1987; McDougall, Arnold, & Nonneman, 1990; Moody & Spear, 1992; Sobrian, Jones, Varghese, & Holson, 2003).

Despite these behavior similarities, a number of drug-induced ontogenetic differences are evident. For instance, younger rats are less sensitive to the cataleptic effects of dopamine antagonists (i.e., SCH 23390 and haloperidol) than are adult rats (Fitzgerald & Hannigan, 1989; Rowlett, Pedigo, & Bardo, 1991). Preweanling rats (PD 11 and PD 17), unlike adults, are also unaffected by the irreversible dopamine receptor antagonist N-ethoxycarbonyl-2-ethoxy-1,2-
dihydroquinoline (EEDQ), since administration of EEDQ did not alter locomotor, sniffing, or grooming responses induced by dopamine agonist drugs (McDougall, Crawford, & Nonneman, 1992; Mestin & McDougall, 1993).

In some cases, adult behavioral patterns are not expressed until the third or fourth postnatal week. In particular, quinpirole does not induce adult-typical biphasic effects (i.e., decreased activity at low doses and increased activity at high doses) until the third or fourth week of postnatal development (Moody & Spear, 1992; Van Hартесвeldt, Meyer, & Potter, 1994). In addition, progressively higher doses of SKF 38393 are required to induce adult-typical grooming behavior in younger rats (McDougall et al., 1990; Moody & Spear, 1992). Taken together, these data indicate that the dopamine system is functional during early ontogeny, however, dopaminergic drugs are capable of inducing both quantitatively and qualitatively different behavioral effects depending on the age of the animal (for a review, see Spear, 1979).

Summary

Dopaminergic drugs affect both motor activity and reward processes by altering the functioning of the
nigrostriatal and mesocorticollimbic pathways. The nigrostriatal pathway is generally associated with motoric function; whereas, the mesocorticollimbic system is associated with locomotor activation and reward processes. The dopamine system undergoes rapid maturation throughout the postnatal period, a process that is responsible for many ontogenetic differences in drug responsiveness. The next chapter will discuss the effects of oral manganese exposure on the dopamine system, as well as the functional interactions between dopamine, glutamate, and GABA neurotransmission within the basal ganglia after manganese exposure.
CHAPTER FOUR

MANGANESE

Introduction

The previous chapters detailed basal ganglia circuitry as well as interactions between dopamine, glutamate, and GABA neurons. The purpose of Chapter 4 will be to discuss the influence of manganese on these neurotransmitter systems. Since the behavioral manifestations of manganese toxicity are mediated by central nervous system functioning, it is important to understand: (1) the mechanisms responsible for transporting manganese into the central nervous system, (2) the actions of manganese at the synapse, and (3) possible explanations for manganese-induced neurotoxicity. The last part of the chapter will detail interactions between manganese and the dopamine, glutamate, and GABA neurotransmitter systems.

Manganese and Central Nervous System Functioning

The toxic effects of many compounds are often associated with their transport mechanisms (Ingersoll, Montgomery, & Aposhian, 1999). Therefore, it is important to understand how manganese is transported into the central
nervous system and throughout the brain. The brain is highly permeable to manganese through transferrin and non-transferrin bound transport. In the former case, manganese binds to transferrin and is transported across the blood-brain barrier via transferrin receptors located on the surface of brain capillary endothelial cells (Aschner & Aschner, 1991; Aschner & Gannon, 1994; Davidsson, Lonnerdal, Sandstrom, Kunz, & Keen, 1989). The greatest percentage of manganese enters the brain independent of transferrin binding (Takeda, Devenyi, & Connor, 1998; Takeda, Ishiwatari, & Okada, 2000). In this case, manganese primarily crosses into the brain as a free ion via several different transport systems (for a review, see Takada, 2003), such as calcium channels (Drapeau & Nachshen, 1984), or by a divalent metal transporter located on brain capillary endothelial cells and choroidal epithelial cells (Gunshin et al., 1997).

Once inside the brain, excess manganese accumulates in iron-rich areas such as the striatum, nucleus accumbens, globus pallidus, substantia nigra, ventral tegmental area and subthalamic nucleus (Erickson, Shihabi, Aschner, & Aschner, 2002, Fell et al., 1996; Ingersoll et al., 1999; Komaki et al., 1999; Komura & Sakamoto, 1994; Nagatomo et
Extracellular manganese is taken up by neurons and glial cells (Takada, 2003). Specifically, neurons take up transferrin-bound manganese via receptor-mediated endocytosis and store it in synaptic vesicles (Takada, 2003). Non-transferrin bound manganese is sequestered in the mitochondria of astrocytes (Aschner et al., 1992; Takada, 2003; Thorley et al., 1990). Manganese is transported via axonal transport along GABAergic striatonigral neurons and dopaminergic nigrostriatal neurons (Takada, 1998). Taken together, manganese is readily transported into and throughout the brain where it is stored in both neurons and glial cells.

Manganese Exposure During Development

Neonatal brains accumulate manganese more extensively than adult brains and are less efficient at maintaining homeostatic levels (Ballatori, Miles, & Clarkson, 1987; Doorman et al., 2000; Fechter, 1999; Kostial, Kello, Jugo, Rabar, & Maljkovic, 1978; Rehnberg, Hein, Carter, & Laskey, 1980; Takada, Ishiwatari, & Okada, 1999). A possible explanation for excess manganese accumulation is that the biliary system of neonatal rats has not fully developed, thus preventing manganese from being excreted in bile.
(Ballatori et al., 1987; Miller, Cotzias, & Everet, 1975). Alternatively, neonatal rats may have an underdeveloped gastrointestinal tract that potentiates manganese absorption (Kostial et al., 1978; Rehnberg, Hein, Carter, & Laskey, 1985). Elevated blood transferrin levels in neonatal rats may also have an impact (Roskams & Connor, 1994), because increased manganese transport across an incomplete blood-brain barrier would result in excess brain manganese (Miller et al., 1975).

**Manganese, Synaptic Transmission, and Neurotoxicity**

The previous sections demonstrated that manganese can accumulate in both neurons and glial cells, with the effect being magnified in young rats. Excess manganese can interfere with synaptic transmission through various mechanisms. First, manganese located in the synaptic cleft may modulate the release of glutamate, aspartate, and GABA from neuron terminals (Tanaka, 2002). Second, manganese can potentiate neurotransmitter release by substituting for calcium in the exocytotic process (Drapeau & Nachshen, 1984). Third, chronic manganese increases the frequency and amplitude of spontaneous excitatory postsynaptic potentials (Centonze, Gubellini, Bernardi, & Calabresi, 2001). Fourth,
manganese potentiates glutamate-induced activation of NMDA receptors located on nigrostriatal dopamine terminals (Cuesta de Di Zio et al., 1995). Taken together, it is clear that manganese can alter neurotransmission of dopamine, glutamate, and GABA neurons in multiple ways.

Although it is known that manganese disrupts neurotransmission, the precise mechanism by which manganese causes neuronal damage is uncertain. Even so, most researchers studying manganese toxicity have focused on three potential mechanisms involving astrocytes (Aschner et al., 1992; Hazell, 2002), extracellular glutamate (Chen & Liao, 2002), and oxidative stress (Chen & Liao, 2002). Astrocytes may be responsible for manganese-induced neurotoxicity, because mitochondria in glial cells store excess manganese (Aschner et al., 1992; Tholey et al., 1987). The excess manganese may disturb astrocytic functioning, thus preventing astrocytes from providing neurons with the necessary substrates for energy and neurotransmitter metabolism (Hazell, 2002; Tomas-Camardiel et al., 2002; Zwingmann, Leibfritz, & Hazell, 2003).

An additional possibility is that manganese may initiate excitotoxicity by indirectly increasing extracellular glutamate. Evidence supporting this idea
includes the finding that manganese treatment decreases the uptake of glutamate in cultured astrocytes, thereby increasing extracellular glutamate (Chen & Liao, 2002). Moreover, intrastriatal injections of manganese cause lesions similar to those produced by N-methyl-D-aspartate (NMDA) agonists (Brouillet, Shinobu, McGarvey, Hochberg, & Beal, 1993). The latter finding is more than coincidental, because it is possible to prevent manganese-induced lesions by either destroying corticostriatal glutamatergic input fibers or pretreating rats with NMDA receptor antagonists (Brouillet et al., 1993).

Lastly, it is possible that manganese can cause neurotoxicity by inducing oxidative stress and free radical formation (Chen & Liao, 2002; Desole et al., 1995; Dobson et al., 2003). Both of these processes can be initiated by excess amounts of extracellular dopamine and glutamate, which is characteristic of early manganese poisoning (Chandra & Shukla, 1981; Chen & Liao, 2002; Desole et al., 1995; Dorman et al., 2000). More specifically, dopamine is catabolized through oxidation, thus increasing free radical formation (Cadet & Brannock, 1998). Consequently, free radicals stimulate the release of additional glutamate which potentiates glutamate excitotoxicity (Cadet &
Additionally, manganese directly induces the oxidation of dopamine, resulting in additional oxidative damage (Chen & Liao, 2002; Desole et al., 1995; Takeda, 2003).

Manganese and Neurotransmitter Systems

Dopamine

Manganese affects dopamine levels complexly, depending upon the length of exposure and the age of the animals. Exposing male rats to manganese (1 mg/ml of water) for 15, 30, 60, or 240 days increased striatal dopamine levels; whereas, exposing rats to the same dose for 300 and 360 days reduced striatal dopamine levels (Chandra & Shukla, 1981). Consistent with the latter finding, chronic exposure to manganese (2 g/kg of food) for 365 days decreased striatal dopamine (Komura & Sakamoto, 1992). Taken together, these findings suggest that manganese exposure of less than 300 days increases striatal dopamine levels, whereas manganese exposure for more than 300 days is associated with striatal dopamine depletions (Chandra & Shukla, 1981). In contrast, a number of other researchers have reported that even a brief exposure to manganese can decrease dopamine levels. For example, subchronic exposure
to manganese (100 mg/kg orally twice a day for 7 consecutive days) decreased dopamine levels in the brainstems of 90- and 120-day-old rats (Desole et al., 1994a), and decreased striatal dopamine levels in 120-, but not 90-, day-old rats (Desole et al., 1994b).

The effects of manganese exposure on preweanling rats is even more uncertain, because neonatal manganese exposure has been reported to increase (Dorman et al., 2000), decrease (Tran, Chowanadisai, Crinella, Chicz-DeMet, & Lonnerdal, 2002; Tran, Chowanadisai, Lonnerdal, Le, Parker, Chicz-DeMet, & Crinella, 2002), or not affect dopamine levels (Kontur & Flechter, 1985; Pappas et al., 1997). Differences in age, dose, and type of dosing method may account for these discrepant results. For example, dopamine levels were enhanced after high-dose oral exposure (25 or 50 mg/kg/day) on PD 1-21 (Dorman et al., 2000), but not after low-dose oral exposure (25 or 50 µg per day) on the same days (Kontur & Flechter, 1985). Age may also influence neurotoxicity because low-dose oral manganese treatment (250 or 500 µg per day) on PD 1-21 resulted in decreased striatal dopamine levels when assayed at PD 40 or PD 62, but not at PD 21 (Kontur & Flechter, 1985; Tran, Chowanadisai, Crinella, Chicz-DeMet, & Lonnerdal, 2002;
Tran, Chowanadisai, Lonnerdal, Le, Parker, Chicz-DeMet, & Crinella, 2002). Interestingly, striatal dopamine levels were not affected when manganese was administered in drinking water prenatally, postnatally, or during early adolescence (Pappas et al., 1997).

In addition to altering dopamine levels, manganese affects the functioning of dopamine autoreceptors, and presynaptic and postsynaptic D₂-like receptors. Manganese disrupts the functioning of dopaminergic autoreceptors in the striatum resulting in a loss of autoreceptor control over dopamine release (Cuesta de Di Zio, Gomez, Bonilla, & Suarez-Roca, 1995). Additionally, manganese-exposed rats exhibit enhanced striatal D₂-like receptor binding without any observable changes in dopamine levels or dopamine metabolites (Seth & Chandra, 1984). This effect is only seen in adult rats, because neonatal manganese exposure reduces striatal D₂-like receptor binding (Seth & Chandra, 1984). Manganese also alters the functioning of D₂-like dopamine receptors in the striatum, because quinpirole-induced excitatory postsynaptic potentials were depressed in manganese-treated rats (Calabresi et al., 2001).

Manganese may damage dopamine transporters. In addition to removing endogenous dopamine and reactive
dopamine metabolites from the synaptic cleft, dopamine transporters bring exogenous compounds into the synaptic terminal, which renders the dopamine transporter vulnerable to damage by multiple toxins (Miller, Gainetdinow, Levy, & Caron, 1999). In particular, it is known that manganese is capable of entering the neuron via dopamine transporters, because cocaine pretreatment blocks manganese absorption (Ingersoll, Montgomery, & Aposhian, 1999). Early manganese exposure may increase the vulnerability of the dopamine transporter and cause damage to dopamine nerve terminals. Consistent with this idea, neonatal exposure to environmental toxins and stimulant drugs affects dopamine transporters. Specifically, cocaine and heptachlor increase dopamine transporter binding, whereas neonatal exposure to PCBs reduce the number of dopamine transporters (Caudle, Richardson, & Miller, 2004; Fang, & Ronnekleiv, 1999; Purkerson-Parker, McDaniel, & Moser, 2001; Richardson, & Miller, 2004). It has yet to be determined if early manganese exposure causes lasting changes to dopamine transporters that persist into adulthood.

**Glutamate and γ-aminobutyric Acid**

Previous chapters discussed how dopamine, glutamate, and GABA neurons interact in the basal ganglia. Therefore,
it is not surprising that manganese alters glutamate and
GABA system functioning, as well as dopaminergic
functioning. Several research reports indicate that GABA
neurotransmission is altered by manganese exposure
(Bonilla, 1978; Erikson & Aschner, 2003; Gianutsos &
Murray, 1982; Gwiazda, Lee, Sheridan, & Smith, 2002; Lipe,
Duhart, Newport, Slikker, & Ali, 1999). Specifically, long-
term exposure to high doses of manganese in drinking water
(10 mg/ml for 60 days), food (4% of total diet for 180
days), or subcutaneous injection (80 mg/kg, alternating
days for 3 weeks) enhanced GABA levels in the striatum,
while reducing dopamine content (Bonilla, 1977; Gianutsos &
Murray, 1982). Consistent with these findings, repeated
treatment with low doses (4.8 mg/kg, 3 times a week for 5
weeks) of manganese increased striatal GABA levels, while
leaving dopamine unaffected (Gwiazda, Lee, Sheridan &
Smith, 2002). However, the effects of manganese are not
uniform, because a manganese regimen of 6 mg/kg (for 30
consecutive days) caused a decrease in whole brain GABA
levels (Chandra, Malhotra, & Shulka, 1982). Likewise,
manganese (200 nM) perfused into the striatum and
hippocampus during in vivo microdialysis also reduced GABA
concentrations (Takeda, Sotogaku, & Oku, 2002, 2003). When
these results are considered together it appears that manganese differentially affects GABA neurotransmission depending on route of administration, dose, and brain areas investigated.

Researchers have also examined the effects of manganese on glutamate neurotransmission. This is of particular importance because extracellular glutamate causes excitotoxicity, which is one of the mechanisms responsible for manganese-induced neurotoxicity (Brouillet et al., 1993). Specifically, manganese increases extracellular glutamate by altering the rate of glutamate uptake by astrocytes (Chen & Liao, 2002; Erikson & Aschner, 2002). In particular, manganese down-regulates glutamate/aspartate (GLAST) transporter expression, thus reducing the ability of astrocytes to remove extracellular glutamate (Erikson & Aschner, 2002).

Manganese also disrupts glutamate and GABA neurotransmission in developing rats (Erikson, Zakariya, Shihabi, Aschner, & Aschner, 2002; Lipe et al., 1999). Exposing weanling rats to high doses of manganese (20 mg/kg via oral gavage for 30 days) increased striatal GABA levels (Lipe et al., 1999). However, exposing iron-deficient weanling rats to manganese (100 mg/kg in food for 42 days)
decreased GABA levels in both the striatum and globus pallidus, while increasing glutamate in the cortex (Erikson et al., 2002). In the latter study, manganese concentrations were negatively correlated with striatal GABA levels (Erikson et al., 2002). These manganese-induced effects have only been observed in iron-deficient weanling rats, so it has yet to be determined whether similar effects will occur after neonatal or perinatal manganese exposure in non-iron deprived rats.

Interaction Between Manganese and the Dopamine, Glutamate, and γ-aminobutyric Acid Neurotransmitter Systems

It has been well established that long-term or high-dose exposure to manganese causes dopamine depletion in the nigrostriatal pathway. However, prior to actual cell loss or degradation of this pathway, neuropsychiatric symptoms and neuromotor impairment are exhibited by humans, non-human primates, and rodents (Aschner, 2000; Beuter et al., 1999; Dorman et al., 2000; Mergler, 1999; Mergler et al., 1994; Newland & Weiss, 1999; Olanow et al., 1996; Witholt, Gwiazda, & Smith, 2000). For this reason it is possible that the initial motor impairment occurring after manganese exposure may be caused by GABAergic, rather than
dopaminergic, dysfunction (Chandra, Malhotra, & Shukla, 1982; Erikson & Aschner, 2003; Gwiazda et al., 2002; Witholt et al., 2000). In light of this, Erikson and Aschner (2003) have suggested that disruptions of GABAergic and glutamatergic neurotransmission may precede disruptions of the dopaminergic system. Moreover, neurochemical alterations of glutamate and GABA may be primarily responsible for the behavioral manifestations of early manganese toxicity (Erikson & Aschner, 2003). Specifically, manganese exposure (4.8 mg/kg, 3 times a week for 5 weeks) increased striatal GABA levels, suppressed overall motor activity, and impaired neuromuscular coordination without altering dopamine or dopamine metabolites (Gwiazda et al., 2002; Witholt et al., 2000).

The interaction between the dopamine, glutamate, and GABA neurotransmitter systems involves basal ganglia circuitry (see Figure 3). For example, manganese increases cortical glutamatergic input to the striatum (Brouillet et al., 1993; Calabresi et al., 2001; Cuesta de Di Zio et al., 1995) and decreases inhibitory GABA input to the substantia nigra and globus pallidus (Erikson et al., 2002). Manganese accumulation in the globus pallidus also decreases the firing rate of GABA neurons projecting to the subthalamic
Figure 3. Schematic representation of basal ganglia circuitry during manganese exposure (Adapted from Erikson & Aschner, 2003; Verity, 1999). Bold lines represent an increase in transmission; whereas, broken lines represent a decrease in transmission.
nucleus (Erikson & Aschner, 2003). As a result, glutamate neurotransmission from the subthalamic nucleus to the substantia nigra is disinhibited (Erikson & Aschner, 2003). In summary, high-dose or long-term manganese exposure disrupts the functioning of dopaminergic, glutamatergic, and GABAergic neurons. Interestingly, low-dose manganese exposure can alter glutamate and GABA levels without changing dopamine levels. Therefore, Erikson and Aschner (2003) suggest that manganese-induced alterations in glutamate and GABA neurotransmission may precede changes involving dopamine neurons.
CHAPTER FIVE
MANGANESE AND BEHAVIORAL ALTERATIONS

Introduction

The previous chapters described manganese toxicity in humans, the function of the basal ganglia, dopaminergic transmission, and the effects of manganese within the central nervous system. Not surprisingly, manganese also causes behavioral alterations in laboratory animals. Chapter 5 will discuss behavioral changes in neonatal and adult rats that result from manganese exposure.

Manganese and Locomotor Activity

Since manganese toxicity is associated with dysfunctions of the dopamine neurotransmitter system, research has focused on alterations in spontaneous locomotor activity as a measure of manganese toxicity. The effects of manganese on motoric function are not uniform, however, because there are conflicting reports regarding manganese exposure and locomotor activity (Dorman et al., 2000; Normandin et al., 2002; Salehi et al., 2003). For example, inhaled manganese (300 µg/m³ a day for 13 weeks) has been alternately reported to leave motoric activity unaffected or increase motoric activity (Normandin et al.,
2002; Salehi et al., 2003). Likewise, spontaneous locomotor activity has been reported to decline after exposure to manganese during the neonatal period or in adulthood (Talavera, Arcaya, Giraldoth, Suarez, & Bonilla, 1999; Torrente, Colomina, & Domingo, 2002). Conversely, locomotor activity of 17-day-old rats has been reported to increase after perinatal exposure to manganese (Pappas et al., 1997) and enhanced locomotor activity was evident in adult rats exposed to manganese during adolescence (Calabresi et al., 2000; Nachtman, Tubben & Commissaris, 1986).

Manganese poisoning progresses in stages, so it is possible that these discrepant behavioral effects reflect manganese-induced changes in underlying neural systems. For example, manganese administered in drinking water increases motor activity during the first month of exposure, followed by five months of no change, and then causes a decrease in locomotor activity (Bonilla, 1984). It is possible that the initial increase in locomotor activity is due to the increased dopamine levels often associated with short-term manganese exposure (Bonilla, 1984; Chandra & Shukla, 1981; Nachtman et al., 1986). The subsequent decline in locomotor activity is consistent with nigrostriatal dopamine
depletions often associated with long-term exposure (Bonilla, 1984; Chandra & Shukla, 1981).

Because manganese alters neuronal functioning at a synaptic level, a pharmacological challenge may be necessary to "unmask" manganese-induced motoric deficits that are not observed during basal conditions (Adams, Buelke-Sam, Nelson, Reiter, Sobotka, Tilson, & Nelson, 1985; Hughes & Sparber, 1978; U.S. EPA, 1995). For example, exposing rats to manganese (10 mg/ml in drinking water) from conception to PD 80 did not affect locomotor activity; however, an amphetamine challenge (1 mg/kg) attenuated locomotor activity in manganese-exposed rats (Leung et al., 1982). In another study, amphetamine potentiated manganese-induced locomotor activity. In particular, manganese-exposed rats (1 mg/kg in drinking water for 14-29 weeks) challenged with amphetamine (1.25 mg/kg) exhibited enhanced locomotor activity relative to saline-challenged rats (Nachtman et al., 1986). Interestingly, this manganese-induced effect declined in magnitude by 29 weeks and dissipated with subsequent testing (Nachtman et al., 1986). Taken together, these data indicate that amphetamine differentially affects the neuronal functioning of
manganese-exposed rats by alternately potentiating and suppressing dopamine-mediated locomotor activity.

Manganese and Learning

Several different learning paradigms have been used to investigate the effects of manganese on learning and memory. For example, neonatal manganese exposure impaired performance on a passive avoidance task at PD 32, but not at PD 21, or in adulthood (Dorman et al., 2000; Torente et al., 2002; Tran, Chovanadisai, Lonnerdal, Le, Parker, Chicz-DeMet, & Crinella, 2002). In adult rats, manganese also impaired the acquisition of an avoidance response to both conditioned and unconditioned stimuli (Shukakidze, Lazriev, & Mitargwvriya, 2003).

Manganese also influences performance on maze learning, however the effects are dependent upon age of exposure and manganese regimen. Rats exposed neonatally to manganese were unaffected on the elevated plus maze, Morris water maze, or radial arm maze (Pappas et al., 1997). During adolescence, acquisition rates on the radial arm maze were similar for manganese-exposed (20 mg/ml in water for 10 weeks) and control rats, indicating that manganese does not affect spatial learning (Calabrasi et al., 2001).
However, manganese-exposed adolescent rats correctly completed an eight arm radial maze faster than controls, thus indicating that manganese causes motor impairments rather than cognitive dysfunction (Calabrasi et al., 2001). Adult rats exposed chronically to high doses of manganese (20 or 50 mg/kg in drinking water for 30 days) prior to maze training showed severe learning impairment and motoric dysfunction on a multipath maze (Shukakidze et al., 2003).

Manganese and Functional Observational Batteries

A functional observational battery (FOB) is comprised of a variety of assessments to evaluate developmental landmarks, sensory and motor development, and autonomic functioning (U.S. EPA, 1995; Verity, 1997). Exposing rats to manganese during gestation (1 or 2 mg/kg/day, s.c.) caused a subsequent increase in infant mortality between PD 0 and PD 4, and delayed both eye opening and incisor eruption (Torrente et al., 2002). Manganese treatment did not affect surface righting, although it did decrease the latency to complete a 180° turn on a negative geotaxis task (Torrente et al., 2002). On an olfactory discrimination task, manganese increased the latency for 10-day-old rats to find their home bedding (Tran, Chowanadisai, Crinella, 57
Chicz-DeMet, & Lonnerdal, 2002). Taken together, these data indicate that early manganese exposure is capable of interfering with normal physical, sensory, and motor development.

While FOBs are primarily used in developmental neurotoxicological studies, adult female rats exposed chronically to low doses of manganese (4.8 mg/kg, 3 days a week for 5 weeks) exhibited increased motor activity, rearing, balance beam falls and general balance impairment, as well as gait abnormalities and enhanced behavioral arousal (Witholt, Gwiazda, & Smith, 2000). In contrast, adult male rats exposed to manganese (0, 25, or 50 mg/kg) for 18 consecutive days did not show any differences on a FOB (Dorman et al., 2000). Taken together, these results indicate that manganese exposure regimen, sex, and age at testing may impact the behavioral manifestations of manganese toxicity.

Summary

This chapter detailed behavioral alterations that result from manganese exposure. The most intriguing findings include: 1) the effects of early manganese exposure (gestational through adolescents) on physical,
sensory, and motor development, and 2) manganese-induced changes in responsiveness to dopamine agonist drugs.
CHAPTER SIX
DIFFERENTIAL REINFORCEMENT OF
LOW RATES OF RESPONDING

Introduction

Schedule controlled operant behaviors are often used to assess learning and memory processes. Performance on these tasks depends on training and motivational variables, which may be disrupted by environmental neurotoxicants (U.S. EPA, 1995). Alterations of typical response patterns associated with specific reinforcement schedules are potential evidence of a neurotoxic effect (U.S. EPA, 1995). These alterations in operant responding may include increases in overall response rates, temporal processing, and response distributions. The differential reinforcement of low rate responding (DRL) task is a response contingent behavioral task designed to assess both impulsivity and temporal processing. The next chapter will provide information about the DRL task, neural substrates involved in DRL performance, and the role of dopamine in DRL performance.
Differential Reinforcement of Low Rates of Responding

The DRL reinforcement schedule is an interval reinforcement schedule which requires that a specified amount of time pass before reinforcement is available. The DRL schedule differs from a traditional fixed interval schedule because premature responses reset the fixed interval. It often takes multiple trials to perform this task accurately because there are both extinction and partial reinforcement components to DRL learning (Reynolds, 1975; Zeigler, 1977). For example, at the beginning of training the majority of responses are not reinforced, therefore operant responding begins to extinguish. However, before the behavior is extinguished, a response generated after the fixed interval will be reinforced. This reinforced response will generate increased responding. This pattern will be repeated until the organism learns that reinforcement is only available after a fixed interval of time has passed between responses (Reynolds, 1975).

In neurotoxicity or drug studies, performance on a DRL schedule is primarily defined by overall response rates and response patterns. Overall response rates on a DRL schedule can be assessed by general endpoint measurements,
including: total bar press responses, number of reinforcements received, and efficiency scores (reinforcements received/bar press responses). However, while these measures can provide general information about performance, subtle differences in response patterns often go unnoticed (Thompson & Grabowski, 1972). In neurotoxicity studies, examining response patterns is important because it is necessary to differentiate between rapid bar press responses (burst responses) and mistimed responses.

Typically, response patterns are identified by an analysis of inter-response-time (IRT) (Thompson & Grabowski, 1972). IRT is the amount of time occurring between operant responses (Thompson & Grabowski, 1972). In an IRT analysis, operant responses are categorized into time bins (see Figure 4). For example, bin 1 represents the number of bar press responses occurring between 0-2 s; bin 2 represents bar press responses occurring between 2-4 s; and bin 3 represents bar press responses occurring between 4-6 s, etc. The typical IRT distribution on a DRL reinforcement schedule is bimodal. The first peak in the distribution represents short IRTs (i.e., bin 1 responses). The second peak occurs in the time bin representing
Figure 4. Sample of a traditional differential reinforcement of low rates of responding (DRL) intertrial response time (IRT) distribution. Typical performance has a bimodal response pattern with peak bar press responding being represented in the first time bin (i.e., b1) and reinforcement time bin (i.e., b9).
reinforced responses (bin 9, in this case). Alterations in the characteristic IRT distribution of a DRL schedule may indicate behavioral abnormalities. In particular, increased bar presses in bin 1 is indicative of increased impulsivity (Nalwa & Rao, 1985; 2001; Sokolowski & Salamone, 1994; Wiley, Compton, & Golden, 2000). Additionally, changes in the number of bar press responses occurring immediately prior to reinforcement (i.e., bin 6-8, in this case) or after reinforcement (i.e., bin 10-12, in this case) indicates a disruption of temporal processing (Compton et al., 2001; Wiley, Compton, & Golden, 2000).

Neural Substrates Involved in Differential Reinforcement of Low Rates of Responding Performance

Optimal performance on the DRL task requires response inhibition and accurate timing ability, both of which involve corticostriatal circuitry. Response inhibition is mediated by the prefrontal cortex, since damage to this area typically increases impulsive responding (Broersen & Uylings, 1999; Christakou, Robbins, & Everitt, 2001; Granon, Hardouin, Courtiere, & Poucet, 1998). Not surprisingly, lesions to the prefrontal cortex and striatum increase impulsive responding on a DRL schedule (Dunnett &
Iverson, 1982; Kolb, Nonneman, & Singh, 1974; Nalwa & Rao, 1985, 2001; Numan, Seifert, & Lubar, 1975; Rosenkilde & Divac, 1975; Schmartz & Isaacson, 1967). Taken together, these studies imply that intact corticostriatal circuitry is necessary to inhibit impulsive responding on a DRL task.

DRL performance also requires accurate estimation of time duration and this complex process involves basal ganglia-thalamocortical circuitry (Matell & Meck, 2000; Meck, 1996; Meck & Benson, 2002). Human neuroimaging studies show increased activation of basal ganglia-thalamocortical circuitry during the performance of timing tasks (Rao, Harrington, Haaland, Bobholz, Cox, & Binder, 1997; Rao, Mayer, & Harrington, 2001). Within the basal ganglia, the nigrostriatal pathway is particularly important because dopamine cell loss in the substantia nigra disrupts temporal processing (Malapani, Rakitin, Levy, Meck, Deweer, Dubois, & Gibbon, 1998). Since DRL performance requires both response inhibition and temporal processing, it is not surprising that the striatum mediates DRL performance via cortical connections (Neil, 1967).
The Role of Dopamine in Differential Reinforcement of Low Rates of Responding Performance

The dopamine system appears to be important for DRL performance because enhancing or depressing dopamine neurotransmission alters overall response rates and temporal processing. Specifically, 6-OHDA lesions of the frontal cortex increases impulsive responding, as indicated by an increase in bin 1 responses on the DRL task (Sokolowski & Salamone, 1994). Likewise, 6-OHDA lesions of the striatum cause both an increase in impulsive responding (i.e., bin 1 responses) and a decrease in overall efficiency scores (Dunnett & Iverson, 1982). Dopamine agonists speed up DRL performance (i.e., they shift the peak IRT bin to the left), while dopamine antagonists slow down DRL performance (i.e., they shift the peak IRT bin to the right) (Paule et al., 1999). More precisely, treatment with various indirect dopamine agonists (i.e., methamphetamine, amphetamine, and methylphenidate) decreases efficiency scores, increases the number of short IRTs, and increases overall responses (Bizot, 1998; Emmett-Oglesby, 1980; Paule et al., 1999; Sabol, Richards, & Yung, 2000; Seiden, Andresen, & Mac Phail, 1979). These effects appear to be mediated by dopamine receptors located in the
striatum (Neil, 1967). In contrast, dopamine antagonists (e.g., haloperidol, clozapine, and pimozide) decrease response rates, which results in longer IRTs (Britton & Koob, 1989; Compton et al., 2001; Seiden, Dahms, & Shaughnessy, 1985; Wiley et al., 2000).

Differential Reinforcement of Low Rates of Responding Performance and Environmental Toxins

The adverse effects of environmental toxins can be identified using the DRL task. Specifically, organophosphates, trimethyltin, and lead acetate all disrupt DRL performance (Bizot, 1998; Dietz, McMillan, Grant, & Kimmell, 1978; McDonough, Smith, & Smith, 1986; Rice, 1992; Rice, & Gilbert, 1985). In the former case, the number of overall responses and the number of reinforcements earned on the DRL task were reduced after exposure to organophosphates (Bizot, 1998; McDonough et al., 1986; Wenger et al., 1985). Likewise, trimethyltin, which impairs cellular functioning, transiently alters DRL performance at low doses and permanently decreases DRL responding at high doses (Wenger et al., 1985; Woodruff, Baisden, & Nonneman, 1991). Lead acetate, a mineral that alters dopaminergic functioning, also alters DRL

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performance in rodents and non-human primates (Dietz et al., 1978; Jason, & Kellog, 1981; Rice, 1992; Rice, & Gilbert, 1985). In particular, neonatal lead exposure increases the number of bar presses with short IRTs and decreases the number of reinforcements earned (Dietz et al., 1978; Rice, 1992; Rice & Gilbert, 1985). Therefore, while no studies have investigated the ability of manganese to alter DRL performance, there is an abundance of evidence suggesting that a variety of neurotoxicants, some of which affect the dopamine system, alter DRL performance.

Summary

The DRL task is a sensitive measure of impulsivity and temporal processing (Paule et al., 1999; Reynolds, 1975; Sokolowski & Salamone, 1994; Thompson & Grabowski, 1972). Intact functioning of the prefrontal cortex and basal ganglia is necessary for successful performance on the DRL task, thus this task is sensitive to alterations of dopamine neurotransmission (Compton et al., 2001; Neil, 1967; Sokolowski & Salamone, 1994). Not surprisingly, neurotoxin-induced alterations of the dopamine system adversely affect DRL performance.
CHAPTER SEVEN

CONDITIONED PLACE PREFERENCE

Introduction

Chapter 6 discussed the use of the DRL task to measure neurotoxicity because environmental toxins often affect impulsivity and temporal processing. It is also possible that environmental neurotoxicants are capable of disrupting reward functioning. At present, it is uncertain whether neonatal manganese exposure alters the rewarding properties of psychostimulant drugs. Chapter 7 will discuss the CPP paradigm, the neural substrates mediating cocaine place preference, and the effects of environmental toxins on CPP.

Conditioned Place Preference

In the CPP paradigm, the animal learns an association between a particular compartment of a testing apparatus and the rewarding properties of food, water or a drug (Beninger & Miller, 1998; Wise & Hoffman, 1992). In most CPP studies the environmental context becomes associated with the rewarding aspects of a particular drug. The associations formed during the CPP procedure are based on Pavlovian learning principles. Specifically, a previously neutral stimulus (i.e., the testing compartment) is paired with an
unconditioned stimulus (i.e., the drug). After repeated pairings of the testing compartment (conditioned stimulus) with the drug (unconditioned stimulus), the animal forms an association between the two. In the case of CPP, the animal prefers to spend time in the drug-paired compartment because the test compartment has come to elicit feelings of reward or drug craving (Beninger & Miller, 1998; Wise & Hoffman, 1992).

Neural Substrates Involved in Cocaine-Induced Conditioned Place Preference

Cocaine- and amphetamine-induced CPP involves multiple neural substrates including the nucleus accumbens, ventral tegmental area, globus pallidus, and prefrontal cortex (for a review, see Bardo, 1998). Together these brain areas compose the mesolimbic, mesocortical, and mesopallidal dopamine circuits that were detailed in Chapter 3. The CPP paradigm has provided evidence implicating each of these circuits with the reinforcing properties of dopaminergic drugs.

Mesolimbic Circuitry and Conditioned Place Preference

The mesolimbic dopamine pathway consists of dopaminergic cell bodies located in the ventral tegmental
area and projecting to the nucleus accumbens (Ikemoto & Panksepp, 1999; Oades & Halliday, 1987; Ungerstedt, 1971). Microinjecting both direct (SKF 38393 and quinpirole) and indirect (amphetamine) dopamine agonists into the nucleus accumbens increases the amount of time rats spend in the drug-paired compartment (Carr & White, 1983; 1986; White, Packard, & Hiroi, 1991). Not surprisingly, intra-accumbens microinjections of D₁-like and D₂-like antagonists (SCH 23390 and sulpiride) block amphetamine-induced CPP (Hiroi & White, 1991). Conversely, cocaine-induced CPP is only blocked when a D₁-like receptor antagonist (SCH 23390), and not a D₂-like receptor antagonist (sulpiride), is microinjected into the nucleus accumbens (Baker, Fuchs, Specio, Khroyan, & Neisewander, 1998; Baker, Khroyan, O’Dell, Fuchs, & Neisewander, 1996). Therefore, D₁-like receptor stimulation in the nucleus accumbens is more important for the rewarding properties of cocaine CPP than is D₂-like receptor stimulation (Baker et al., 1996, 1998).

Mesocortical Circuitry and Conditioned Place Preference

The mesocortical dopamine pathway is composed of dopaminergic cell bodies located in the ventral tegmental area and projecting to the prefrontal cortex (Ikemoto &
Several lines of evidence suggest that the prefrontal cortex is associated with the rewarding properties of cocaine. First, rats will self-administer cocaine directly into the prefrontal cortex (Goeders & Smith, 1983, 1986). Second, destruction of dopamine terminals in the prefrontal cortex prevents a CPP response to cocaine, but not amphetamine (Bardo, 1998; Carr & White, 1986; Isaac, Nonneman, Neisewander, Landers, & Bardo, 1989; McBride, Murphy, & Ikemoto, 1999). Taken together, these data suggest a dissociation between those brain areas mediating the effects of psychostimulant drugs, with the prefrontal cortex being more important for cocaine CPP, whereas the nucleus accumbens is critical for amphetamine CPP (Carr & White, 1986; Isaac et al., 1989).

**Mesopallidal Circuitry and Conditioned Place Preference**

Emerging research suggests that mesopallidal circuitry may also be important for the rewarding actions of psychostimulant drugs (Berridge, 2003; Gong, Neill, & Justice, 1996; McBride et al., 1999; Panagis et al., 1997). This pathway consists of dopamine cell bodies located in the ventral tegmental area and projecting to the globus
pallidus (Klitenick, Deutch, Churchill, & Kalivas, 1992). Lesions to the globus pallidus attenuate the reinforcing effects of cocaine and food reward (Gong et al., 1997; McAlonan, Robbins, & Everitt, 1993). Additionally, microinjecting amphetamine and cocaine into the globus pallidus induces CPP (Gong, Neill, & Justice, 1996). These data are consistent with electrophysiology studies showing that pallidal neurons are responsive to systemic cocaine administration independent of activation of the nucleus accumbens (Johnson & Napier, 1996).

Conditioned Place Preference and Environmental Toxins

Very few studies have investigated the effects of environmental toxins on reward processes. Available studies indicate that exposure to environmental toxins can lead to changes in the rewarding properties of psychostimulant drugs. For example, cadmium attenuates place preference conditioning to low, but not high, doses of cocaine and apomorphine (Miller, Palme, Najvar, Caudill, & Nation, 1999). In addition, perinatal lead exposure attenuates cocaine- and morphine-induced CPP in adult rats (Miller, Nation, & Bratton, 2000; Valles, Cardon, Heard, Bratton, & Nation, 2003). Taken together, these studies indicate that
environmental toxins are capable of causing long-lasting alterations in reward function.
CHAPTER EIGHT
THE EFFECTS OF NEONATAL MANGANESE EXPOSURE ON IMPULSIVITY, UNLEARNED MOTORIC FUNCTION, AND REWARD

Summary

Manganese is an essential trace mineral that can damage the central nervous system. There is increasing evidence that children may be particularly prone to manganese neurotoxicity, because immature brains accumulate manganese more extensively than adult brains and are less efficient at maintaining homeostatic levels (Takada, Ishiwatari, & Okada, 1999). Therefore, manganese exposure during development may amplify the risk of neuronal damage (Cawte, 1985; Lonnerdal, 1994; Weiss, 1999). Of special concern are infant formulas, because manganese levels in commercially available infant formulas may be sufficient to cause lasting behavioral deficits and neurological dysfunctions that are similar to symptoms observed in ADHD (Lonnerdal, 1994; Tran, Chowanadisai, Crinella, Chicz-DeMet, & Lonnerdal, 2002; Tran, Chowanadisai, Lonnerdal, Le, Parker, Chicz-DeMet, & Crinella, 2002). Thus, children fed these formulas may be at risk for lasting brain changes.
that result in long-term behavioral dysfunction (Cawte, 1985; Lonnerdal, 1994; Mergler, 1999; Weiss, 1999).

Based on this rationale, I designed a series of experiments to explore the effects of low-dose manganese exposure on behaviors that are sensitive to disruption of the corticostriatal pathway, and the nigrostriatal and mesocorticolimbic dopaminergic systems. Additionally, I investigated the effects of early manganese exposure on developmental landmarks, sensory and motor development, and neurochemical indices of dopamine transporters.

**Experiment One**

Recent research has found that moderate levels of manganese, consistent with those found in infant formulas, are able to produce behavioral and neurochemical alterations in rats (Tran, Chowanadisai, Crinella, Chicz-DeMet, & Lonnerdal, 2002; Tran, Chowanadisai, Lonnerdal, Le, Parker, Chicz-DeMet, & Crinella, 2002). Specifically, these reports show that manganese decreases striatal dopamine levels and impairs performance on tasks that assess sensory and motor development (Tran, Chowanadisai, Crinella, Chicz-DeMet, & Lonnerdal, 2002; Tran, Chowanadisai, Lonnerdal, Le, Parker, Chicz-DeMet, &
Crinella, 2002). In these studies, however, ADHD symptomology, including impulsivity, was not measured.

Therefore, the purpose of this experiment was to determine whether early manganese exposure affects impulsivity of adult rats. The DRL paradigm will be used for two reasons: First, the DRL task is a sensitive measure of drug-induced changes in impulsivity; and, second, alterations in dopamine system functioning are known to affect DRL performance. Thus, if manganese enhances impulsivity by modulating the dopamine system, the DRL task should be sensitive to these changes.

Specifically, overall DRL performance was assessed by examining the total number of bar press responses and efficiency scores (reinforcement received/total responses) of saline- and manganese-exposed rats. Impulsivity, timing, and learning were further examined by analyzing IRT distributions. I hypothesized that early manganese exposure would enhance impulsivity of adult rats, as evidenced by reduced efficiency scores, increased amounts of total responding, bin 1 responding (responses occurring 0-2 s after a previous bar press), and burst responding (number of consecutive responses occurring within 1 s of the previous bar press). Additionally, manganese-exposed rats
should have had an increased number of responses in the time bins preceding the reinforcement bin, indicating a disruption of temporal processing. I also hypothesized that manganese-exposed rats should show impairments in acquisition (learning) of the DRL task as evidenced by fewer reinforced responses than control rats and a slower shift of bin responses (i.e., from early time bins to latter time bins over training days).

Experiment Two

Several lines of evidence suggest that neonatal manganese exposure can have deleterious behavioral and neurochemical effects. First, early manganese exposure is capable of impacting physical maturation, sensory, and motor development, as well as locomotor activity (Pappas et al., 1997; Tran, Chawanadisai, Crinella, Chicz-DeMet, & Lonnerdal, 2002; Torrente et al., 2002). Second, neonatal manganese exposure causes atypical responding to indirect dopamine agonists, which is indicative of manganese-induced neuronal impairments (Leung et al., 1982). These effects are not uniform, however, so it is not clear if moderate levels of manganese will induce short- or long-term changes in motoric development, motor activity, or neuronal
function. Third, manganese interferes with dopamine transporter functioning (Aposhian et al., 1999).

Therefore, the purpose for this experiment was three-fold. The first purpose was to assess the effects of early manganese exposure on standard developmental landmarks (e.g. physical development, motor development, sensory function, and activity levels) during the neonatal period. Balance and coordination was assessed during adulthood. The second purpose was to assess the effects of early manganese exposure on basal and cocaine-induced locomotor activity in preweanling and adult rats. The third purpose was to assess the effects of early manganese exposure on striatal dopamine transporters.

I hypothesized that early manganese exposure would delay the appearance of developmental landmarks, interfere with neonatal sensory and motor development, and impair balance and coordination in adulthood. I also hypothesized that manganese-exposed rats would exhibit an attenuated locomotor response to cocaine challenge. Finally, I hypothesized that manganese would decrease striatal dopamine transporter binding sites.
**Experiment Three**

The rewarding properties of cocaine are mediated by dopamine release in the nucleus accumbens, prefrontal cortex, and globus pallidus (Bardo, 1998; Hiroi & White, 1990). This is interesting because ADHD has been hypothesized to result from an immature reward system (Casey et al., 1997; Johansen et al., 2002; Sagvolden et al., 1998; Solanto et al., 1997). There are two reasons to suspect that manganese may disrupt psychostimulant-induced reward processes. First, manganese potentiates glutamate-induced activation of NMDA receptors and NMDA receptors are implicated in the acquisition of cocaine CPP (Cervo & Samanin, 1995; Cuesta de Di Zio et al., 1995). Second, manganese accumulates in brain areas associated with the mesopallidal and mesolimbic reward circuits, including the globus pallidus, the ventral tegmental area, and the nucleus accumbens (Berridge, 2003; Fell et al., 1996; Gong, Neill, & Justice, 1996; 1997; Komaki et al., 1999; McBride et al., 1999; Panagis et al., 1997).

Currently, most research on neonatal manganese exposure has focused on alterations of unlearned motor performance, which is predominantly mediated by the
nigrostriatal dopamine system. The effect of neonatal manganese exposure on the mesocorticolimbic dopamine system has not been fully explored. Therefore, the purpose of this study is to investigate the effects of neonatal manganese treatment on drug reward by utilizing a CPP paradigm. I hypothesized that manganese exposure would reduce the rewarding properties of cocaine, as evidenced by a decreased amount of time spent in the drug-paired compartment.
Subjects

Subjects were 208 male and female rats of Sprague-Dawley descent (Harlan, San Diego), born and raised at California State University, San Bernardino. Rats were housed in a colony room maintained on a 12:12 light:dark cycle at 22°-24°C. Litters were culled to 10 on postnatal day (PD) 1, weaned on PD 24, and group housed until the start of behavioral testing. Food and water was provided ad lib. Rats were treated in accordance with the National Institutes of Health guidelines ("Principles of Laboratory Animal Care", NIH Publication #85-23) under a research protocol approved by the Institutional Animal Care and Use Committee of California State University, San Bernardino.

Drugs and Chemicals

Manganese chloride (Sigma Chemicals, St. Louis) was dissolved in 10% sucrose solution and administered orally via micropipette. (-)-Cocaine hydrochloride (Sigma) was dissolved in saline and injected intraperitoneally at a volume of 5 ml/kg for preweanling rats and 1 ml/kg for adult rats.
Statistics

Behavioral data was analyzed using repeated measures analyses of variance (ANOVA). Both manganese condition (0, 250, 500, or 750 μg) and sex (male and female) were between-subject variables. Repeated measure variables were day, trial, or time block depending upon the experiment and behavioral task. Significant higher order interactions were further analyzed using one- or two-way ANOVAs. Neurochemical data were analyzed using nonlinear regression to determine $B_{\text{max}}$ and $K_d$ values. Group differences were assessed using one-way (manganese treatment) ANOVAs. All post hoc comparisons were done using Tukey tests ($p < .05$).
CHAPTER TEN

EXPERIMENT ONE: THE EFFECTS OF NEONATAL MANGANESE EXPOSURE ON A DIFFERENTIAL REINFORCEMENT OF LOW RATES OF RESPONSE TASK DURING ADULTHOOD

The purpose of Experiment 1 was to assess the effects of neonatal manganese exposure on the impulsivity of adult rats by using a DRL-20 task. It was predicted that early manganese exposure would amplify impulsivity of adult rats, as evidenced by increased amounts of total responding, bin 1 responding, and burst responding. Additionally, it was expected that manganese-treated rats would have fewer reinforced responses and reduced efficiency scores than control rats.

Methods

Manganese Treatment

A total of 32 rats (n = 8 per group) were used in this experiment. On PD 1-21, male and female rats were given daily supplements consisting of 0, 250, 500, or 750 μg manganese.

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Apparatus

Standard operant chambers (Coulborn Instruments, Allentown, PA) were used. The chambers were located inside sound attenuated cubicles and were controlled by an IBM compatible computer interfaced with Graphic State Notation software and data collection program. Operant chambers (30 x 30 x 25 cm) included one response lever, a house light, and a feeder.

Procedures

On PD 83, rats began food restriction (90% of normal body weight). On PD 90-94, rats were shaped to bar press for food reward. On PD 95, rats began the DRL procedure. Specifically, on PD 95 and PD 96 rats were placed on a DRL-5 schedule of reinforcement where they must withhold a response for more than 5 s to gain a food reward. Responses prior to the fixed interval reset the time clock. On PD 97-106, rats were advanced to a DRL-10 schedule for 10 consecutive days. Then for the following 30 days, rats were trained on a DRL-20 schedule in which they received a food reward after withholding a response for 20 s. Daily sessions terminated after acquisition of 50 pellets or until 50 min had elapsed.
DRL performance was assessed using several different measures. Total daily bar press responses, total reinforcements earned, and efficiency scores (reinforcements received/total responses) were used to assess overall performance. An analysis of IRTs was used to measure differences in overall patterns of responding. Specifically, responses occurring prior to the 20 s interval were recorded in ten 2-s time bins. Reinforced responses were recorded in an eleventh time bin. Responses occurring in the 0-2 s time bin were used as a measure of impulsivity. Multiple rapid bar press responses occurring within 1 s of each other were grouped and termed burst responses.

**Statistics**

Total responses, efficiency scores, reinforcements received, bin 1 responses, and burst responses were analyzed using separate $4 \times 2 \times 10$ (manganese condition $\times$ sex $\times$ time block) repeated measures ANOVAs. The manganese independent variable consisted of four levels of manganese pretreatment (0, 250, 500 or 1000 µg manganese per day); the sex independent variable consisted of male and female rats; and the repeated measure was over 30 days of DRL
performance divided into 10 three-day time blocks. Post hoc comparisons were done using Tukey tests \((p < .05)\).

\section*{Results}

\textbf{Differential Reinforcement of Low Rates of Responding: 5 - Second Delay}

Early manganese exposure did not impact acquisition of bar press shaping on an FR 1 schedule of reinforcement. During the subsequent two days of DRL 5 training, manganese interacted with sex to affect total number of bar press responses \([\text{manganese} \times \text{sex interaction}, F(3,47)=3.09, P < 0.05]\. However, post hoc comparisons using Tukey tests did not reveal any significant differences between groups. Additionally, when males and females were analyzed separately, manganese did not affect total bar press responses.

\textbf{Differential Reinforcement of Low Rates of Responding: 10 - Second Delay}

During the 10 days of DRL 10 training, manganese exposure did not alter performance on any behavioral measures \(\text{(efficiency scores, total number of responses, or burst responding). Additionally, manganese-induced effects were not apparent on an analysis of inter trial response} \)
Figure 5. Analysis of intertrial response time (IRT) bin distributions of male (upper graph) and female (lower graph) rats (n = 6-8). Time bins (2 s) are totaled over the 10-day testing session. Rats were pretreated with manganese from PD 1-21.

aSignificant difference between manganese-treated rats and vehicle controls (P < 0.05).
times for male or female rats (Fig. 5). In general, male rats (329.15 ± 24.13) bar pressed significantly more than female rats (245.00 ± 13.79) [sex main effect, $F(1,47)=10.43, P < 0.05$], with this effect reaching significance on days 1 - 4 [sex x day interaction, $F(9,423)=2.86, P < 0.05$]. Additionally, male rats (65.71 ± 7.11) made more 1 s burst responses than female rats (42.75 ± 3.59) on the DRL 10 schedule of reinforcement [sex main effect, $F(1,47)=8.77, P < 0.05$].

Differential Reinforcement of Low Rates of Responding: 20 - Second Delay

Over 30 consecutive days of DRL 20 training, efficiency scores did not differ according to manganese exposure or sex. Manganese exposure also did not affect total number of bar press responses. Overall, male rats (234.97 ± 20.94) had more bar press responses than female rats (183.09 ± 11.78) [sex main effect, $F(1,53)=4.915, P < 0.05$]. To further evaluate differences between controls and manganese-exposed rats, interaction comparisons were performed on total number of bar press responses. Data were combined across manganese treatments (250, 500, and 750 µg) and harmonic means were used to adjust for uneven sample size. The total number of bar press responses of manganese-
Figure 6. Mean total bar press responses (± S.E.M.) of adult male and female rats (n = 6-8) tested on a differential reinforcement (DRL) 20 s schedule for 30 consecutive days. Rats were treated with manganese from PD 1-21. Inset shows the average total bar press responses. 

aSignificantly different from manganese-treated rats (P < 0.05).

bSignificantly different from male rats (P < 0.05).
exposed rats varied according to sex (Fig. 6, inset) [manganese x sex interaction, \( F(9,459)=2.98, P < 0.05 \)]. For male rats, manganese caused a significant reduction in total number of bar press responses across the first 12 days (time blocks 1 - 4) of DRL 20 testing (Fig. 6, top graph) [manganese x time block interaction, \( F(9,216)=2.75, P < 0.05 \); and Tukey tests, \( P < 0.05 \)]. For female rats, manganese did not alter total bar press responses (Fig. 6, bottom graph).

Responses occurring within 1 s of each other were grouped and termed burst responses. Male rats (40.41 ± 7.08) made more 1 s burst responses than female rats (22.40 ± 3.67) [sex main effect, \( F(1,47)=6.23, P < 0.05 \)]. Burst responses varied by manganese exposure and sex over the 30 days of DRL 20 training (Fig. 7) [manganese x sex x day interaction, \( F(27,423)=1.95, P < 0.05 \)]. Interaction comparisons were conducted by combining manganese exposure groups. The number of 1 s burst responses varied for manganese-exposed rats according to sex (Fig. 7, inset) [manganese x sex interaction, \( F(1,51)=7.32, P < 0.05 \)]. Comparisons between manganese exposed groups (250, 500, or 750 μg) and controls showed that male rats exposed to
Figure 7. Mean (± S.E.M.) 1 s burst responses made by adult male and female rats (n = 6-8) tested on a differential reinforcement (DRL) 20 s schedule for 30 consecutive days. Rats were treated with manganese from PD 1-21. Inset shows the average total number of 1 s burst responses. 

aSignificantly different from manganese-treated rats (P < 0.05).

bSignificantly different from male rats (P < 0.05).
manganese committed fewer 1 s burst responses than controls during time blocks 1 - 6 (Fig. 7, top graph) [manganese \times time block interaction, F(9, 216)=4.32, P < 0.05; and Tukey tests, P < 0.05]. The 1 s burst responses of female manganese-exposed rats did not differ from vehicle-treated rats (Fig. 7, bottom graph).

An analysis of intertrial response times showed that differences in the bin distribution of manganese-treated rats varied according to sex throughout the 30 days of DRL testing (Fig. 8) [manganese \times sex \times response bin, F(30,470)=2.59, P < 0.05]. For male rats, manganese reduced bin 1 responses during time blocks 1 - 7 (Fig. 9) [manganese \times time block interaction, F(27,198)=5.48, P < 0.05; and Tukey tests, P<0.05]. In contrast, female rats exposed to manganese pressed the bar more than controls (Fig. 10, upper graph) [manganese \times time block interaction, F(27,225)=1.67, P < 0.05; and Tukey tests, P < 0.05]. Specifically, female rats pretreated with 500 \mu g manganese had more bin 7 responses on days 4 - 6 (i.e., time block 2); whereas, female rats pretreated with 750 \mu g manganese had more bin 7 responses than vehicle-treated rats on days 7 - 15 (i.e., time blocks 3 - 5) (Fig. 10, upper graph).
Figure 8. Analysis of intertrial response time (IRT) bin distributions of male (upper graph) and female (lower graph) rats (n = 6-8). Time bins (2 s) are totaled over the 30-day testing session. Rats were pretreated neonatally with manganese from PD 1-21. *Significant difference between manganese-treated rats and vehicle controls (P < 0.05).
Figure 9. Mean bar (± S.E.M.) press responses occurring in the 0-2 s time bin (i.e., time bin 1) for male rats (n = 6-8) during the 30 days of testing divided into ten 3-day time blocks. Rats were pretreated neonatally with manganese from PD 1-21.

*Significantly different from manganese-treated rats (P < 0.05).
Figure 10. Mean (± S.E.M.) number of bar press responses of female rats (n = 7-8) occurring in bin 7 (upper graph) and bin 11 (lower graph). The 30 days of testing are divided into ten 3-day blocks. Rats were pretreated with manganese (0, 250, 500, or 750 μg) on PD 1-21.

*Significantly different from vehicle-treated rats (P < 0.05).
Manganese exposure also affected the number of reinforced responses of female rats in bin 11 [manganese × time block interaction, $F(27,225)=1.56$, $P < 0.05$; and Tukey tests, $P < 0.05$]. Specifically, vehicle-exposed rats received more reinforcements on days 10 - 12 than rats pretreated with 50 μg manganese (i.e., time block 4) (Fig. 10, lower graph).

Discussion: Experiment One

Accurate performance on the DRL task requires precise estimation of time duration and the ability to inhibit a response, consequently this task is commonly considered a sensitive measure of impulsivity (Monterosso & Ainslie, 1999; Wiley et al., 2000). In addition, the DRL task is sensitive to alterations of the dopamine neurotransmitter system, therefore this task can be used to assess the effects of environmental neurotoxins on dopamine neurons. In the current study, neonatal exposure to manganese was evaluated on DRL 5, 10, and 20 schedules of reinforcement. It was originally hypothesized that manganese treatment would increase the impulsive responding of both male and female rats. However, the pattern of results reflects an overall reduction of responding in male rats and a subtle response increase in female rats.
Manganese-exposed rats were shaped over four days to press a lever for food reward and there were no differences in the ability of rats to acquire the operant response. During DRL training, female rats made fewer non-reinforced responses than male rats on the DRL 10 and 20 tasks. These findings are consistent with previous studies showing sexually dimorphic response rates on the DRL task (van Hest, van Haaren, & van de Poll, 1987). Moreover, the present experiment demonstrated that neonatal manganese exposure impacted male and female rats differently. Specifically, manganese reduced total bar press responding, burst responding, and bin 1 responses of adult male rats. Female rats did not differ on these measures; however, an analysis of the IRT bin distribution for female rats showed that manganese exposure caused increased responses in time bin 7 and decreased responses in time bin 11.

In the current study, manganese-exposed male rats had fewer total bar press responses and 1 s burst responses relative to vehicle-exposed rats. An analysis of the IRT bin distribution demonstrated that the decrease in bar press responding for male rats was predominately caused by reduced responses in time bin 1. Responses occurring in the first time bin are indicative of the ability to delay or
inhibit a voluntary behavior (Monterosso & Ainslie, 1999; Wiley, Compton, & Gorden, 2000). Taken together, it appears as if neonatal manganese treatment caused a decrease in impulsivity. In fact, this pattern of manganese-exposed effects is reminiscent of the behavioral response patterns induced by dopamine antagonist drugs. Dopamine antagonists slow down DRL performance, thus decreasing response rates and causing longer IRTs (Britton & Koob, 1989; Compton et al., 2001; Paule et al., 1999; Seiden, Dahms, & Shaughnessy, 1985; Wiley et al., 2000).

Based on these considerations, it is probable that neonatal manganese treatment attenuates dopamine transmission in a way that depresses motor, reward, or attentional functioning on the DRL task. For instance, the reduced bar press responding exhibited by manganese-treated male rats could result from manganese-induced alterations of the nigrostriatal dopamine pathway. Since manganese toxicity is commonly associated with extrapyramidal motor impairments, the generalized slowing of responses observed in manganese-exposed rats could be indicative of subtle long-term deficits in motoric ability. Similar to the effects of dopamine antagonists, manganese-exposed rats may have taken longer to terminate individual responses,
thereby reducing the number of response opportunities available during a testing session (Ettenberg, 1989). Decreased responses consisted primarily of rapidly executed burst responses and responses with short IRTs (i.e., 1 s burst and bin 1 responses), which further suggests that manganese may cause subtle motor difficulties. Consistent with this idea, manganese-exposed (5 and 10 mg/kg, i.p.) adult cebus monkeys had multiple failed attempts to complete a lever moving task on a variable ratio schedule of reinforcement (Newland & Weiss, 1992). Moreover, manganese exposure caused these monkeys to increase the length of IRT's, to decrease overall responses, and to eliminate burst responses (Newland & Weiss, 1992). Thus, manganese-induced motor impairment is a probable explanation for the reduced responses observed on the DRL 20 task.

Alternately, manganese may depress reward system functioning in male rats, hence the decreased bar press responding may reflect a lack of motivation for the food reinforcer. Because food reward is mediated, in part, by mesolimbic dopamine transmission (Fell et al., 1996; Gong, Neill, & Justice, 1996; 1997; Zhang, Balmadrid, & Kelly, 2003), accumulation of manganese in the mesolimbic pathway
may depress dopaminergic mediation of food reward. More specifically, manganese- and vehicle-exposed rats performed similarly on DRL 5 and DRL 10 tasks; however the increased difficulty of the DRL 20 task may have revealed a reduction in the saliency of food reward. Thus, manganese-exposed rats may be less inclined to bar press on a more difficult schedule of reinforcement.

Another possible explanation for the manganese-induced reduction in bar press responses is that manganese could have adversely affected attentional processes of male rats (i.e., the animals were easily distracted). For example, mesocortical dopamine transmission is important for modulating associative processes involved in decision-making, attentional control, and working memory, processes collectively referred to as “executive functions” (Barkley, 2003; Sullivan & Brake, 2003). Mesocortical dopamine is essential for an organism to focus more precisely on specific stimuli controlling behavior at a given time (Robbins, 2000; Sullivan & Brake, 2003). Since DRL performance is dependent on both cortical and striatal dopamine function, reductions in mesocortical dopamine could adversely affect DRL performance (Neil, 1967; Sokolowski & Salamone, 1994).
In contrast to male rats, manganese-exposed female rats did not show a decrease in rapidly executed burst responses, but instead showed subtle response increases in time bin 7. The increased responses observed prior to the fixed interval can be explained in two ways. First, manganese may have impaired the timing ability of female rats. However, this is unlikely since the number of responses in those time bins immediately prior to reinforcement (i.e., bins 9 and 10) did not vary. Alternately, manganese may have disrupted the ability of female rats to adapt to the new response requirements (i.e., a 20 s withholding period), because prior performance on a DRL 10 schedule may have interfered with later performance. Specifically, manganese-exposed female rats were unable to transfer learning from a DRL 10 schedule to a DRL 20 schedule. For example, manganese caused a dose-dependant increase in bar press responses sequentially surrounding the 10 s delay (i.e., bins 5 - 8), with the manganese effect reaching statistical significance on time bin 7. The transient nature of this effect (occurring on days 2 - 15) is suggestive of a delayed adaptation to the new response contingency, because performance was consistent with controls during the final
testing sessions. The decreased number of reinforcements earned by manganese-exposed females supports this idea, because increased responding during the 20 s delay would lead to a decline in the number of opportunities to earn reinforcement. A similar response pattern was observed in female rats exposed to polychlorinated biphenyls (PCB) (Holene et al., 1999). Specifically, PCB-treated females had difficulty adapting from a variable interval/extinction paradigm to a DRL 14 task. Holene and colleagues (1999) suggested that the PCB-exposed females exhibited a temporary delay in acquisition of the new reinforcement schedule.

When considered together, the differential responding of male and female manganese-exposed rats supports previous research demonstrating that developmental neurotoxicants are capable of producing sex-dependant effects (Hojo et al., 2002; Holene et al., 1999; Weiss, 2002). Because the developing endocrine system is vulnerable to exogenous compounds, alterations in gonadal hormones may contribute to the disparities observed in male and female rats (Weiss, 2002). At this point, it is important to note that male rats are particularly sensitive to neurotoxicants that damage the nigrostriatal dopamine system (Miller, Ali,
O’Callaghan, & Laws, 1998). This is largely attributed to the neuroprotective effects of estrogen (Dluzen, 2000; Miller et al., 1998). Although preweanling females have higher concentrations of serum estradiol than males (Banu, Govindarajulu, & Aruldhas, 2002), it is uncertain whether these levels are sufficient to produce neuroprotective effects during manganese exposure. Conversely, testosterone is known to affect performance on operant tasks (van Haaren, van Hest, & Heinsbroek, 1990; van Hest, van Haaren, & van de Poll, 1989), so it is possible that the differential responding of male and female rats may have been due to the presence of testosterone rather than the neuroprotective effects of estrogen.

In conclusion, early manganese exposure impacted the DRL performance of adult rats. Interestingly, many of these effects varied according to sex. These manganese-induced effects may involve several different dopamine-mediated systems: motor, reward, attention, and/or learning. Nevertheless, the present results indicate that neonatal manganese exposure has long-term behavioral consequences when performance is assessed on a DRL task.
CHAPTER ELEVEN

EXPERIMENT TWO: THE EFFECTS OF NEONATAL MANGANESE EXPOSURE ON UNLEARNED BEHAVIORS AND DOPAMINE TRANSPORTERS

The purpose of Experiment 2 was threefold. The first purpose was to examine the effects of early manganese exposure on general motoric ability of neonatal and adult rats. It was expected that neonatal manganese treatment would cause impairments in neonatal sensory and motor development, and impair balance and coordination in adulthood. The second purpose was to investigate the effects of neonatal manganese on basal and cocaine-induced locomotor activity of preweanling and adult rats. It was predicted that manganese-exposed rats would exhibit an attenuated locomotor response to cocaine challenge. The final purpose was to assess the effects of early manganese exposure on dopamine transporter binding sites in the striatum. It was expected that early manganese exposure would decrease the number of striatal dopamine transporters.
Methods

Manganese Treatment

A total of 72 rats \( (n = 8 \text{ per group}) \) were used in this experiment. On PD 1-21, rats were given supplements consisting of 0, 250, or 750 \( \mu \text{g} \) manganese dissolved in 25 \( \mu \text{l} \) of 10% sucrose solution.

Apparatus

Locomotor activity was measured using commercially available activity chambers (Coulbourn Instruments, Allentown, PA). For neonatal and preweanling animals the chambers measured 25.5 × 25.5 × 41 cm and for adult animals the chambers measured 41 × 41 × 41 cm. Both sized chambers consisted of acrylic walls, a plastic floor, and an open top. Each chamber utilized an X-Y photobeam array with 16 photocells and detectors to determine the total distance traveled (a measure of horizontal locomotor activity).

Olfactory orientation was measured in a Plexiglas enclosed runway (12 × 28 × 12 cm) with soiled home bedding at one end and clean bedding at the other end. Negative geotaxis was tested on a 25° Plexiglas inclined plane. The surface of the inclined plane was covered with sandpaper. Adult balance and coordination was evaluated on an elevated
balance beam (49 x 22 x 150 cm) at a 15° incline. A darkened goal box was placed at the elevated end of the beam. For male rats the beam width was 1.9 cm and the beam width for females was 1.3 cm.

**Procedures**

**Developmental Landmarks.** Pups were fed manganese daily between PD 1-21 and evaluated daily for developmental landmarks (i.e., eye opening, pina detachment, and incisor eruption). Specifically, eye opening was recorded when both eyes lids were completely separated. Pina detachment was recorded when the tip of the ear is separated from the head. Incisor eruption was recorded on the first appearance of upper incisors. Body weights were recorded on alternate days.

**Neonatal Sensory and Motor Development.** Negative geotaxis was used to measure the development of motor ability and motor reflex. On PD 8, PD 10, and PD 12 neonatal pups were placed head down on the 25° inclined plane and latency to rotate 180° on the inclined plane was measured during a 120 s testing session. If the rat fell, crawled off the plane, or braced (no movement), the rat failed the task and a maximum time of 120 s was assigned.
Olfactory discrimination was assessed as a measure of sensory development. On PD 9, PD 11, and PD 13 all pups were tested individually for olfactory discrimination of their home cage bedding. Specifically, 5-day old soiled bedding from their home cage was placed at one end of the runway while fresh bedding was placed at the other end. Each pup was placed on a center line with the head facing the Plexiglas wall. Latency to reach the home bedding was recorded. If the pup did not complete the trial in 120 s or if the pup reached the clean bedding, the pup was considered to have failed the task and a maximum time of 120 s was assigned.

Locomotor activity was used to as a measure of motor development. On PD 10, PD 12, and PD 14 motor activity for each pup was recorded for 1 min in the activity chambers. Total distance traveled and total number of movements per episode was recorded.

Adult Balance and Coordination. On PD 90, manganese-exposed adult rats were tested for balance and coordination using a balance beam task. Each rat was given three trials. On trial 1, rats were placed in the goal box for 30 s. Then an experimenter blind to the treatment condition placed the rat on the low end of the balance beam. On trials 2 and 3,
centrifuged at 20,000 × g at 4°C for 20 min. The pellet was resuspended in ice-cold buffer and centrifuged again at 20,000 × g at 4°C for 20 min. The final pellet was suspended in 12 vol of buffer. Protein concentrations for the final pellet were determined using the Bio-Rad Protein Assay with BSA as the standard.

Saturation binding assays were conducted in duplicate. Each assay tube contained 430 µg of buffer, varying levels of [³H]GBR 12935 (12 final concentrations, 0.1 - 37 nM), and tissue suspensions (50 µg/protein). Non-specific binding was determined in the presence of 10 µg GBR 12909 which selectively inhibits dopamine transporter function. The incubation period lasted 90 min at 4°C. Incubation was terminated by vacuum filtration (Brandell) over glass filter fibers (Brandell) previously soaked in 0.1% polyethylenimine. Filters were washed three times with ice-cold Krebs-HEPES buffer and radioactivity was measured by liquid scintillation spectrometry.

Statistics

Behavioral Data. Separate between subject (manganese condition × sex) ANOVAs were used to analyze eye opening, pina detachment, and incisor eruption. Body weights
throughout the neonatal period were analyzed using $3 \times 2 \times 11$ (manganese condition $\times$ sex $\times$ day) repeated measures ANOVA. Negative geotaxis, olfactory orientation, and neonatal locomotor activity were analyzed using $3 \times 2 \times 3$ (manganese condition $\times$ sex $\times$ day) repeated measures ANOVAs. To control for litter effects, means were calculated on each dependant measure for manganese treatment groups (0, 250, or 750 $\mu$g manganese) for both male and female rat pups. Thus, only one score was used for each level of manganese pretreatment per litter per sex. On the balance beam task, time to travel to the goal box, freezes and foot slips were analyzed with separate $3 \times 2 \times 3$ (manganese condition $\times$ sex $\times$ trial) repeated measures ANOVAs.

For preweanling rats, saline and cocaine-induced locomotor activity was analyzed using a $3 \times 3 \times 2 \times 12$ (manganese condition $\times$ cocaine dose $\times$ sex $\times$ time block) repeated measures ANOVA. Pre-injection body weights were analyzed using a $3 \times 2$ (manganese condition $\times$ sex) ANOVA. For adult rats, locomotor activity was analyzed using a $3 \times 3 \times 2 \times 12 \times 5$ (manganese condition $\times$ cocaine dose $\times$ sex $\times$
time block x days) ANOVA. All post hoc comparisons were done using Tukey tests ($p < .05$).

**Neurochemical Data.** Dopamine transporter binding sites ($B_{\text{max}}$) and affinity ($K_d$) were determined using nonlinear regression with Prism (GraphPad Software, San Diego, CA). $B_{\text{max}}$ and $K_d$ data from homogenate ligand-binding assays were analyzed using separate randomized block (manganese condition) between-subject ANOVAs. Each of the 7 assays were treated as separate blocks. This statistical procedure allows for the variance between assays to be partitioned out of the error term. All post hoc comparisons were done using Tukey tests ($p < .05$).

**Results**

**Neonatal Assessments**

Manganese exposure caused a significant reduction in body weights [manganese main effect, $F(2,90)=3.93$, $P < 0.05$]. This effect was only evident during the later part of the exposure phase, because rats exposed to 750 µg manganese weighed less than rats exposed to vehicle or 250 µg manganese on PD 15-21 (Fig. 11) [manganese x day interaction, $F(20,900)=1.88$, $P < 0.05$]. Overall, male pups (21.32 g ± .28) weighed more than female pups
Figure 11. Mean (± S.E.M.) body weight data of preweanling rats (n = 31-33) treated from PD 1-21 with daily oral supplements of manganese (0, 250, or 750 µg).

*Significant difference between rats exposed to 250 µg or 750 µg manganese (P < 0.05).

*Significant difference between rats exposed to vehicle or 750 µg manganese (P < 0.05).
(20.60 g ± .26) regardless of manganese condition [sex main effect, $F(2,90)=4.15$, $P < 0.05$].

Manganese exposure did not alter developmental landmarks including: pina detachment, incisor eruption, or eye opening (Table 1). Manganese exposure did affect performance on the negative geotaxis task, because rats exposed to manganese exhibited an increased latency to turn 180° on a 25° inclined plane (Table 2) [manganese main effect, $F(2,44)=4.45$, $P < 0.05$]. Performance on the olfactory discrimination task and motor activity task was not affected by manganese exposure (Table 3).

**Acute Cocaine Challenge: Preweanling Rats**

On PD 23, body weights did not vary according to manganese treatment or sex (Table 4). Manganese exposure did not alter basal or cocaine-induced locomotor activity of preweanling rats (Fig. 12); however, rats challenged with cocaine on PD 23 (10 and 20 mg/kg) showed enhanced distance traveled scores when compared to vehicle-treated rats (Fig. 12) [cocaine main effect, $F(2,134)=159.25$, $P < 0.05$]
Table 1. Mean (± S.E.M.) Age (postnatal day, PD) at Expression of Developmental Landmarks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Developmental Landmarks</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pina Detachment</td>
<td>Incisor Eruption</td>
<td>Eye Opening</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.52 ± 0.16</td>
<td>9.30 ± 0.22</td>
<td>15.60 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>250 µg Manganese</td>
<td>3.60 ± 0.17</td>
<td>9.19 ± 0.11</td>
<td>15.58 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>750 µg Manganese</td>
<td>3.57 ± 0.16</td>
<td>9.25 ± 0.18</td>
<td>15.53 ± 0.10</td>
<td></td>
</tr>
</tbody>
</table>

Note: Rats were exposed to vehicle or manganese on PD 1-21.
Table 2. Mean (± S.E.M.) Latencies (s) of Rat Pups to Complete the Negative Geotaxis Task.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Negative Geotaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PD 8</td>
</tr>
<tr>
<td>Vehicle</td>
<td>27.15 ± 6.78</td>
</tr>
<tr>
<td>250 µg Manganese</td>
<td>19.16 ± 3.87</td>
</tr>
<tr>
<td>750 µg Manganese</td>
<td>28.86 ± 6.08</td>
</tr>
</tbody>
</table>

Note: Rats were exposed to vehicle or manganese on PD 1-21. *Significantly different from rats exposed to vehicle or 250 µg (P < 0.05).
Table 3. Mean (± S.E.M.) Latencies (s) and Distance Traveled Scores (cm) of Rat Pups Tested on the Olfactory Orientation and Motor Activity Tasks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sensory and Motor Development</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Olfactory Orientation</td>
</tr>
<tr>
<td>Vehicle</td>
<td>34.32 ± 3.11</td>
</tr>
<tr>
<td>250 µg Manganese</td>
<td>36.11 ± 3.12</td>
</tr>
<tr>
<td>750 µg Manganese</td>
<td>33.78 ± 3.12</td>
</tr>
</tbody>
</table>

Note: Rats were exposed to vehicle or manganese on PD 1-21. Olfactory orientation was measured on PD 9, 11, and 13, while distance traveled was measured on PD 10, 12, and 14.
Table 4. Mean (± S.E.M.) Body Weights (g) of Male and Female Rats on PD 23 and 91.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 μg Mn</th>
<th>250 μg Mn</th>
<th>750 μg Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD 23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>59.56 ± 2.02</td>
<td>58.09 ± 1.92</td>
<td>57.01 ± 2.71</td>
</tr>
<tr>
<td>Females</td>
<td>57.94 ± 1.69</td>
<td>57.35 ± 1.49</td>
<td>52.93 ± 1.81</td>
</tr>
<tr>
<td>PD 91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>382.37 ± 4.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>390.45 ± 4.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>389.64 ± 3.99&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Females</td>
<td>237.75 ± 2.38</td>
<td>238.95 ± 3.09</td>
<td>237.96 ± 2.83</td>
</tr>
</tbody>
</table>

Note: Rats were exposed to vehicle or manganese on PD 1-21.<sup>a</sup>Significantly different from female rats (P < 0.05).
Figure 12. Mean (± S.E.M.) distance traveled scores of rats (*n* = 16-17) challenged with cocaine (10 or 20 mg/kg) or saline on PD 23. Rats were injected after a 5 min habituation period and immediately placed into the chamber where locomotor activity was assessed for 60 min. Rats had been exposed to vehicle or manganese (250, or 750 μg) on PD 1-21.

*a*Significantly different from rats given a challenge injection of 0 mg/kg cocaine (*P* < 0.05).
Adult Balance and Coordination

When tested on the balance beam, latency to reach the goal box did not differ in male rats exposed to vehicle (48.48 s ± 9.42), 250 µg (47.70 s ± 10.75) or 750 µg (46.76 s ± 5.64) manganese. Similarly, female rats exposed to vehicle (48.88 s ± 9.85), 250 µg (55.75 s ± 10.52) or 750 µg (60.58 s ± 10.68) manganese did not differ on the balance beam.

Acute Cocaine Challenge: Adult Rats

On PD 91, body weights of vehicle- and manganese-exposed rats did not differ, however male rats weighed significantly more than female rats (Table 4) [sex main effect, $F(1,140)=2554.99, P < 0.05$]. Distance traveled scores of manganese-exposed rats did not interact with cocaine and sex (Fig. 13) [manganese × cocaine × sex interaction, $F(2,128)=2.37, P = 0.056$]; however, the cocaine variable interacted with sex to affect behavior [cocaine × sex interaction, $F(2,128)=14.81, P < 0.05$]. More specifically, an acute challenge injection of cocaine caused a dose-dependant increase in the distance traveled scores of male and female rats, with both doses of
Figure 13. Mean (± S.E.M.) distance traveled scores of rats \((n = 8-9)\) challenged with cocaine \((0, 10 \text{ or } 20 \text{ mg/kg})\) on PD 91. Rats were injected after a 5 min habituation period and immediately placed into the chamber where locomotor activity was assessed for 60 min. Rats had been exposed to manganese \((250 \text{ or } 750 \mu g)\) or vehicle on PD 1-21.
cocaine causing significantly greater distance traveled scores in female rats than male rats (Fig. 14).

Cocaine also interacted with the manganese variable to affect distance traveled scores [cocaine × manganese interaction, $F(4,128)=3.59$, $P < 0.05$]. When challenged with saline, distance traveled scores of vehicle- and manganese-exposed rats did not differ (Fig. 15, upper graph). In contrast, when challenged with 10 mg/kg cocaine, rats previously exposed to 250 µg manganese had smaller distance traveled scores than vehicle controls during time blocks 2 and 3 (Fig 15, middle graph) [manganese × time block interaction, $F(14,322)=2.30$, $P < 0.05$]. When challenged with 20 mg/kg cocaine, rats exposed to 750 µg manganese had smaller distance traveled scores than rats exposed to vehicle or 250 µg manganese [manganese main effect, $F(2, 43)=3.44$, $P < 0.05$] (Fig 15, lower graph).

Additionally, cocaine caused a dose-dependent increase in the distance traveled scores of rats exposed to vehicle or 250 µg manganese, but not for rats exposed to 750 µg manganese (Fig. 16).
Figure 14. Mean (± S.E.M) distance traveled scores of rats (n = 24-25) challenged with cocaine (0, 10 or 20 mg/kg) or saline on PD 91. Rats were injected after a 5 min habituation period and immediately placed into the chamber where locomotor activity was assessed for 60 min. Rats had been given daily oral supplements of manganese (250 or 750 µg) or vehicle on PD 1-21.

*Significantly different from same-sexed rats given a challenge injection of 0 mg/kg cocaine (P < 0.05).

*Significantly different from male rats given the identical dose of cocaine (P < 0.05).
Figure 15. Mean (± S.E.M) distance traveled scores of male and female rats (n = 16-17) challenged with cocaine (0, 10, or 20 mg/kg) on PD 91. Rats were injected after a 5 min habituation period and immediately placed into the chamber where locomotor activity was assessed for 60 min. Rats had been exposed to manganese (250 or 750 μg) or vehicle on PD 1-21.

*Significantly different from rats pretreated with 0 μg manganese and challenged with the identical dose of cocaine (P < 0.05).
Figure 16. Mean (± S.E.M) distance traveled scores of rats (n = 16-17) challenged with cocaine (0, 10 or 20 mg/kg) on PD 91. Rats were injected after a 5 min habituation period and immediately placed into the chamber where locomotor activity was assessed for 60 min. Rats had been exposed to manganese (250 or 750 µg) or vehicle on PD 1-21.

a Significantly different from rats exposed to the same dose of manganese and given a challenge injection of 0 mg/kg cocaine (P < 0.05).

b Significantly different from rats exposed to the same dose of manganese and given a challenge injection of 10 mg/kg cocaine (P < 0.05).
Dopamine Transporter Binding Sites

Neonatal manganese exposure caused a decrease in striatal dopamine transporter binding sites that persisted into adulthood [manganese main effect, $F(2, 16)=3.72, P < 0.05$] (Fig. 17). Specifically, rats exposed to 750 µg manganese had smaller $B_{\text{max}}$ values than control rats (Fig. 18). Manganese did not significantly affect $K_d$ values, although there was a trend for manganese to cause a dose-dependant decline in $K_d$ (Table 5).

Discussion: Experiment Two

Evidence from other studies has suggested that exposure to manganese can result in behavioral and neurological changes to the developing nervous system. However, it is not clear if chronic exposure to low or moderate doses of manganese during the neonatal period is capable of inducing long-term changes in behavior. Thus, one purpose of this experiment was to assess the effects of manganese on standard measures of physical, sensory, and motor development during the neonatal period and then to assess balance and coordination in adulthood. Exposure to environmental toxins can alter behavioral responses to dopaminergic drugs. Therefore, another purpose of this
Figure 17. Representative [³H]GBR 12935 saturation curve of dopamine transporter binding in the striatum of male rats exposed to manganese (250 or 750 µg) or vehicle from PD 1-21.
Figure 18. Mean (± S.E.M.) $B_{\text{max}}$ values for $[^{3}\text{H}]\text{GBR 12935}$ binding in the striatum of rats ($n = 5-7$) exposed to manganese (250 or 750 µg) or vehicle on PD 1-21.

*aSignificantly different from rats exposed to vehicle ($P < 0.05$).*
Table 5. Mean (± S.E.M.) $B_{\text{max}}$ and $K_d$ Values for $[^3]$H$\text{GBR 12935}$
Binding in the Striatum of Rats Exposed to Manganese (250 or 750 µg) or Vehicle on PD 1-21.

<table>
<thead>
<tr>
<th></th>
<th>$B_{\text{max}}$ (fmol/mg protein)</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>932.15 ± 185.47</td>
<td>44.07 ± 12.47</td>
</tr>
<tr>
<td>250 µg Manganese</td>
<td>743.37 ± 105.74</td>
<td>31.78 ± 7.70</td>
</tr>
<tr>
<td>750 µg Manganese</td>
<td>355.92 ± 100.86$^a$</td>
<td>16.34 ± 7.01</td>
</tr>
</tbody>
</table>

$^a$Significantly different from rats exposed to vehicle ($P < 0.05$).
experiment was to determine if early manganese exposure was capable of altering dopamine-mediated locomotor activity in preweanling and adult rats. We found that manganese did not affect the expression of developmental landmarks, but did reduce the normal pattern of weight gain during the last week of manganese exposure. Manganese (750 µg) also impacted the development of motor coordination (i.e., negative geotaxis) at PD 8 - 12 without concomitant effects on olfactory orientation or motor activity. Additionally, when tested on PD 23, basal and cocaine-induced motor activity was not affected by manganese.

On PD 91, neonatal manganese exposure did not affect basal motor activity of adult rats. However, when injected with cocaine, manganese-exposed rats had smaller distance traveled scores than controls. Additionally, cocaine caused a dose-dependent increase in the locomotor activity of both male and female rats, with female rats exhibiting more robust cocaine-induced locomotor activity than male rats. In control rats, manganese also reduced the density of dopamine transporters in the striatum (B_{max}), while causing a non-significant decline in affinity (K_d).

Although exposing mice to manganese during late gestation has been reported to cause a delay in the
appearance of developmental landmarks (e.g., pina detachment, incisor eruption, eye opening) (Torrente et al., 2002), such changes were not evident in the current study. The most obvious explanation is the method of manganese exposure, because during gestation the fetus is directly exposed to manganese crossing the placenta via active transport (Krachler, Rossipal, & Micetic-Turk, 1999). Although fetal rats are more susceptible to the neurotoxic effects of manganese than are neonatal and preweanling rats, the current study demonstrates that neonatal manganese exposure is capable of causing short-term changes in the dopamine system that are apparent during the preweanling period. Manganese delayed the ability of rat pups (PD 8, 10, and 12) to coordinate the movements required to turn 180° on a 25° inclined plane. Proper execution of this task typically reaches asymptotic levels by PD 8 and is dependant upon the vestibular system (Adams et al., 1985). Dopamine is involved in modulating the vestibular system either by directly stimulating vestibular neurons or by modulating GABAergic neurons in this area (Vibert, Serafin, Crambes, Vidal, & Muhlethaler, 1995). Manganese-induced changes in balance and coordination were restricted to the preweanling period,
because vehicle- and manganese-exposed adult rats performed similarly on the balance beam task. In addition to measuring negative geotaxis, vehicle- and manganese-exposed rats were tested on olfactory discrimination and motor movement tasks. Manganese did not affect performance on either of these tasks. This pattern of results was surprising, because both olfactory orientation and motor movement are typically affected by manipulations of the dopamine system (Adams et al., 1985; Tran, Chowanadisai, Lonnerdal, Parker, Chicz-DeMet, & Crinella, 2002; Vorhees et al., 1995).

Consistent with prior research, the high dose of manganese decreased weight gain during the neonatal period (Dorman et al., 2000; Pappas et al., 1996). This effect dissipated with the cessation of manganese treatment, because body weights of vehicle- and manganese-exposed rats were similar on PD 23 and PD 91. Feeding behavior during neonatal development is sensitive to manipulations of the dopamine system, with both increased and decreased dopaminergic transmission inducing anorexia (Raskin & Campell, 1981; Zhou & Palmiter, 1995). The anorexia evidenced by rat pups in the current study is suggestive of an increase in dopamine transmission because a high dose of
manganese increases both striatal dopamine and DOPAC levels (Dorman et al., 2000). Of course, it remains possible that the manganese-induced weight loss was mediated by non-dopaminergic mechanisms.

Basal motor activity was not affected by neonatal manganese exposure on PD 23 or PD 91. When challenged with cocaine on PD 23, neither manganese treatment nor sex impacted cocaine-induced behavior. However, when challenged with 10 or 20 mg/kg cocaine on PD 91, male and female manganese-exposed rats demonstrated a difference in the response magnitude to cocaine. In particular, female rats had greater distance traveled scores than male rats after being treated with either dose of cocaine. In fact, female rats treated with 10 mg/kg cocaine had distance traveled scores that were similar to male rats treated with 20 mg/kg cocaine. These data are consistent with previous reports demonstrating that the efficacy of cocaine differs between male and female rats. In particular, male rats require an increased amount of cocaine to achieve the behavioral intensity of female rats (Festa et al., 2004).

Overall, early manganese exposure caused an atypical response pattern to the locomotor activating effects of cocaine in adulthood. Specifically, manganese was
responsible for a pronounced reduction in the behavioral response to cocaine. Similar findings have been reported in rats exposed to manganese from gestation to PD 80 and then challenged with amphetamine (Leung et al., 1982). Several explanations for these manganese-induced effects exist, including 1) functional changes in postsynaptic receptors, 2) adaptive changes in the dopamine transporter, and 3) biphasic alterations in neurotransmitter levels.

The marked reduction in distance traveled scores of manganese-exposed rats suggests that manganese causes long-term changes in the sensitivity of dopamine receptors. This line of reasoning is primarily supported by studies examining the effects of direct dopaminergic drugs on postsynaptic terminals. In particular, manganese decreases the frequency and amplitude of quinpirole-induced excitatory postsynaptic potentials, suggesting a decreased efficacy or decreased sensitivity of D_2-like receptors (Calabrassi et al., 2000). Additionally, manganese may alter the functioning of D_1-like dopamine receptors. The attenuated motor activity observed in the present study could be due to a dysfunction of the D_1-like receptor, because manganese decreases ligand binding to postsynaptic D_1-like receptors (Eriksson, Gillberg, Aquilonius, Hedstrom,
In the current study, rats exposed to 750 µg manganese did not show the traditional dose-dependant increase in cocaine-induced locomotor activity that was apparent in control rats. In particular, 20 mg/kg cocaine did not increase locomotor activity beyond that of the 10 mg/kg cocaine group. It is possible that a manganese-induced dysfunction of the D₁-like receptor may interfere with the enabling role that D₁-like receptors play in D₂-like mediated behaviors. Specifically, it is possible that a manganese-induced subsensitivity of D₂-like receptors, in combination with dysfunctional D₁-like receptors, may hamper the synergistic coupling of D₁-like and D₂-like receptors during development.

A more parsimonious explanation for the attenuated response to cocaine is that manganese caused neurotoxicity. This hypothesis is supported by the finding that dopamine transporter binding sites were reduced in rats exposed to manganese (750 µg) from PD 1 - 21. Loss of dopamine reuptake sites is indicative of damage to dopamine terminals (Frost & Cadet, 2000, Wagner, Ricaurte, Johanson, Schuster, & Seiden, 1980). Most commonly, reductions in dopamine transporters are associated with high doses of psychostimulant drugs. A variety of environmental toxins,
including manganese, are also transported into the intracellular environment via dopamine transporters, thus leaving the neuron vulnerable to toxicity (Bridges & Zalups, 2005; Erikson, Byron, & Beard, 2000; Ingersoll, Montgomery, & Aposhian, 1999; Miller et al., 1999).

Alternatively, reductions in the number of dopamine transporter binding sites may reflect functional changes, including declined affinity. Conformational changes (i.e., structural changes) decrease the affinity of dopamine transporters to cocaine and similar reuptake blockers depending upon the affected transmembrane domain (Goldberg, Beuming, Soyer, Goldstein, Weinstein, & Javitch, 2003; Loland, Granas, Javitch, & Gether, 2004; Loland, Norgaard-Nielsen, & Gether, 2003). For example, changes in structure can alter the distribution of conformational states as well as interfering with the “gating” mechanism between the inter- and extracellular environment (Goldberg, Beuming, Soyer, Goldstein, Weinstein, & Javitch, 2003). In regards to the present experiment, manganese-exposed rats showed a trend towards decreased affinity. Since manganese exposure occurred during a sensitive period (i.e., PD 1 - 21) for the development of dopamine transporters (Galineau et al., 2004; Tarazi, Tomasini, & Baldessarini, 1998) it is
possible that the decline in affinity may result from manganese-induced changes in the conformation of the dopamine transporter. This possibility is unlikely, however, because manganese has been shown to cause neurotoxicity with prolonged exposure, while not interfering with $^3$H-dopamine uptake in the striatum (Defazio, Soleo, Zefferino, & Livrea, 1996) The latter finding is important because $^3$H-dopamine uptake is a direct indicator of the functionality of dopamine transporters.

In the current study, the pattern of behavioral results is consistent with the biphasic progression of manganese toxicity reported in neurochemical studies. During the early stages of manganese poisoning, increases in dopamine transmission have been reported (Dorman et al., 2000; Seth & Chandra, 1984). In neonatal rats, manganese exposure decreased [$^3$H]-spiroperidol binding (Seth & Chandra, 1984), which is consistent with reports of manganese-induced increases in dopamine levels during the neonatal period (Dorman et al., 2000; Seth & Chandra, 1984). As manganese toxicity progresses, damage to the nigrostriatal pathway and the globus pallidus causes behavioral abnormalities characteristic of manganese poisoning. A dosing regimen of manganese, similar to the
levels of manganese used in the current study, caused a reduction in striatal dopamine levels at PD 40 and 62 (Tran, Chowanadisai, Crinella, Chicz-DeMet, & Lonnerdal, 2002; Tran, Chowanadisai, Lonnerdal, Le, Parker, Chicz-DeMet, & Crinella, 2002). Thus, the manganese regimen used in the present study is sufficient to cause long-term depletions of striatal dopamine levels and a reduction in dopamine transporter binding sites. When the present data are combined together (i.e., the decreased body weights during the neonatal period along with the pronounced reduction in cocaine-induced behaviors on PD 91), it appears that exposing rats to 750 μg manganese on PD 1 - 21 is sufficient to induce the biphasic alterations in dopamine transmission that are commonly associated with manganese toxicity (Aschner, 2000; Chandra & Shulka, 1981; Mergler, 1999).

In summary, the results of Experiment 2 are consistent with previous studies showing that exposure to manganese during the preweanling period causes lasting alterations in cocaine-induced behaviors. In addition, weight gain and coordination were transiently affected by manganese exposure. The behavioral changes observed in the current experiment support the biphasic symptology
evidenced during manganese toxicity. The behavioral effects of manganese toxicity could be attributed to loss of dopamine neurons, evidenced by decreased dopamine transporters. Importantly, the levels of manganese used in the current study are capable of causing long-term damage to these membrane proteins, which may account for the decreased locomotor activating effects of cocaine in adulthood.
CHAPTER TWELVE

EXPERIMENT THREE: THE EFFECTS OF NEONATAL MANGANESE EXPOSURE ON COCAINE-INDUCED CONDITIONED PLACE PREFERENCE

Methods

The purpose of Experiment 3 was to investigate the effects of neonatal manganese treatment on drug reward by utilizing a CPP paradigm. It was hypothesized that neonatal manganese exposure would reduce the rewarding properties of cocaine, as evidenced by a decreased amount of time spent in the drug-paired compartment.

Manganese Treatment

A total of 72 rats (n = 8 per group) were used in this experiment. On PD 1-21, rats were given daily supplements consisting of 0, 250, or 750 μg manganese dissolved in 25 μl of 10% sucrose solution.

Apparatus

Conditioned place preference was assessed in a T-shaped plywood chamber that had three compartments separated by removable partitions. The two end compartments measured 24 x 30 x 45 cm high, while the offset compartment measured 24 x 10 x 45 cm high. One end compartment had white
walls, a wire mesh floor, and pine bedding beneath the floor. The other end compartment had black walls, a metal bar floor, and cedar bedding beneath the floor. The offset compartment had gray walls and a solid wood floor. The solid partitions could be replaced with partitions containing 10 x 10 cm openings, which allowed rats free access to the two end compartments.

Procedures

Following acclimation to handling, 90-day-old rats from the three manganese conditions were placed in the offset compartment (the start box) and allowed free access to the two end compartments for 900 s. Initial compartment preference was recorded.

On day 1 of conditioning, half of the rats were restricted to the black compartment and half restricted to the white compartment for 1800 s. Rats placed in the white compartment were injected with cocaine (0, 10, or 20 mg/kg, i.p.), whereas rats placed in the black compartment were injected with saline. On day 2 of conditioning, rats were restricted to the opposite compartment for 1800 s. Rats previously injected with cocaine were given an injection of saline, whereas rats previously given saline were injected
with either 0, 10 or 20 mg/kg cocaine. Conditioning lasted a total of 8 days with alternating daily placements in the black and white compartments.

On the test day (i.e., day 9), rats were placed in the offset compartment and allowed free access to the entire apparatus for 900 s. Time spent in each chamber were recorded.

**Statistics**

Separate between-subject 3 × 3 × 2 ANOVAs (manganese condition × drug dose × sex) were used to analyze place preference data. Initial preference day, acquisition test day data, extinction test day data, and reinstatement data were analyzed separately. Post hoc comparisons were done using Tukey tests (p < .05).

**Results**

**Body Weights**

Overall, manganese exposure caused a reduction in the body weights of rat pups [manganese main effect, 

\[ F(2,62)=3.67, \, P < 0.05 \]. This effect was evident during the later part of the manganese treatment phase, because rats pretreated with 750 μg manganese weighed less than rats pretreated with vehicle or 250 μg manganese on PD 13 - 21
(Fig. 19) [manganese × day interaction, $F(20, 620)=1.94$, $P < 0.05$]. When measured on PD 91, body weights of adult rats were not affected by neonatal manganese exposure. Not surprisingly, adult male rats ($384.40 \pm 4.25$ g) weighed significantly more than adult female rats ($239.4 \pm 2.79$ g) [sex main effect, $F(1, 99)=844.36$, $P < 0.05$].

**Conditioned Place Preference: Conditioning, Extinction and Reinstatement**

Prior to conditioning, a preference for the black side of the apparatus was apparent. This preference did not differ according to manganese exposure or sex (Fig. 20). In control animals, the black preference persisted throughout acquisition, extinction and reinstatement.

On the conditioning test day, rats that received cocaine showed a robust preference for the white (drug-paired) compartment (Fig. 21, upper right graph). Specifically, conditioning with 10 or 20 mg/kg cocaine caused an increase in time spent on the drug-paired side relative to saline controls [cocaine main effect, $F(2, 83)=45.84$, $P < 0.05$]. The place preference for cocaine disappeared by the extinction test day, because rats conditioned with cocaine or saline spent similar amounts of time in the white (drug-paired) compartment (Fig. 21, lower
Figure 19. Mean (± S.E.M.) body weights of preweanling rats (n = 22-24) treated from PD 1-21 with daily oral supplements of manganese (250 or 750 µg) or vehicle.
*Significant difference between rats treated with vehicle and 750 µg manganese (P < 0.05).
Figure 20. Mean (± S.E.M.) time (s) spent in the white compartment on the initial preference day (PD 90) for male and female rats (n = 13-16) previously exposed to manganese (250 or 750 µg) or vehicle from PD 1-21.
Figure 21. Mean time spent (± S.E.M.) in the drug-paired compartment on the Pre-Conditioning day, Conditioning, Extinction, and Reinstatement test days. Rats \((n = 27-33)\) were initially given four alternating daily injections of cocaine (10 or 20 mg/kg) or vehicle prior to placement on the white side and four alternating injections of saline prior to placement on the black side. During eight days of extinction training, rats were injected with saline and given alternating daily placement in the black and white compartments. On the Reinstatement test day all rats were primed with 20 mg/kg cocaine immediately prior to the testing session. Rats were previously exposed to manganese (250 or 750 μg) or vehicle from PD 1-21.

*Indicates a significant difference from rats conditioned with saline \((P < 0.05)\).
left graph). The cocaine-induced preference was reinstated on the final test day. Overall, a priming dose of cocaine reinstated a place preference in those rats originally conditioned with either 10 or 20 mg/kg cocaine (Fig. 21, lower right graph) [cocaine main effect, $F(2,82)=18.7$, $P < 0.05$].

There was no evidence that manganese caused any alterations in the acquisition, extinction, or reinstatement of cocaine CPP (Fig. 22). Likewise, sex did not interact with manganese or cocaine to influence CPP.

Discussion: Experiment Three

Manganese accumulates in areas of the brain (e.g., nucleus accumbens, prefrontal cortex, and globus pallidus) associated with the rewarding properties of psychostimulant drugs (Berridge, 2003; Fell et al., 1996; Gong, Neill, & Justice, 1996; 1997; Komaki et al., 1999; McBride et al., 1999; Panagis et al., 1997). However, it was unknown whether exposure to manganese during neonatal development would cause lasting changes in drug reward. For this reason, the acquisition, extinction, and reinstatement of cocaine-induced CPP was examined in male and female rats exposed to manganese from PD 1-21. Consistent with data
Figure 22. Mean time spent (± S.E.M.) in the drug-paired compartment on the Conditioning, Extinction, and Reinstatement test days. Rats (n = 9-12) were exposed to manganese (250 or 750 µg) or vehicle from PD 1-21 and conditioned with cocaine (0, 10, or 20 mg/kg) on four alternating days. The place preference was extinguished across eight daily sessions, and reinstated with a priming dose of 20 mg/kg cocaine.

*Indicates a significant difference from rats conditioned with saline (P < 0.05).
from our laboratory (see Experiment. 2) and others (Dorman et al., 2000; Pappas et al., 1996), rat pups treated with 750 μg manganese had reduced body weights during the later part of manganese treatment (PD 13-21). As expected, these initial differences in body weight were not apparent prior to the beginning of CPP conditioning (i.e., PD 90). Originally, it was hypothesized that manganese exposure would decrease the rewarding properties of cocaine. Male and female rats in this study showed a robust preference for the cocaine-paired side after the conditioning period, and this preference was successfully extinguished and reinstated with subsequent testing. However, there was no indication that manganese treatment interfered with the acquisition, extinction or reinstatement of cocaine CPP.

Male and female rats exhibited similar patterns of responding through-out CPP acquisition, extinction and reinstatement. Recently, it has been demonstrated that gonadal hormones have a role in modulating the rewarding effects of cocaine, thus causing an increased sensitivity to the drug in female rats (Russo, Festa, Fabian, Gazi, Kraish, Jenab, & Quinones-Jenab, 2003). However, this effect primarily results from female rats, when compared to male rats, showing place conditioning to lower amounts of
the drug after fewer cocaine pairings (Russo et al., 2003). It has been reported that 10 mg/kg cocaine reliably induces place preferences in both male and female rats, while 20 mg/kg cocaine may be the preferential dose for male rats (Russo et al., 2003). In the present study, 20 mg/kg cocaine produced robust CPP in both male and female rats. Russo et al. (2003) used a four-day conditioning period whereas we used an eight-day conditioning procedure, perhaps indicating that sex differences associated with cocaine-induced CPP are dependant on fewer conditioning sessions.

The current study shows that the CPP responding of female rats, like male rats, can be extinguished and reinstated with a priming dose of cocaine. This finding indicates that male and female rats are capable of forming associations between contextual stimuli and the rewarding effects of cocaine, and that the salience of these contextual cues can be extinguished and subsequently reinstated with a priming injection of cocaine (Mueller & Stewart, 2000). These results are not consistent with cocaine self-administration studies, which demonstrated that female rats respond more robustly to a priming injection of cocaine than male rats (Lynch & Carroll,
In self-administration studies, however, rats are required to engage in an operant response to obtain the drug, while the CPP procedure relies on Pavlovian associations formed between contextual stimuli and the rewarding effects of the drug (i.e., an operant response is not required). Moreover, in the former situation delivery of the drug is controlled by the animal, whereas in the latter situation drug administration is controlled by the experimenter. Differences between drug-self administration and CPP tasks may mean that separate neuronal mechanisms underlie the reward processes associated with the two behavioral paradigms (Bardo & Bevins, 2000). Therefore, sex-differences observed in the reinstatement of cocaine self-administration may be attributed to differences in the reinforcing effects of cocaine; whereas, the similar reinstatement patterns of male and female rats in a CPP paradigm may indicate a lack of sex differences in those mechanisms responsible for forming the association between contextual stimuli and the rewarding properties of cocaine. It is not known whether sex differences in the reinstatement of cocaine CPP would emerge if a less optimal CPP paradigm were used (i.e., fewer sessions or lower doses of cocaine).
Given recent reports demonstrating that environmental neurotoxins can interfere with reward function (Guillot, Richardson, & Miller, 2004), it is surprising that manganese exposure did not impact the acquisition, extinction, or reinstatement of cocaine-induced CPP. The most likely explanation is that manganese differentially affects the nigrostriatal and mesolimbic dopamine systems. Although manganese accumulates in brain areas that make up the mesolimbic dopamine system, manganese preferentially damages dopamine neurons in the nigrostriatal pathway (Eriksson, Lenngren, & Heilbronn, 1987; Erikson, Shihabi, Aschner, & Aschner, 2002; Fell et al., 1996; Komaki et al., 1999). Thus, the nigrostriatal system is more susceptible to manganese toxicity than the mesolimbic reward system. Alternatively, the dosing paradigm used in this experiment may not have been sufficient to alter the mesolimbic dopamine system. Behavioral manifestations of manganese toxicity are dependant on age of exposure and testing, route and dose of administration, and the biphasic progression of toxicity (see Chapter 5). Therefore, it is unknown if a more aggressive manganese exposure paradigm would interfere with reward system functioning.
In conclusion, the findings of this experiment show that neonatal manganese exposure did not impact the acquisition, extinction, or reinstatement of cocaine CPP. However, manganese exposure from PD 1-21 did cause a transient decrease in body weights during the neonatal period. In addition, this experiment showed that place preference to cocaine can be extinguished and later reinstated in response to a priming dose of cocaine in female rats as well as male rats. Experiment 3 also demonstrated that sex differences are not observed when cocaine-induced CPP is measured after an eight day conditioning period (four cocaine pairings and four saline pairings).
Manganese is a complex trace mineral that is an essential nutrient but, at high levels, may also be a neurotoxicant. It is generally believed that children and infants may be at a particular risk for manganese neurotoxicity due to an immature central nervous system (Cawte, 1985; Mergler, 1999; Takada et al., 1999; Weiss, 1999). Importantly, infant formulas contain substantially more manganese than the recommended daily allowance, so this sub-population of infants may be at particular risk for developing lasting brain changes that result in long-term behavioral dysfunction (Lonnerdal, 1994). There is even evidence that the amount of manganese contained in soy-based infant formula may be sufficient to induce brain changes responsible for ADHD (Tran, Chowanadisai, Crinella, Chicz-DeMet, & Lonnerdal, 2002; Tran, Chowanadisai, Lonnerdal, Le, Parker, Chicz-DeMet, & Crinella, 2002). Therefore, the purpose of this thesis was to examine the effects of low to moderate doses of manganese (0, 250, or 750 μg per day from PD 1-21) on a comprehensive battery of behaviors that are sensitive to disruption of the dopamine
system. The three experiments comprising this thesis evaluated behavior during the neonatal period, preweanling period, and adulthood.

Manganese exposure did not affect the appearance of developmental landmarks, nor did it alter neonatal motor activity or olfactory orientation. Manganese did, however, impair neonatal motor coordination and depressed body weight gain on PD 13-21. After cessation of manganese treatment on PD 21, body weights returned to control levels by PD 23. Importantly, manganese exposure did not affect basal or cocaine-induced locomotor activity on PD 23. When tested at PD 90, however, manganese-exposed rats exhibited both reduced responding on a DRL 20 task and a pronounced reduction in cocaine-induced motor activity. Early manganese exposure did not affect basal motor activity, balance, or coordination at PD 90. Additionally, manganese did not alter the rewarding properties of cocaine, as measured by the acquisition, extinction, and reinstatement of CPP.

When considered together, the present results suggest that early manganese exposure causes a long-term suppression in the initiation of motor movement, without altering reward or learning processes. Manganese-induced
reductions in bar press responsivity are not likely due to a learning impairment, because manganese did not interfere with acquisition of either a response contingent behavioral task (Experiment 1) or CPP (Experiment 3). These learning paradigms differ because the former task requires the performance of an overt voluntary behavior, while the later task relies on the pairing of two previously unrelated stimuli. Since manganese-induced deficits impacted the execution of rapidly occurring bar press responses, and not the learning of response contingencies, it is likely that the behavioral differences were due to motor impairment. Moreover, the inability of manganese to alter the acquisition, extinction, and reinstatement of cocaine-induced CPP further suggests that learning mechanisms are not affected by neonatal manganese exposure. However, it is possible that manganese may cause learning impairments that are not sensitive to the DRL or CPP paradigms.

The pronounced decrease in cocaine-induced locomotor activity observed in manganese-exposed rats (Experiment 2) is also consistent with a motor impairment hypothesis rather than a reward hypothesis. This conclusion is supported by the finding that early manganese exposure did not impact the acquisition, extinction, or reinstatement of
cocaine CPP in adulthood (Experiment 3). Cocaine-induced locomotor activity and CPP are mediated, at least partially, by the mesocorticolimbic dopamine system (Bardo, 1998; Dickson, Lang, Hinton, & Kelly, 1994; Mele, Thomas, & Pert, 1998; Wise & Bozarth, 1987). Motor movement, on the other hand, is primarily mediated by the nigrostriatal dopamine system, because damage to this system impairs various indices of motoric function in both humans and animals (Arnt, 1987; Calne et al., 1994; Carey, 1986; Goldberg et al., 2003; Olanow et al., 1996). Importantly, chronic manganese exposure causes cell loss in the nigrostriatal pathway, which is characterized by bradykinesia, dystonia, and widespread muscular rigidity (Aschner, 2000; Calne et al., 1994; Mergler, 1999). Therefore, the most parsimonious conclusion is that early manganese exposure causes motoric deficits that result from dysfunction of the nigrostriatal dopaminergic system. In particular, manganese-induced reductions in striatal dopamine transporters is suggestive of neurotoxicity. Motoric deficits are evidenced by decreased bar press responding on a DRL schedule of reinforcement and attenuation of psychostimulant-induced locomotor activity.
Motor impairments observed in ADHD children involve dopaminergic transmission in the nigrostriatal pathway (Johansen et al., 2002; Sagvolden et al., 1998). However, manganese-induced motor impairments evidenced in this study are not consistent with established rodent models of ADHD (i.e., 6-OHDA lesions, spontaneously hypertensive rats, and PCB-exposed rats) (for a review, see Davids, Zhang, Tarazi, & Baldessarini, 2003). For example, in one rodent model of ADHD, 6-hydroxydopamine (6-OHDA) lesions are used to reduce dopamine transporter binding in the striatum and nucleus accumbens (Davids, Zhang, Tarazi, & Baldessarini, 2002). These 6-OHDA-treated rats exhibit robust increases in basal motor activity, that return to control levels with methylphenidate treatment (Davids et al., 2002). A second rodent model of ADHD utilizes spontaneously hypertensive rats. These rats display hyperactive behaviors consistent with ADHD, show decreased dopamine and serotonin turnover in the striatum, and respond more quickly than controls to an acute challenge of methylphenidate (Amini, Yang, Swann, & Dafny, 2004; de Villiers, Russell, Sagvolden, Searson, Jaffer, & Taljaard, 1995; Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 2000). A third rodent model involves exposing neonatal rats to PCBs. These rats show increased
hyperactivity when compared to controls (Holene, Nafstad, Skaare, & Sagvolden, 1998). In the present study, manganese-exposed rats did not exhibit behaviors consistent with established rodent models of ADHD. Specifically, the basal locomotor activity of manganese- and vehicle-exposed rats was similar and manganese-treated rats showed a lessened responsivity to cocaine. Manganese-exposed rats also exhibited reduced bar pressing, bin 1 responses, and burst responses. In contrast, both spontaneously hypertensive rats and PCB-exposed rats exhibit an increase in burst responses, which is suggestive of the impulsivity associated with ADHD (Biox, Qiao, Kolpus, & Sagvolden, 1998; Holene et al., 1998). In summary, manganese-exposed rats do not respond in a manner similar to the established rodent models of ADHD.

Dysregulation of the dopamine system causes ADHD-type symptomology. The dopamine transporter has been implicated as a source of dysregulation by both genetic studies (Barr et al., 2001; Cook et al., 1995; Gill, Daly, Heron, Hawi, & Fitzgerald, 1997; Waldman et al., 1998) and neuroimaging studies (Cheon et al., 2003; Jucaite, Fernell, Halldin, Forssberg, & Farde, 2005; Krause, Dresel, Krause, la Fougere, & Ackenheil, 2003). Generally, neuroimaging
studies show increased striatal dopamine transporters in ADHD patients (Krause et al., 2003; Cheon et al., 2003). The expression of more dopamine transporters suggests increased dopamine reuptake in ADHD cases. The result is a decrease of extracellular dopamine concentrations that is consistent with the dopamine hypothesis of ADHD (Swanson et al., 2000). In rodent models, spontaneously hypertensive rats also show an increase in striatal dopamine transporters (Watanabe et al., 1997). These findings are in distinct contrast with the present study, in which I found that early manganese exposure causes a persistent reduction of dopamine transporters. Taken together, these findings are consistent with the idea that neonatal manganese exposure produces neurotoxicity that is unrelated to the development of ADHD-type behaviors.

Although the results from this thesis suggest that neonatal manganese exposure does not induce ADHD-type symptoms, manganese does appear to have long-term behavioral and neuronal consequences that are not expressed until adulthood. Exposure to other neurotoxic substances can also have long latency periods resulting in “silent” damage (Weiss, Clarkson, & Simon, 2002). This is important because pre-exposure to neurotoxicants may exacerbate or
cause the early expression of neurodegenerative disorders (i.e., Parkinson's Disease) that result from age-related cell loss (Calne, Eisen, McGeer, & Spencer, 1986; Weiss, Clarkson, & Simon, 2002). In the case of manganese exposure, neurobehavioral and neuropsychiatric symptomology are apparent long after blood levels and body burden of manganese have returned to normal (Hochberg et al., 1996; Huang et al., 1993; 1998). Moreover, neurological and behavioral changes have been detected in humans exposed to manganese even in the absence of overt symptoms of manganese toxicity (Mergler, 1999).

In conclusion, this thesis demonstrates that exposure to manganese during neonatal development causes both short- and long-term changes in behavior. Although, this thesis does not provide data supporting a relationship between early manganese exposure and ADHD, it does provide evidence that manganese causes both long-term behavioral changes and reductions in striatal dopamine transporters.
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