2006

The Effect and Chronic Acute Pre-Treatment with Methylphenidate on Recovery of Cognitive Function Following Experimental Traumatic Brain Injury In Rats

Katharine Coryell Eakin
Virginia Commonwealth University

THE EFFECTS OF CHRONIC AND ACUTE PRE-TREATMENT WITH METHYLPHENIDATE ON THE RECOVERY OF COGNITIVE FUNCTION FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY IN RATS

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

Katharine Coryell Eakin
Bachelor of Science, Virginia Commonwealth University, 1999

Director: Robert Hamm, Ph.D.
Professor, Department of psychology

Virginia Commonwealth University
Richmond, Virginia
May, 2006

Table of Contents

Page

List of Figures

Page

List of Abbreviations

2DG	[11C] raclopride and 2-deoxyglucose
5-HT	Serotonin
AA	Arachidonic acid
ACh	Acetylcholine
ACTH	Adrenocorticotropic Hormones
ADHD	Attention Deficit Hyperactivity Disorder
AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of variance
APA	American Psychological Association
atm	Atmospheres of pressure
ATP	Adenosine triphosphate
BBB	Blood brain barrier
°C	Celsius
Ca^{2+}	Calcium
cAMP	Cyclic AMP
CCI	Controlled cortical impact

CDC	Centers for Disease Control and Prevention
CNS	Central nervous system
CO_2	Carbon dioxide
COMT	Catechol-O-methyltransferase
CPP	Cerebral perfusion pressure
CSF	Cerebral spinal Fluid
D1-R	Dopamine receptor subtype 1
D2-R	Dopamine receptor subtype 2
DA	Dopamine
DAI	Diffuse axonal injury
DAT	Dopamine transporter
DHECP	dihydroergocryptine
DNA	Deoxyribonucleic acid
DOPAC	Dihydroxyphenylacetic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders 4^{th} edition
EAA	Excitatory amino acid
ED	Emergency Department

EPI	Epinephrine
F	F statistic
FP	Fluid percussion
h	Hours
H^+	Hydrogen ion
HCl	Hydrochloride
HII	Hypoxic-ischemic injury
HIV	Human Immunodeficiency Virus
HVA	Homovanillic acid
i.p.	Intraperitoneal
i.v.	Intravenously
ICP	Intracranial pressure
IEG	Immediate early genes
K^+	Potassium ion
kg	Kilogram
L-DOPA	L-dihydroxyphenylalanine
M	Mean

mABF	Mean arterial blood flow
MAO	Monoamine oxidase
Mg^{2+}	Magnesium ion
ml	Milliliters
mm	Millimeter
MPH	Methylphenidate
MPH HCl	Methylphenidate hydrochloride
MRI	Magnetic resonance imaging
ms	Milliseconds
MWM	Morris water maze
Na^{2+}	Sodium ion
NE	Norepinephrine
ng	Nanogram
NINDS	National Institute of Neurological Disorders and Stroke
nM	Nanomole
NT	Neurotansmitter
p	Probability

pH	Partial pressure hydrogen
p.o.	Per os (oral)
PB	Phosphate buffer
PBS	Phosphate buffer solution
PET	Positron emission tomography
PID	Post injury day
RFD	Remote functional depression
S-N-K	Student-Newman-Keuls
SPECT	Single photon emission computed tomography
TBI	Traumatic brain injury
UCSF	University of California San Fransico
VTA	Ventral tegmental area

Abstract

THE EFFECTS OF CHRONIC AND ACUTE PRE-TREATMENT WITH METHYLPHENIDATE ON THE RECOVERY OF COGNITIVE FUNCTION FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY IN RATS

By Katharine Eakin, B.S.

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2006

Major Director: Robert Hamm, Ph.D. Professor, Department of Psychology

Adolescent and young adult males are at a higher risk for traumatic brain injury (TBI) compared to the general population. Diagnosis of Attention Deficit Hyperactivity Disorder (ADHD) is also more prevalent for males in these age groups. The most commonly prescribed medication for ADHD is methylphenidate (MPH). Based on the increase in the number of new diagnoses of ADHD and the number of children who continue taking MPH into adulthood, it is important to evaluate how chronic or acute

MPH administered prior to injury may influence recovery following TBI. In both studies, cognitive abilities of male Sprague-Dawley rats were assessed on post-injury using the Morris Water Maze. There was no effect of chronic MPH treatment on cognitive outcome following TBI. In contrast, acute MPH pre-treatment improved cognitive outcome as measured by the MWM. The MPH + injury group reached sham-injury levels on days 4 and 5 in the MWM.

Introduction

Human Traumatic Brain Injury

Traumatic brain injury (TBI) is a serious public health problem in the United States (Langlois, Rutland-Brown, & Thomas, 2004). TBI is one of the primary causes of mortality and morbidity among children, young adults and the elderly (Thurman, Alverson, Browne, Dunn, Guerrero, et al., 1999; Sosin, Sniezek, & Thurman 1996). According to the Centers for Disease Control and Prevention (CDC) approximately 1.4 million Americans sustain a TBI each year (Langlois et al., 2004). This is larger than the number of new cases of breast cancer (212,920 cases) (American Cancer Society) and HIV/AIDS (43,700) (CDC) combined. In the United States TBI accounts for one-third of all injury- related deaths. TBI is also a growing issue for the world population as well. It is reported that by 2020 TBI will be the 3rd leading cause of death and disability in the world (Murray & Lopez, 1997; Povlishock & Katz, 2005).

Of the 1.4 million Americans who sustain traumatic brain injuries each year and seek medical attention approximately 1.1 million will receive care from an emergency department (ED), 230,000 will require hospitalization, and 50,000 people will die. This data does not take into account individuals seen in private practice or those who did not seek medical attention. National Hospital Discharge Survey data from the CDC indicate that 80,000-90,000 individuals become disabled from TBI each year. A conservative

estimate is that 5.3 million individuals (2% of the U.S. population) live with long-term disabilities due to TBI (Thurman, Alverson, Dunn, Guerrero, & Sniezek 1999; Langlois et al., 2004).

The most common causes of TBI are falls, followed by accidents involving motor vehicles or traffic accidents. The third most common cause of TBI is violence associated with firearms or assault (Langlois et al., 2004). Data compiled by the CDC from ED records, hospitalization records, and mortalities associated with TBI from 1995-2001 indicate that children aged 0-4 years and young adults aged 25-34 have the highest rates of TBI. The population with the highest incidence of TBI is children 0-4 years of age. Falls are the most common cause of injury for that age group. It is estimated that the yearly impact of TBI on children aged 0-14 years in the United States can account for approximately 475,000 ED visits, 37,000 hospitalizations, and 27,000 deaths (Langlois et al., 2004).

In almost every age group males are more likely to incur a TBI compared to females. The only age group where men and women have similar rates of TBI are individuals aged 55-64 (Langlois et al., 2004). Based on average annual numbers of TBI-related ED visits, hospitalizations, and deaths combined for males (835,000) and females (561,000) of all age groups, males are 1.5 times more likely to suffer a TBI compared to females. The difference between the sexes is exaggerated when comparing mortality rates. Combining mortality rates across all age groups males are 2.8 times more likely to die as a result of TBI compared to females. Additionally in the young adult population between the ages of 15-24, mortality rates are 3.6 times higher in males than in females.

The increased risk is attributed to a higher incidence of motor vehicle accidents, use of firearms, and risk-taking behavior associated with that population (Langlois et al., 2004).

In addition to the physical and emotional havoc that TBI can wreak on an individual, their family, and friends there is also a tremendous financial burden associated with TBI. Based on data obtained from the CDC, it is estimated that 56 billion dollars are spent in the U.S. each year on the direct and indirect costs associated with TBI (Langlois et al., 2004). Of particular concern are the younger victims of TBI who may require long-term financial and/or personal care as well as a reduced ability of these individuals to provide for their families (Povlishock & Katz, 2005).

Pre-Injury Treatment and Selective Vulnerability

The majority of TBI research deals with post-traumatic interventions. However, data obtained by investigating pre-injury factors contributes to a better appreciation of the whole picture associated with TBI. Understanding how pharmacological alterations in brain neurochemistry prior to injury can lead to increased vulnerability or neuroprotection is an important component of TBI research.

In the study by Brown and colleagues (2000) it was hypothesized that pre, post, and combined nicotine treatments would improve cognitive outcomes following TBI (Brown, Gonzalez, & Kolb, 2000). It was speculated that pre-injury treatment with nicotine may offer neuroprotective effects. Nicotine had already been shown to improve memory and cognitive performance when administered to patients diagnosed with Alzheimer's disease (Samuels & Davis, 1998). Improvements were also observed in the cognitive functioning of normal human subjects when nicotine was administered either

before or after behavioral training (Warburton, Wesnes, Shergold, & James, 1986). In the pre-injury group, nicotine was administered for 11days prior to injury. The lesion volume and behavioral analysis showed significant improvement as a result of pre-injury treatment with nicotine (Brown et al., 2000).

In the study by Micale and colleagues, short-term (sub-acute) administration of DA agonists for seven days prior to hyperbaric hypoxia was found to reverse the effects of hyperbaric hypoxia induced amnesia (Micale, Incognito, Ignoto, Rampello, Spartà, et al., 2006). The drugs that were tested include the non-ergoline D2-R agonist ropinirole as well as the ergot-derived selective D2-R agonist drugs bromocriptine, cabergoline, pergolide and dihydroergocryptine (DHECP). The results from the above study support the previous findings of Medico and colleagues, that ropinirole and DHECP were effective in ameliorating hyperbaric hypoxia induced amnesia when administered for seven-days prior to injury (Medico, DeVivo, Tomasello, Grech, Nicosia, et al., 2002).

Other studies have utilized acute pre-treatment methodologies to assess post-injury cognitive outcome. For instance, in the study by Enomoto and colleagues (2005) Mg^{2+} was administered between 20min and 5min pre-injury and was found to improve performance on working and reference memory tasks as measured by the radial arm maze (Enomoto, Osugi, Satoh, McIntosh, & Nabeshima, 2005). In another study, bromocriptine was administered 15 min prior to injury. Similar to the findings from the sub-acute studies above, bromocriptine improved cognitive performance following TBI (Kline, Massucci, Ma, Zafonte, & Dixon, 2004). Jiang and colleagues (1994) administered the muscarinic antagonist scopolamine prior to injury and found that it

significantly reduced spatial memory deficits (Jiang, Lyeth, Delahunty, Phillips, & Hamm, 1994).

Although the majority of TBI research is focused on post-injury treatments, the aim of this study is to investigate the selective vulnerability of the brain to pre-injury drug-induced alterations in neurochemistry. Cognitive functioning following TBI will be evaluated as a function of pre-injury exposure to methylphenidate (MPH), a widely prescribed pharmacotherapy used as a treatment for Attention Deficit Hyperactivity Disorder (ADHD). Thus it will be determined if chronic or acute pre-treatment with MPH increases the vulnerability of the brain to TBI-induced pathology.

Biomechanics of Human Traumatic Brain Injury

The CDC defines TBI as a sudden physical assault to the brain. TBI can result from rapid acceleration or deceleration of the head and neck; blunt trauma to the head caused by an object striking the head; or an object penetrating the skull (McIntosh, Smith, Meaney, Kotapka, Gennarelli, et al., 1996; Povlishock & Christman, 1994). Holbourn (1943) was the first to discover the effects of shearing strains, specifically rotational acceleration forces, as a primary cause of predictable injury in the brain. The effect of acceleration or deceleration forces on the brain is varied and largely depends on the presence of rotational forces applied to the head and neck. Individuals who sustain a sagittal (front to back) injury have the best likelihood of recovery, lateral (side to side) injuries have the worst percentage for recovery, and oblique injury outcomes are somewhere in between. These forces are sufficient to produce brain injury without any accompanying contact injury.

The mechanical forces that produce TBI can be attributed to either static or dynamic loading. In order to assess the severity of a TBI resulting from dynamic loading, it is important to account for inertial, acceleration and impact forces. The greater the force acting on the head and neck the more damage is inflicted (Gaetz, 2004; Ommaya & Gennarelli, 1974). By understanding the biomechanical events associated with TBI researchers are able to better replicate injuries in various models. This research can ultimately lead to the development of new treatments for TBI.

Static Loading

Static loading occurs when forces are applied to an unmoving head over an extended time course taking more than 200 milliseconds to develop. This type of force generally produces multiple, comminuted, or eggshell fractures of the skull. This type of mechanical force does not normally produce the characteristic symptoms commonly associated with TBI. For example, symptoms like coma or neurological signs of injury are generally not seen unless the force is sufficient to cause deformation of the skull and brain tissue (Graham, McIntosh, Maxwell, & Nicoll, 2000).

Dynamic Loading

Dynamic loading is the mechanical force that is most commonly associated with the sequelae following TBI. Dynamic loading generally occurs in a much faster timeframe (less than 50 ms) compared to static loading. Dynamic loading can be further classified as either impact or impulsive (Graham et al., 2000; McIntosh et al., 1996).

Impact loading. Impact loading occurs when a blunt object strikes the head, typically producing both contact and inertial related injuries. The amount of damage

produced is directly related to the amount of force applied to the skull. Contact forces can generate stress waves that radiate through the skull and can cause additional skull fractures separate from those at the point of impact (Graham et al., 2000; McIntosh et al., 1996).

Impulsive loading. Impulsive loading occurs when the head is set into motion or when a moving head is suddenly stopped, either without it striking anything or by contact with an object. This type of injury can be created when the head moves indirectly as a result of impact to the body in another area (McIntosh et al., 1996). Primary damage to the brain parenchyma is caused by nonuniform distribution of pressure and strain (Graham et al., 2000; McIntosh et al., 1996). Biological tissue is more resilient to slower strains compared to fast strains (Graham et al., 2000).

Pathobiology of Human Traumatic Brain Injury

TBI is characterized by two distinct phases, the primary injury phase and a secondary injury phase. The primary injury phase is very short, lasting only a few hours (McIntosh et al., 1996). The primary injury typically results from lacerations, surface contusions, skull fractures, diffuse axonal injury, hematomas, and excitotoxicity (Graham et al., 2000; McIntosh et al., 1996; Povlishock & Becker, 1985). The secondary phase is a chain of events that occurs following the primary insult but does not manifest clinically for a period of time after injury. The damage that occurs during this phase of injury can be caused by increased intracranial pressure, swelling, edema, ischemia, hypoxia, alterations in the brains neurochemistry and infection (Graham et al. 2000, McIntosh, 1996).

An alternate classification of TBI has been characterized using neuroimaging techniques that allow researchers to correlate structural damage to functional outcomes. Under this system, researchers adopted the terms focal and diffuse to describe the types of brain damage. Focal brain damage includes surface contusions and lacerations, intracranial hematoma, and increased intracranial pressure. Diffuse brain damage includes ischemic injury, diffuse axonal injury (DAI), and swelling (McIntosh et al., 1996). There are some cases where focal and diffuse pathologies can coexist. This is seen more often in severely head-injured patients than in patients who received mild or moderate head injuries (Graham et al. 2000; Povlishock & Katz, 2005). Recently a third category of generalized abnormalities has been recognized that also affects the mortality and morbidity associated with TBI. Generalized changes that occur in the brain include neuroexcitation, abnormal agonist-receptor interactions and a multitude of vascular irregularities (Povlishock & Christman, 1994; Povlishock & Katz, 2005).

Focal Injury

Primary focal injury such as a missile or puncture wound is characterized by the presence of contusions or direct disruptions of brain tissue and can include hemorrhaging and hematomas in the extradural, subarachnoid, subdural, and intracranial areas (Gaetz, 2004; Gennarelli, 1993). Secondary damage from focal injuries includes delayed neuronal injury to neighboring regions, microvascular injury, focal hypoxic-ischemic injury, herniation, and regional and diffuse hypometabolism. Focal injuries produce regions of significantly reduced cerebral blood flow creating ischemic conditions that

promote inflammation and cytotoxicity in addition to neuronal necrosis (Bullock, Maxwell, Graham, Teasdale, & Adams, 1991; Gaetz, 2004).

Contusions. The presence of a contusion is extremely common in patients with focal TBI; however, TBI can occur without the presence of a contusion. Contusions cannot be used as an explanation for loss of consciousness at the time of injury or as a factor in the maintenance of a comatose state. Contusions are linked to focal seizures and/or functional deficits in the language centers of the brain (Ribas & Jane, 1992; Povlishock & Christman, 1994). In most cases contusions are the byproduct of hemorrhagic lesions within the gray matter or at the gray-white interface and contribute to neuronal damage and ischemia (Povlishock & Katz, 2005). Generally "traditional" contusions are located on the frontal and temporal poles, the lateral and inferior surfaces of the frontal and temporal lobes, and above the Sylvian fissure (Gaetz, 2004; Gennarelli & Graham, 1998; Gurdjian, 1976; McIntosh et al., 1996; Povlishock & Christman, 1994). They are commonly seen at the apex of gyri and can appear as punctate hemorrhages or streaks of hemorrhage usually accompanied by progressive bleeding into adjoining white matter (Gennarelli & Graham, 1998).

There are several types of contusions that include coup contusions that occur directly beneath skull fractures, contracoup that occur some distance (not always directly opposite) from the fracture, and gliding contusions. Gliding contusions are more associated with diffuse brain injuries. The term "gliding contusions" is used to describe hemorrhagic lesions in the parasagittal cortex. Gliding contusions are produced by cortical gray matter moving in opposition to the underlying white matter causing shearing

strains that damage the penetrating vessels located at the gray/white interface (Adams, Doyle, Graham, Lawrence, & McLellan, 1986; Povlishock & Christman, 1994). In addition, through the use of MRI's, nonhemorrhagic contusions were identified. These lesions are not associated with hemorrhaging but are located on the cortical surface where one would expect to see "traditional" contusions. Another type of nonhemorrhagic contusion was identified within the subcortical white matter, leaving the superficial cortex unharmed. These lesions are correlated with shearing forces, causing diffuse axonal injury (Povlishock & Christman, 1994).

Hematomas. There are several kinds of hemorrhaging that include intracranial hematomas, extradural hematoma, and acute subdural hematomas (Gaetz, 2004; Gennarelli & Graham, 1998; Povlishock & Christman, 1994). One possible explanation for the formation of hematomas is the rupturing of cerebral arterioles caused by the shearing and tensile forces generated by the injury (Povlishock & Christman, 1994). Intracranial hematomas are located deep within the parenchyma and are associated with the rupturing of a blood vessel. Extradural hematomas are associated with a skull fracture. Acute subdural hematomas are caused by the rupturing of the bridging veins within the dura or cortical arteries (Genneralli & Graham, 1998). Cerebral hematomas are usually formed at the time of injury; however, there is evidence of delayed hematoma formation that has been observed in patients with injuries ranging from mild to severe (Soloniuk, Pitts, Lovely, & Bartowski, 1986; Povlishock & Christman, 1994). Hemorrhaging and contusions are associated with secondary ischemic damage and

subsequent necrosis from the excess of blood affecting the adjacent tissue (Genneralli & Graham, 1998).

Diffuse Injury

Primary diffuse injury consists of diffuse axonal injury (DAI) and petechial white matter hemorrhage. Secondary diffuse injury is associated with delayed neuronal injury, microvascular injury, diffuse hypoxic-ischemic injury (HII), and diffuse hypometabolism (Povlishock & Katz, 2005). In addition to secondary injuries, delayed pathology resulting from a brain injury can lead to DAI characterized by axonal swelling and degradation and followed by axonal separation from its downstream segment and characteristic formation of a retraction bulb (Povlishock & Christman, 1995).

Diffuse cell death is among the sequelae commonly observed following TBI and can be attributed to apoptotic and necrotic cascades. Necrotic cell death occurs following degradation of the cell membrane thereby disrupting the ionic homeostasis leading to the rapid destruction of the cytoskeleton and its cytoplasmic components (Povlishock & Katz, 2005). Rapid cell death is linked to the activation of the cysteine proteases, calpain and caspase, causing degeneration of the membrane, making it more porous, and ultimately resulting in the swift demise of the soma. The pathobiology of apoptotic cell death is not as well defined and there is some dispute over the cause of the observed apoptotic events following TBI. The main theories for the initiation of the apoptotic events are excessive neuroexcitation, radical-mediated injury, or a dysregulation of calcium homeostasis (Raghupathi, 2004; Raghupathi, Graham, & McIntosh, 2000; Yakovlev & Faden, 2004, Povlishock & Katz, 2005). Apoptotic events are mediated by

internucleosomal DNA strand breaks with nuclear condensation. It is these changes that cause the cell to slowly die (Povlishock & Katz, 2005).

Diffuse axonal injury. Diffuse axonal injury (DAI) can occur for months post-injury. Other aspects commonly associated with DAI are edema, petechial hemorrhages, non-hemorrhagic macroscopic white matter lesions and small subarachnoid and intraventricular hemorrhages (Povlishock & Katz, 2005). Retraction bulbs and microglia scars are the trademark of DAI. DAI was first described by Strich (1956) as the tearing of axons throughout the brain caused by shearing forces generated at the time of injury. This was thought to be the primary cause of axonal damage following injury because when the damaged tissue was visualized postmortem using histological techniques, the injured axons appeared reactive and swollen (Strich, 1956; Adams, Graham, Murray, & Scott, 1982). However, later research proved that DAI was not the result of immediate shearing of axons as was originally thought. It was determined that reactive axons were undetectable using histological techniques unless the patient had survived for a minimum of 12 hours post-injury (Pilz, 1983). Further postmortem analysis utilized antibodies targeted to neurofilaments at various timepoints post-injury. Focal accumulation of neurofilaments was tied to further swelling of the axon cylinder and ultimately leading to detachment from its downstream segment at approximately 12 hours post-injury (Povlishock and Christman, 1994).

Generalized Changes

In addition to changes produced following focal and diffuse brain injury there are generalized changes that occur following TBI. Generalized changes include alterations in

the permeability of the blood brain barrier (BBB), neurotransmitter responses, CO_2 levels, and cerebral blood flow (Povlishock & Christman, 1994).

Findings from clinical studies have found elevated neurotransmitter levels in the cerebral spinal fluid (CSF) of brain-injured patients. Studies have also shown that increases in the levels of excitatory amino acids (EAA) such as glutamate and aspartate in the extracellular fluid surrounding the contusion area can remain elevated for as many as four days post-injury (Povlishock & Christman, 1994). EAA release is determined by the severity of the initial injury combined with any secondary events that may have occurred prior to stabilization at the hospital (Zauner & Bullock, 1995). Most of the research supporting the presence of generalized changes comes from preclinical studies and as such will be discussed in greater detail in the experimental injury section.

Human TBI Outcome

Despite the distinctions made between diffuse, focal, and generalized injuries, it is highly likely that an individual who sustains a TBI will have characteristics of all three. An individual's outcome following TBI is related to several factors, including the age of the individual, pre-injury abilities, personality, and severity of the injury (CDC, website on TBI). Individuals who have sustained a TBI are likely to suffer cognitive and/or behavioral impairments following the injury. The most common deficit attributed to TBI is cognitive impairment. The CDC estimates that each year 1.1 million or 75% of individuals who suffer a TBI are diagnosed with mild brain injury. Patients who are diagnosed with mild TBI do not typically lose consciousness; however, cognitive and neurological impairments due to diffuse axonal injury are likely (Povlishock & Katz,

2005). Even mild TBI can result in the loss of gainful employment. Researchers have found that the physical impairment attributed to the injury is not a significant factor when determining an individual's ability to return to work. What is significant are the cognitive, behavioral, and personality changes that can impact an individual's ability to maintain their employment (Wehman, Targett, West, & Kregel, 2005). TBI patients who suffer a moderate to severe injury usually experience unconsciousness and/or post-traumatic amnesia following TBI (Povlishock & Katz, 2005). Permanent memory loss is associated with severe head injuries. TBI can also bring about psychiatric conditions. The frequency of mood disorders, such as major depression and anxiety, are significantly greater in TBI populations (Jorge & Robinson, 2003).

Experimental Traumatic Brain Injury

Models and Mechanics of Experimental TBI

Experimental brain injury models must generate similar injuries to those observed following human TBI. Regardless of the injury outcome measure such as physiological, behavioral, or anatomical, the results must be reproducible and quantifiable, clinically relevant, and produce a continuum of injury severities (Lighthall, Dixon, & Anderson, 1989). There is no one model that can replicate the complex mechanisms that can occur following human TBI. This necessitated the development and implementation of several preclinical models of TBI to properly characterize its underlying pathology.

There are four models that can be used to investigate the effects of TBI. They are physical, computational, cell culture, and animal. All of the models listed have provided important data used in the understanding and treatment of TBI. However, to date only

animal models are able to represent how a living organism responds to trauma. Animal models have been able to reliably reproduce the sequelae associated with human TBI. Animal models of injury include dynamic closed head injury, penetration, ablation, lesioning, and quasistatic injury. Because the present study utilizes a closed head model of head injury, the other models of TBI will not be discussed further. An interesting aspect that must be taken into consideration when evaluating experimental data is the time course of events following trauma. The pathophysiological mechanisms that occur following experimental injury occur in a faster timeframe compared to what is observed following human TBI (Zauner & Bullock, 1995). This is also important when evaluating the effects of pharmacological treatments in animal models of injury, such as the rate of metabolism of the selected drug.

Fluid percussion injury. Fluid percussion (FP) injury is the most commonly used rodent model of TBI. This model requires that a small diameter (4.8mm) craniotomy be made, which exposes the underlying dura mater. The injury is produced by applying a brief fluid pulse directly on the surface of the dura via the craniotomy. The injury can be delivered either centrally or laterally. Central FP delivers the fluid pulse along the central suture midway between bregma and lambda. Lateral FP delivers the injury to the parietal lobe midway between the coronal and lambdoid sutures. The FP model is able to replicate the cognitive and histological changes similar to those seen in human head injury (Dixon, Lyeth, Povlishock, Findling, Hamm, et al., 1987). In rodent models FP has been shown to produce cognitive deficits that can last for weeks or months post-injury (Hamm, Lyeth, Jenkins, O'Dell, & Pike, 1993). Other aspects of human TBI are

also generated following FP injury such as hemorrhaging at the gray/white interface, acute hypertension, bradycardia, increased plasma glucose levels, and suppression of electroencephalogram amplitude that is related to the magnitude of the head injury (Cortez, McIntosh, & Noble, 1989; Dixon, Lighthall, & Anderson, 1988).

Both central and lateral FP injury models are capable of producing cognitive deficits via damage to the hippocampus which is a region of the brain that is known to be selectively vulnerable in human TBI. This ability is essential for cognitive recovery-based research. The two models differ in the type of damage inflicted on the hippocampus. Central FP does not produce the same magnitude of cell loss that is typically seen following lateral FP; however, it does produce hippocampal damage (Hamm et al., 1993; Lyeth, Jenkins, Hamm, Dixon, Phillips, et al., 1990). The memory impairment observed following central FP is not believed to be due to cell death but instead due to neuronal dysfunction in the hippocampus (Lyeth, et al., 1990; Hayes, Jenkins, & Lyeth, 1992). Lateral FP injury is known to cause injury to the CA3 region as well as bilateral cell loss in the hilar region of the hippocampus (Cortez et al., 1989; Hicks, Smith, Lowenstein, Saint, & McIntosh, 1993; Smith, Okiyama, Thomas, Claussen, & McIntosh, 1991). Memory dysfunction observed following lateral FP is directly related to the amount of cell death in the dentate hilar region (Smith, Lowenstein, Gennarelli, & McIntosh, 1994). Because of the similarities lateral FP has with injuries observed following human TBI, the lateral FP model was selected for use in the present study.

Controlled cortical impact. Controlled cortical impact (CCI) uses a pneumatic impactor to impact exposed brain tissue. The advantage of this model is that the biomechanical events contributing to the injury can be quantified. Force, velocity, and tissue deformation can be compared to the amount of tissue damage and/or functional impairment. The CCI model has been shown to produce cognitive deficits similar to those observed following human TBI (Hamm, Dixon, Gbadebo, Singha, Jenkins, et al., 1992). CCI is able to simulate the neuropathology of severe human head injury more effectively than FP injury (Dixon, Clifton, Lighthall, Yaghmai, & Hayes, 1991). Hoffman and colleagues developed a bilateral model of frontal cortical contusion that was able to reproduce deficits typically observed after frontal lobe damage in humans (Hoffman, Fulop, & Stein, 1994). A pneumatically-controlled cortical impactor was used to create bilateral contusions of the medial prefrontal cortex in male Sprague-Dawley rats. Both CCI models were able to produce cognitive deficits as measured by the Morris Water Maze in addition to producing neurological, histological, and physiological deficits (Hamm et al., 1992; Hoffman et al., 1994). CCI can also cause direct hemorrhage within the cortical gray matter and produce significant edema and damage to the blood brain barrier (BBB) (Beaumont, Hayasaki, Marmarou, Barzo, Fatouros, et al., 2001).

Weight drop model. In this model, a weight is dropped from a predetermined distance onto the cranium of the animal. This model is described in detail in the article by Marmarou and colleagues (1994). The weight drop model utilizes a free falling brass weight that is released in a Plexiglas guide tube and impacted on a stainless steel disk affixed on the top the head of the rat. The stainless steel disk is cemented onto the

calvaria and functions to prevent the formation of skull fractures. The disk allows for higher impact-acceleration levels that have been shown to elicit diffuse brain damage (Marmarou, Foda, van den Brink, Campbell, Kita, & Demetriadou, 1994). Observed outcomes following weight drop injury are apnea, convulsions, subarachnoid hemorrhage, intraventricular hemorrhage, and in severely injured animals there was evidence of petechial hemorrhage. This model also produces microscopic damage to neurons, axons, astrocytes, and small blood vessels in mild and severely damaged groups. Neuronal changes were present in both mild and severely damaged groups and were directly related to the severity of the injury. It was demonstrated through the use of this model that brain stem damage is not a necessary component of severe head injury (Foda & Marmarou, 1994).

Pathobiology of Experimental TBI

Focal. The FP (Dixon et al., 1987, 1988) and CCI models (Lighthall, 1989) are capable of eliciting focal contusions and hemorrhaging in various animal models including primates, rodent and nonrodent models. In the most severe injuries, hemorrhaging can lead to further destruction of the cortical gray matter followed by the formation of a cystic cavity surrounded by glial cells. Precontusoinal changes observed in animal models of TBI correlate with nonhemorrhagic contusions observed in human TBI. Hemorrhaging in the injured areas of the cortex can expand over time and produce a larger hemorrhagic mass that can facilitate secondary ischemia and infarction (Povlishock & Christman, 1994).

Intraparenchymal hemorrhaging can be generated following FP injury but it is typically seen only following severe injuries. Like human TBI, the presence of a contusion is not always related to mortality and the presence of a contusion does not necessarily have a direct correlation with behavioral pathologies. Only when the contusion spans a large area of nervous tissue and/or involves a discrete functional area does it have a direct relationship to behavioral outcome (Povlishock & Christman, 1994).

Diffuse. Not all animal models of TBI are able to mimic the pathologies of diffuse axonal injury. FP injury and CCI models are only able to produce focally confined axonal damage. However, these models have been used to obtain the majority of the data regarding traumatically-induced DAI (Povlishock & Katz, 2005). The model that most closely replicates the pathology associated with DAI was described in the study by Gennarelli and colleagues (1982). In this study nonhuman primates sustained DAI as a result of rapid acceleration of the head in one of three directions (sagittal, oblique, or lateral) without impact (Gennarelli, Thibault, Adams, Graham, Thompson, et al., 1982). However, due to the difficulty and expense associated with nonhuman primate studies, new models utilizing optic nerve stretch have been developed (Maxwell, Irvine, Watt, Graham, Adams, et al., 1991).

It was through the use of animal studies that the nature of DAI could be fully investigated. Povlishock and colleagues used anterograde tracers in the major conducting pathways prior to applying varying levels of experimental injury to determine if the axons were disconnecting at the time of injury or if there was another process occurring within the axon that was facilitating axonal degradation. Through these experiments it

was shown that within 1-2 hours post-injury there was a change in the axon length and an accumulation of the anterogradely transported tracer which caused local swelling of the axon. Within 3- to 6-hours post-injury the axonal swelling increased to form a retraction bulb and ultimately resulted in axonal separation (Cheng & Povlishock, 1988; Erb & Povlishock, 1988; Povlishock, Becker, Cheng, & Vaughan, 1983; Povlishock & Becker, 1985; Povlishock & Kontos, 1985). This proved that axonal injury was not attributed to tearing of the axon by external forces at the time of injury, but rather that it was the result of injury-induced changes within the axon. In the review article by Povlishock and Katz (2005) it was described that while previous research, including their own, has focused solely on investigating the effects of injury on large caliber myelinated axons, recent findings have shed light on the importance of injury to myelinated and unmyelinated fine caliber fibers and how this aspect of injury may be vastly more important to an individual's outcome following TBI (Reeves, Phillips, Walker, & Povlishock, 2004).

Generalized. Following FP injury in animals it has been shown that there is an immediate increase in extracellular release of neurotransmitters, including catecholamines, acetylcholine, and glutamate (Faden, Demediuk, Panter, & Vink, 1989; Hayes et al., 1992; Zauner & Bullock, 1995). Shearing injuries can initiate widespread changes in neurotransmitter functioning and ionic homeostasis. These changes set off widespread depolarization of cells allowing an influx of Na^{2+} and Ca^{2+} ions into the cell and an efflux of K^{+} to the extracellular space (Katayama, Becker, Tamura, & Hovda, 1990). These cellular changes are related to neuronal and glial swelling that can lead to edema and increases in intracranial pressure (ICP) (Zauner & Bullock, 1995). EAA's are

known to be elevated following experimental TBI (Faden et al., 1989; Katayama et al., 1990). There are several excitatory neurotransmitters; however, glutamate is the primary neurotransmitter involved in TBI-induced neurotoxicity (Rothman & Olney, 1986). Increased release of EAA's such as glutamate and aspartate are released from the hippocampus following moderate to severe TBI, with increased neurotransmitter release as injury severity increases (Faden et al., 1989; Hayes & Dixon, 1994). Through the use of animal studies it has been found that multiple agonist-receptor interactions are involved in TBI pathologies. Based on this information it was discovered that treatment with receptor antagonists offers a neuroprotective effect. Povlishock and Christman (1994) discuss several studies that used EAA antagonists to elicit a neuroprotective effect. When multiple EAA antagonists are combined they offer greater neuroprotection than when used individually (Jenkins, Lyeth, Lewelt, Moszynski, Dewitt, et al., 1988).

In brain tissues somewhat affected by reduced regional cerebral blood flow (rCBF), glutamate excitotoxicity may be involved in secondary ischemic damage. Hypoxia-related neuronal depolarization is related to increased extracellular levels of glutamate due to increased release and decreased reuptake of glutamate. High levels of glutamate can cause depolarization of cell membranes thereby activating voltage dependent Ca^{2+} channels that in turn activate the release of more glutamate via a positive feedback loop resulting in glutamate neurotoxicity and ultimately cell death (Gennarelli, 1993). Other amino acid neurotransmitters such as glycine are reported to be involved in seizure activity and toxicity from secondary damage (Nilsson, Ronne-Engstrom, Flink, Ungerstedt, Carlson, et al., 1994). Glutamate antagonists have been found to be effective

in reducing intracranial pressure produced by edema (Schroder, Muizelaar, Bullock, Salvant, & Povlishock, 1995).

Edema is the accumulation of serous fluid within a body cavity or tissue and is a significant factor related to secondary injury. Edema can be caused by a multitude of events and is the endpoint of several pathological processes. There are two primary types of edema, vasogenic and cytotoxic. Vasogenic edemas are related to the BBB. Vasogenic edemas can occur at tight junctions of endothelial cells that limit the transfer of macromolecules across the BBB. Compromises in this region can facilitate the passage of neurotoxic vascular components into the parenchyma (McIntosh et al., 1996). Cytotoxic edema is brought on by acute ischemic events and characterized by swelling of neurons, glia, and endothelial cells. The lack of oxygen prevents adenosine triphosphate (ATP)-dependent Na^{+2} and K^{+} ion transport. ATP levels can be disrupted by ischemic reduction in cerebral blood flow or mitochondrial dysfunction. Sodium accumulates within the cells disrupting osmotic equilibrium forcing excessive amounts of water into the cell. Intracellular calcium levels are also increased and lead to the activation of phospholipases and the subsequent release of arachidonic acid followed by the release of oxygen-derived free radicals and infarction (Kandel, Schwartz, & Jessell, 2000).

Disruption of the BBB can be brought on by several different mechanisms. Hypertensive responses following moderate or severe injury are known to disrupt the BBB (Hayes & Dixon, 1994). Mild and moderate focal TBI have also been linked to alterations in the permeability of the BBB that has been shown to persist for up to 15 hours post-injury (Cortez et al., 1989).

Vascular abnormalities in preclinical research are very similar to those seen following human TBI and include impairment or loss of autoregulation (Lewelt, Jenkins, & Miller, 1980), impaired physiologic cerebral vascular responsiveness to changes in arterial blood gases (Wei, Dietrich, Povlishock, Navari, & Kontos, 1980), and altered cerebral blood flow (DeWitt, Jenkins, Wei, Lutz, Becker, et al., 1986; Povlishock & Christman, 1994; Yamakami & McIntosh, 1989). Abnormally low levels of CO_2 in the blood stream have been found following experimental TBI. It is hypothesized that shear and tensile strains may produce functional and structural changes in cerebral blood vessels (Povlishock & Christman, 1994).

Biphasic Hypothesis

The biphasic model of brain injury deals with the sequelae associated with secondary injuries and is divided into the acute and chronic phases. The acute phase is marked by cerebral hypermetabolism and increases in the extracellular release of several neurotransmitters leading to neurotoxicity. The chronic phase is characterized by a hypofunctional state with reduced cerebral metabolism that in humans is maintained in the days and weeks following TBI. Experimental therapies to treat injury are designed to target either the acute or chronic phase. It is within these two time points that treatments can be implemented. Typically treatments that are effective in the excitotoxic acute phase are ineffective when administered during the hypofunctional chronic phase and vice versa. The time sensitive nature of treatment intervention can make the transitioning from acute treatments in preclinical models to clinical use extremely difficult. It is for this reason that it is important to investigate not only therapies geared towards post-injury

timepoints but also to examine how pre-injury states may influence an individual's treatment or recovery. Due to the difficulties stated above regarding the time post-injury interventions are administered, having a better understanding of the preexisting neurological states could eventually enable therapies to be selected based on the individual's specific pathology.

Remote Functional Depression (RFD) is a hypothesis proposed by Feeney (1991) to explain the apparent biphasic condition associated with the brain's response to trauma-induced injury. RFD is derived from the idea of diaschisis, first proposed by von Monakow in 1905. Diaschisis is a term used to describe how focal injury to one area of the brain can produce damage to a morphologically separate area via common neural pathways. Von Monakow attributed the remote damage to a loss of excitatory input from the injured area. It was speculated that spontaneous recovery from this state was due to the resolution of the dysfunctional state (von Monakow, 1969).

Dopamine

Overview

Dopamine (DA) is a vitally important neurotransmitter that is involved in several processes including learning and memory, executive functioning, planning and execution of movement, and hormonal regulation. The primary actions of DA are the activation or inhibition of cyclic AMP (cAMP) pathways and modulation of Ca^{2+} signaling (Vallone, Picetti, & Borrelli, 2000). DA also plays an important role in TBI pathology as well as post-injury pharmacological treatments (Zhu, Hamm, Reeves, Povlishock, & Phillips,

2000). Of particular interest to this study is methylphenidate (MPH), a DA agonist that is used in the treatment of ADHD.

Synthesis

The neurotransmitter dopamine is classified as a catecholamine and is located primarily in the central nervous system. The neurotransmitter group classified as the catecholamines is comprised of dopamine (DA), norepinephrine (NE), and epinephrine (EPI). Dopamine is a modulatory neurotransmitter that is both inhibitory and excitatory. Dopamine is synthesized in the cytoplasm of dopaminergic neurons. The amino acid tyrosine is converted to L-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase. Tyrosine hydroxylase is the rate-limiting enzyme in the synthesis for DA and NE. L-DOPA is converted into DA by the enzyme DOPA decarboxylase (or aromatic L-amino acid decarboxylase). Once the DA has been synthesized it is transported into synaptic vesicles by a monoamine-H^+ transporter (Haines, 1997).

Metabolism

Following an action potential, catecholamines that were released into the synapse by the presynaptic neuron can be taken back into the presynaptic neuron by the DA transporter (DAT) located in the membrane of the presynaptic neuron. DA can then be taken back into a synaptic vesicle to be re-used or metabolized by monoamine oxidase (MAO) or catechol-O-methyltransferase (COMT) located only in the cytoplasm. When catecholamines are broken down by MAO the result is 3,4,-dihydroxyphenyacetic acid (DOPAC). The enzyme COMT breaks down catecholamines into 3-methoxytyramine.

When both enzymes act on a catecholamine the result is the formation of homovanillic acid (HVA) (3-methoxy-4hydroxy-phenylacetic acid) (Haines, 1997).

Dopaminergic Pathways

The pathways associated with the dopamine system are the corticostriatal, mesolimbic, mesocortical, nigrostriatal, and hypothalamic-pituitary (UCSF Medical School website). The corticostriatal pathway is comprised of pathways that originate from the motor and premotor cortex and the orbitofrontal areas and terminate in the basal ganglia. The corticostriatal pathway is not specifically a dopaminergic pathway but it is involved in regulating DA functioning within the basal ganglia. The premotor and motor cortex projects to the putamen and is involved in planned movements and also regulates automatic and involuntary aspects of movement that originate in the putamen. The projections that originate in the orbitofrontal cortex and project to the caudate are involved in regulating impulsive aspects of behavior that originate within the caudate and related structures. The mesolimbic pathway is a dopaminergic pathway that originates in the ventral tegmental area (VTA) of the midbrain and projects to the nucleus accumbens and amygdala. This pathway is related to emotional behavior, motivation, pleasure, and reward. Like the mesolimbic pathway the mesocortical pathway also originates in the VTA. From there it projects to the prefrontal cortex, especially the dorsolateral prefrontal cortex. The dorsolateral prefrontal cortex is involved in attention, initiative, motivation, planning, decision making, working memory, and other higher order cognitive functions. The dopamine projections from the mesocortical pathway regulate these functions. Lesions to the mesocortical pathway result in increased DA activity

within the mesolimbic tract. Both the mesolimbic and mesocortical pathways work together to balance activity within the limbic system. The nigrostriatal pathway involves the extrapyramidal motor system and is important in coordination and maintenance of movement. This pathway originates from cell bodies in the substantia nigra, particularly in the substantia nigra pars compacta, and projects to the dorsal striatum. This pathway ascends via the medial forebrain bundle and then in the internal capsule to innervate the caudate nucleus, putamen, and the globus pallidus. The basal ganglia regulate automatic aspects of body movement particularly in sequencing actions such as placing one foot in front of the other to walk forward in an even and coordinated manner. The hypothalamic-pituitary pathway originates in the periventricular area of the hypothalamus and projects to the anterior pituitary. Within this pathway the release of DA from the hypothalamus regulates the release of prolactin by the pituitary (UCSF Medical School website).

DA is the most abundant catecholamine in the brain, and it is estimated that it comprises 80% of the total catecholamine content. However, the total number of DA cells in the brain is quite low. The human brain contains roughly 1 million dopaminergic cells, a small number compared to the 10 billion cells found within the cortex. DA-containing neurons are located predominantly within the more rostral parts of the brain such as the midbrain, hypothalamus, and olfactory bulbs (Feldman, Meyer, & Quenzer, 1997).

Receptors

There are five known DA receptor subtypes identified as D1-D5. These receptors are further classified into subfamilies based on biochemical and pharmacological criteria and are referred to as D1-like receptors and D2-like receptors. D1-like receptors include D1 and D5 receptor subtypes, D2-like receptors include D2, D3, and D4 receptor subtypes. It is generally accepted that D1-like and D2-like receptors work in concert in regulating DA-mediated actions (Maltais, Côté, Drolet, & Falardeau, 2000). The D1 receptor (D1-R) and D2 receptor (D2-R) subtypes are the most abundant of the DA receptor subtypes. In humans the D1-R is primarily expressed in the caudate-putamen, nucleus accumbens, olfactory tubercle, cerebral cortex, and amygdala. In the substantia nigra pars reticulata there has been binding of D1-R specific ligands although no mRNA has been detected. This finding indicates that D1-R's are synthesized in striatal neurons that send their projections to the substantia nigra via the direct nigrostriatal pathway (Vallone et al., 2000). The D2-R is mainly located in the caudate-putamen, olfactory tubercle, and nucleus accumbens. There is also expression in the substantia nigra pars compacta, and VTA. Since these regions give rise to DA neurons, it is believed that the D2-R is located on the presynaptic neuron; alternately the D1-R is exclusively located on postsynaptic neurons (Maltais et al., 2000).

Dopamine receptors in the rat brain. In the rat, the distribution of D1-R's and D2-R's are very similar to what is seen in humans. Rats have strong immunoreactivity for DA receptors in the caudate-putamen, nucleus accumbens, olfactory tubercle, substantia nigra, periventricular nucleus of the hypothalamus, dentate gyrus, and the endopiriform

cortex. Some reactivity was detected in the amygdaloid complex including the intercalated nuclei, anterior part of the basolateral nucleus, anterior part of the cortical nucleus, and supraoptic nucleus (Maltais et al., 2000). D1 and D2 receptors play opposing regulatory roles. The D1-R stimulates the release of cyclic AMP (cAMP) and phospholipase C while the D2-R inhibits those events. DA receptors also have a modulatory effect on arachidonic acid (AA). The D2-R stimulates the release of AA whereas the D1-R inhibits it. Increases in Ca^{2+} levels activate the D2-R and promote the synthesis of AA (Vallone et al., 2000). TBI-induced increases in intracellular Ca^{2+} can trigger the breakdown of AA into harmful compounds that are associated with neuronal death and poor post-injury outcome (McIntosh et al., 1996).

Dopamine Transporter

The dopamine transporter (DAT) acts to modulate the activity of DA in the synapse by quickly taking up DA following release from a presynaptic neuron. DAT levels were assessed four weeks post-injury using a Western blot and it was discovered that DAT protein expression was reduced (Yan, Kline, Ma, Li, & Dixon, 2002). Yan and colleagues (2002) hypothesize that this reduction in DAT protein is a compensatory mechanism to improve DA transmission chronically after TBI by reducing the number of reuptake sites.

Dopamine and TBI

DA is a key mediator in determining post-traumatic functioning following TBI. DA agonists administered in the hypofunctional chronic phase post-injury have been shown to improve cognitive outcome following TBI in experimental and clinical settings

(Kline, Yan, Bao, Marion, & Dixon, 2000; Gualtieri, Chandler, Coons, & Brown, 1989; Whyte, Vaccaro, Grieb-Neff, & Hart, 2002; Zhu et al., 2000). Both the D1-R and D2-R subtypes are involved in memory dysfunction following brain injury. Long-term memory dysfunction has been attributed to depleted dopamine levels in the hippocampus (Tang, Noda, & Nabeshima, 1997; Tang, Noda, & Nabeshima, 1997a).

Massucci et al., (2004) examined DA concentrations in the frontal cortex and striatum following severe lateral CCI injury in rats. The frontal cortex and striatum were analyzed because of their major DA projections. DA concentrations were assessed at 1 hour and 1 day post-injury. At 1 hour striatal DA concentrations were elevated in both the ipsilateral and contralateral sides. Frontal cortex DA levels were elevated on the contralateral side only. At 1 day post-injury frontal cortex levels were elevated on the ipsilateral side only (Massucci, Kline, Ma, Zafonte, & Dixon, 2004). In contrast to this data McIntosh et al., 1996 demonstrated that moderate level FP injury did increase striatal DA concentrations; however, this elevation was only observed at 6 hours post-injury and only in the ipsilateral cortex (McIntosh et al., 1996). Assessments were made at 1h, 6h, 24h, 1 week, and 2 weeks post-injury. DA concentrations in the ipsilateral striatum had returned to baseline levels within 24 hours post-injury. It was also reported that DA concentrations in the ipsilateral parietal cortex were significantly decreased at 1 hour post-injury and this decrease was observed at each of the experimental timepoints (McIntosh et al., 1996; McIntosh, Yu, & Gennarelli, 1994). There was no change in DA concentration in the contralateral parietal cortex. Measurements taken from the ipsilateral hypothalamus showed DA concentrations were significantly elevated at 1 hour

post-injury and remained elevated at 6 hours and 24 hours post-injury but returned to baseline by 1 week. Levels of NE were also increased in the ipsilateral hypothalamus although they did not reach significant levels until 6 hours post-injury and were significantly elevated at the 24 hour and 1 week timepoints (McIntosh et al., 1996). The differences between the Massucci et al., 2004 and McIntosh et al., 1994 & 1996 studies could be attributed to differences in injury model and severity. What both of these studies do support is the variable role DA plays in brain injury pathology.

MPH treatment following TBI. MPH works in several DA rich areas of the brain and is known to improve cognitive processing speed and abilities. Theses benefits have been demonstrated for not only individuals diagnosed with ADHD but also those who do not meet the diagnostic criteria for diagnosis with ADHD. When MPH is administered to individuals who do not have ADHD they have demonstrated improved performance on spatial working memory (Mehta, Owen, Sahakian, Mavaddat, Pickard, et al., 2000) and mathematical problem solving tasks (Volkow, Wang, Fowler, Telang, Maynard, et al., 2004). Because this drug has been utilized in clinical settings to improve cognitive processing abilities, there have been numerous experimental and clinical studies that have administered MPH to improve cognitive functioning following TBI (Kline et al., 2000; Whyte, Hart, Schuster, Fleming, Polansky, et al., 1997; Whyte et al., 2002).

Numerous studies have shown that MPH in addition to other ADHD treatment medication, such as d-amphetamine, are beneficial for improving cognitive functioning when administered in the chronic phase following TBI (Kline et al., 2000; McIntosh et al., 1996; Whyte et al., 1997). Kline and colleagues found that in rats, treatment with

MPH post-injury was effective in improving spatial memory as measured by the Morris Water Maze (Kline et al., 2000). In human TBI patients MPH is able to improve cognitive processing speed (Whyte et al., 1997). Although there is support for using MPH as a treatment for TBI, the Food and Drug Administration has not approved it for this use.

Attention Deficit Hyperactivity Disorder

Attention Deficit Hyperactivity Disorder (ADHD) is a neuropsychiatric disorder that is commonly diagnosed in childhood (Bolaños, Barrot, Berton, Wallace-Black, & Nester, 2003). According to the Diagnostic and Statistical Manual of Mental Disorders - Fourth Edition (DSM-IV), ADHD is characterized by excessive levels of inattentiveness, impulsivity, and hyperactivity (APA, 1994). It is estimated that 12% of the U.S. population meet the diagnostic criteria for ADHD (Shafritz, Marchione, Gore, Shaywitz, & Shaywitz, 2004; Bolaños et al., 2003). Based on the DSM-IV (1994) there are three recognized types of ADHD. These are: Hyperactive Impulsive Type, Predominantly Inattention Type, and ADHD Combination Type (APA, 1994). The population of individuals with ADHD can be categorized into two groups. The first group is individuals who were diagnosed as children, and the second group is those who have never been diagnosed. The first group is comprised of mostly males who were hyperactive as children. The later group consists of mostly women and/or the inattentive subtype (Wasserstein, 2005).

ADHD is characterized by dysfunction in dopaminergic transmission in the frontal lobes and striatal structures (Filipek, Semrud-Clikeman, Steingard, Renshaw,

Kennedy, et al., 1997). Functional imaging studies have indicated reduced metabolic function in frontal and striatal regions of individuals with ADHD. (Vaidya, Austin, Kirkorian, Ridlehuber, Desmond, et al., 1998; Amen & Carmichael, 1997; Castellanos, Giedd, Eckburg, Marsh, Vaituzis, et al., 1994; Lou, Henrikson, & Bruhn, 1984; Lou, Henriksen, Bruhn, Borner, & Nielsen, 1989; Sieg, Gaffney, Preston, & Hellings, 1995; Zametkin, Nordahl, Gross, King, Semple, et al., 1990). In another study, positron emission tomography (PET) detected irregular dopaminergic presynaptic function in adult males with ADHD (Ernst, Zametkin, Matochik, Pascualvaca, Jons, et al., 1998). MRI studies of ADHD individuals have demonstrated decreased volumes in several brain regions including the striatum and frontal cortex.

Molecular genetic studies have provided further evidence to support the hypothesis that ADHD is related to dopaminergic dysfunction (Swanson, Flodman, Kennedy, Spence, Moyzis, et al., 2000; Cook Jr., Stein, Krasowski, Cox, Olkon et al., 1995; Waldman, Robinson, & Feigon, 1997). In recent studies a correlation has been found between ADHD and the 480-base pair DAT1 allele for the DAT (Cook Jr. et al., 1995; Gill, Daly, Heron, Hawi, & Fitzgerald, 1997; Solanto, 1998; Swanson et al., 2000). Other groups have reported increases in the prevalence of the 7-repeat allele for the D4 gene that has been linked in some studies to novelty-seeking behavior in adults (LaHoste, Swanson, Wigal, Glabe, Wigal, et al., 1996; Solanto, 1998). There is speculation that individuals with ADHD may have a superfluity of DA autoreceptors, which may explain why MPH improves attention in individuals diagnosed with ADHD without producing a "high" feeling (Dougherty, Bonab, Spencer, Rauch, Madras, et al., 1999).

Stimulant Treatment for ADHD

The first observation of the beneficial effects of stimulant medication on behavioral problems in children was made by Charles Bradley in 1937 (Solanto, 1998). Bradley had administered Benzedrine (a racemic mixture of D- and L-amphetamine) for the treatment of postpneumoencephalography headaches. Bradley's theory was that a stimulant would promote the choroid plexus to produce more cerebral spinal fluid to make up for what was taken out during the pneumoencephalography procedure and thereby alleviating the headache. The treatment was not effective on the headaches but the children and their teachers noticed improvement in school performance while on the medication. Bradley did further controlled experiments and showed improved school performance following treatment with Benzedrine and other psychostimulants (Bradley, 1950; Bradley, 1937; Brown, 1998).

Today the majority of individuals who are diagnosed with ADHD are prescribed stimulant medication to manage their symptoms. Over the years, the number of children treated with stimulant ADHD medication has increased dramatically from 300,000 in 1974 to 1.5 million in 1995 (Safer, Zito, & Fine, 1996). It is estimated that 90% of children diagnosed with ADHD in the United States are prescribed MPH (Bolaños et al., 2003; Zito, Safer, dos Reis, Gardner, Boles, et al., 2000). MPH and dextroamphetamine are the most typical pharmacological treatments for ADHD (Rappley, 2005; Solanto, 1998).

Methylphenidate

History

MPH is a central nervous system stimulant and is currently approved by the Food and Drug Administration as a treatment for ADHD and narcolepsy (Challman & Lipsky, 2000). MPH was first synthesized in 1944 and was marketed as Ritalin® in the 1960's by Ciba-Geigy Pharmaceutical Company. In 1956 the Physicians Desk Reference indicated MPH for use as a treatment for lethargy, depressive states, disturbed senile behavior, psychosis associated with depression, and narcolepsy (Leonard, McCartan, White, & King, 2004). MPH was also used as an analeptic to reverse barbiturate-induced coma (Challman & Lipsky, 2000; Wax, 1997). In 1971 there was an epidemic of MPH abuse in Sweden, which prompted the United States to classify MPH as a schedule II controlled substance under the Drug Enforcement Agency classification system (Diller, 1996).

Pharmacokinetics

Methylphenidate is a cyclized derivative of amphetamine with 2 chiral centers (Challman & Lipsky, 2000; Teo, Stirling, Thomas, & Khetani, 2003). Early preparations of the drug were comprised of an 80:20 mixture of the *erythro-* and *threo*-racemates. Further research showed that while both racemates were equipotent in producing hypertensive effects and toxicity, only the *threo*-enantiomers were found to have central nervous system stimulant activity (Challman & Lipsky, 2000; Teo, Stirling, Hoberman, Christian, Thomas, et al., 2003a). The d and l-*threo*-methylphenidate enantiomers

comprise current preparations of the drug in a racemic mixture (Challman & Lipsky, 2000; Ding, Fowler, Volkow, Dewey, Wang, et al. 1997; Teo et al., 2003).

Pharmacological Effects

MPH is a psychostimulant that is pharmacologically distinct from amphetamine. Psychostimulants can be characterized based on whether their activity is attenuated by pre-treatment with reserpine. Reserpine is a drug that disrupts vesicular release by depleting the vesicular stores of catecholamines (Leonard, McCartan, White, & King, 2004). Behavioral effects of amphetamines are not affected by pre-treatment with reserpine because amphetamines release cytosolic not vesicular catecholamine reserves. MPH activity is impaired by reserpine indicating that MPH interacts with catecholamines stored in the synaptic vesicles (Leonard et al., 2004). The precise mechanisms by which MPH treatment enables individuals to reduce hyperactivity and to focus and sustain their attention over long periods of time have not been fully elucidated (Matochik, Liebenauer, King, Szymanski, Cohen, et al., 1994). However, on a molecular level the action of MPH is clearer. MPH is known to have direct effects on the neurotransmitters dopamine, norepinephrine, and to some extent serotonin (Challman & Lipsky, 2000); indirectly MPH has been shown to affect levels of acetylcholine (ACh) (Leonard et al., 2004; Acquas & Fibiger, 1996). MPH administration also alters endocrine functioning, glucose metabolism, cerebral blood flow, and expression of immediate early genes (IEGs) (Leonard et al., 2004).

Dopaminergic activity. MPH is a noncatecholamine sympathomimetic and functions as an indirect dopaminergic agonist (Teo et al., 2003). MPH increases

extracellular dopamine levels by binding to the dopamine transporter (DAT) thereby blocking the reuptake of DA by the presynaptic neuron, allowing DA to remain in the synapse longer (Challman & Lipsky, 2000; Hurd & Ungerstedt, 1989; Volkow, Wang, Fowler, Gatley, Logan, et al., 1998a). Volkow and colleagues (1998a), using positron emission tomography, determined that at therapeutic levels MPH blocked more than half of the brain's DAT's. In humans the brain regions most affected by MPH are the prefrontal cortex, hippocampus, striatum, globus pallidus, subthalamic nucleus, and substantia nigra with the highest concentration of DA found in the striatum (Moll, Heinrich, Trott, Wirth, & Rothenberger, 2000; Mehta et al., 2000; Volkow, Ding, Fowler, Wang, Logan, et al., 1995). In the rat, the brain regions affected by MPH administration are the striatum, nucleus accumbens, olfactory tubercle, and prefrontal (or prelimbic) cortex (Challman & Lipsky, 2000).

Other neurotransmitters. In vitro studies indicate MPH has a high binding affinity for NE transporters (Kuczenski & Segal, 1997; Gatley, Pan, Chen, Chaturvedi, & Ding, 1996). Kuczenski and Segal (1997) showed hippocampal levels of NE were elevated following MPH administration. Using glucose metabolism as a measure of MPH's activity within different brain regions, Volkow and colleagues (1998a) found that glucose metabolism in the cerebellum was increased following MPH treatment. Typically, MPH's effect on glucose metabolism is attributed to activation of D2-R's; however, the cerebellum does not contain D2-R's. It is postulated that the cerebellar increases in glucose metabolism are due to activity on NE (Volkow et al., 1998a; Leonard et al., 2004.)

MPH has been shown to indirectly increase levels of ACh in the prefrontal cortex via stimulation of the D1-R's (Acquas & Fibiger, 1996; Leonard et al., 2004). In general, ACh levels are increased by D1-like receptor activation, whereas D2-R activation decreases ACh release (Berlanga, Simpson, & Alcantara, 2005). Cholinergic interneurons in the striatum express both D5 and D2 receptors. These interneurons are important in associative learning as well as planning and executing movement. It has also been reported that the D1-like and D2-like receptors can have a synergistic effect that is linked to synaptic plasticity and learning (Kashihara, Ishihara, Akiyama, & Abe, 1999; Silkis, 2001).

Regional Brain Glucose Metabolism .

Volkow and colleagues (1998a), used [^{11}C] raclopride and 2-deoxyglucose (2DG) to measure D2-R density and brain metabolism respectively. It was reported that regional glucose metabolism was differentially affected by the density of D2-R's. Regions such as the frontal and temporal cortices showed elevated metabolism if the individual had higher levels of D2-R's whereas individuals with low levels of D2-R's had lower levels of metabolism. These findings indicate that the effect MPH has on the brain depends in part on the state of the dopaminergic system (Leonard et al., 2004). In a separate study by Volkow, Fowler, Ding, et al., (1998) glucose metabolism was compared to baseline levels following either one or two intravenous injections of MPH. The single dose of MPH significantly reduced glucose metabolism as measured by 2DG. Metabolic activity was attenuated in the hippocampus as well as in the frontal, parietal, and occipital cortices. Following the second injection of MPH these same brain regions expressed

increased levels of glucose metabolism above baseline values (Volkow, Fowler, Ding, et al., 1998).

Regional Cerebral Blood Flow

The effects of MPH on regional cerebral blood flow (rCBF) have been investigated in individuals who do not have ADHD (Mehta et al., 2000), those who have been diagnosed with ADHD and are treated with MPH (Schweitzer, Lee, Hanford, Tagamets, Hoffman, et al., 2003; Schweitzer, Lee, Hanford, Zink, Ely, et al., 2004), and individual's who have been diagnosed with ADHD and have not received treatment (Lee, Kim, Kang, Lee, Kim, et al., 2005; Kim, Lee, Cho, & Lee, 2001). The reported effects of rCBF following treatment with MPH are varied. Several studies have indicated that rCBF is altered in individuals diagnosed with ADHD (Kim et al., 2001; Spalletta, Pasini, Pau, Guido, Menghini, et al., 2001). It is speculated that MPH treatment normalizes blood flow to these regions. This is supported by reports that indicate MPH acts on brain regions that are task-specific (Mehta et al., 2000). In contrast, reports by Schweitzer and colleagues (2004) indicate that MPH does not normalize task-related activity in ADHD individuals. They hypothesize that improved performance on cognitive tasks is due to enhanced filtering of non-relevant stimuli via MPH's action on DA release in the prefrontal cortex (Schweitzer et al., 2004).

Most studies have observed reductions in rCBF the prefrontal cortex (Lee et al., 2005; Mehta et al., 2000; Schweitzer et al., 2003 & 2004), parietal cortex (Lee et al., 2005; Mehta et al., 2000; Szobot, Ketzer, Cunha, Parente, Langleben, et al., 2003), and motor cortex (Mehta et al., 2000; Schweitzer et al., 2004) following treatment with MPH.

However, it was reported by Kim et al., (2001) that rCBF in the prefrontal cortex increased following treatment with MPH and there was no change in blood flow to the parietal, occipital, temporal, or cerebellar areas. Increases in rCBF have been reported in the thalamic nuclei (Kim et al., 2001; Schweitzer et al., 2004) and basal ganglia (Kim et al., 2001; Lou, Henriksen, & Bruhn, 1984).

Endocrine Function

MPH administration decreases prolactin release via activation of the hypothalamic-pituitary pathways and increases the secretion of growth hormones. There have been conflicting reports regarding what, if any, effects MPH has on cortisol levels. According to Brown (1977) MPH administration does not increase cortisol levels. Alternatively, Joyce and colleagues did observe increases in cortisol levels (Joyce, Donald, Nicholls, Livesey, & Abbott, 1986) ACTH levels were also increased following administration of MPH. Significant increases in both systolic and diastolic blood pressure (BP) were found following MPH administration. The changes observed in BP as well as heart rate were varied across individuals (Volkow, Fowler, Ding, et al., 1998).

Immediate Early Gene Expression

It has been shown that MPH can effect the expression of the immediate early genes (IEG) c-*fos* and *zif268*. These IEG's were significantly upregulated following a single 10mg/kg dose of MPH to 35 day-old rats 30 min prior to radiograph localization. In contrast, daily treatment with 2mg/kg, 5mg/kg, or 10mg/kg of MPH for 8 days was shown to significantly attenuate expression of c-*fos* and *zif268*. Tissue samples were taken at 30min post-injection on the final day of treatment. Similar to these findings

Chase and colleagues (2002) found that administration of 10mg/kg MPH for 14 days significantly attenuated the expression of c-*fos* expression in the striatum (Chase, Brown, Carrey & Wilkinson, 2003).

Brandon and Steiner (2003) also analyzed levels of dynorphin, an opioid receptor agonist, in the striatum and found they were significantly elevated following seven day treatment with MPH. Increases in dynorphin expression are considered to be a neuroadaptive process related to overstimulation of D1-R's. Attenuation of c-*fos* and *zif268* is also considered a neuroadaptive process that is mediated by D1-R's (Brandon & Steiner, 2003).

In another study MPH administration was shown to have lasting effects on c-*fos* expression 14 days following treatment. Levels of c-*fos* in the striatum were significantly increased following administration of a single dose of either 2mg/kg or 10mg/kg MPH when measured 14 days later (Chase et al., 2003).

MPH-Induced Changes in Neurochemistry

Presynaptic DA-containing neurons release DA following a nerve impulse as well as in a continuous non-pulsatile manner in between nerve impulses. The tonic level of DA represents the existing amount of DA within the synapse whereas the phasic release is the amount of DA released following a nerve impulse (Grace, 1995). The DA system has several safeguards to prevent toxic build up of DA within the synapse, they are: rapid diffusion of DA from the synapse, reuptake of DA by the DAT, and inhibition of additional DA release by stimulating the DA autoreceptors on the presynaptic neuron. The amount of DA that occupies the synapse in between nerve impulses is 4nM.

Following a nerve impulse, extracellular DA levels rise to 250nM although these levels return to normal resting levels within milliseconds. This is primarily due to the rapid diffusion of DA but it is also attributed to the action of the DAT. Tonic levels of DA are elevated following administration of MPH to approximately 24nM. The increase in synaptic DA concentration causes increased activation of the presynaptic D2-R thereby reducing the impulse triggered release of DA to approximately 50nM. The change in presynaptic DA release is hypothesized to attenuate the number or function of postsynaptic DA receptors (Seeman & Madras, 2002). Postsynaptic receptor activity is believed to be regulated by the amount of difference between the tonic levels of DA compared to the phasic release of DA.

There have been no deleterious effects associated with long-term usage of MPH. However, some pre-clinical studies have indicated lasting changes in neurochemistry following treatment with MPH. When MPH was administered to adolescent rats, there were alterations in the activity of their midbrain neurons (Brandon, Martinelli, & White, 2003). Extracellular recordings were taken in four-week-old rats to determine if low doses of MPH administered in adolescence would alter DA neuronal activity in young adulthood. In this study the rats were administered 2.0mg/kg MPH i.p. for 7 days. These rats were divided into two groups. The first was a 1-3 day withdrawal group and the second was a 14-21 day withdrawal group. Measurements were made in the ventral tegmental area (VTA). Rats in the 1-3 day withdrawal group had significantly more spikes emitted in bursts and an increase in burst events. The 14-21 day withdrawal group showed a decrease in the average number of spikes emitted per burst and an increase in

the interspike intervals (Brandon et al., 2003). The study findings indicate DA levels are decreased in the VTA following short-term (sub-acute) administration of MPH. Reduced functioning in the VTA can result in deficits in social emotional processing, emotional blunting, lack of motivation, and anhedonia (UCSF website). In another study, Spronson and colleagues (2001) treated rats with 4mg/kg i.p. MPH twice daily for 4 days. This acute treatment did not produce behavioral dysfunction or long-term alteration in social interaction. However striatal sections examined on post treatment day 18 showed decreased presynaptic striatal dopamine release (Sproson, Chantrey, Hollis, Marsden, & Fonel, 2001).

Another study using sub-acute administration of MPH determined DAT density in the striatum was significantly reduced after early MPH administration in rats. Following termination of treatment with MPH, ligand-binding assay studies showed a reduction in dopamine transporter density by 25% at day 45 post treatment. This decline reached almost 50% at adulthood (day 70). This study indicates the presence of long-term changes in the central dopaminergic system following treatment with MPH during early juvenile life (Moll, Hause, Ruther, Rotherberger, & Huether, 2001).

Bolaños and colleagues treated adolescent rats with 2mg/kg MPH twice a day from postnatal day 20-35. Following treatment, the rats were left undisturbed until postnatal day 90 when behavior was assessed in relation to emotional stimuli. Rats pretreated with MPH were more sensitive to aversive stimuli as assessed by swim stress and anxiogenic challenges. Conversely, pretreated rats had less sensitivity to the naturally rewarding effects of sucrose. These rats were also less active when placed in a

novel environment compared to untreated animals, and demonstrated deficits in initiation and performance of sexual behavior. This study indicates that MPH treatment in adolescence alters DA functioning in the striatum and mesolimbic brain regions (Bolaños et al., 2003).

Study Rationale

Dose Selection

In humans, therapeutic concentrations of MPH are reached when plasma levels are between 8-10 ng/ml (Swanson & Volkow, 2002). These levels are typically reached within 1 to 1.5 hours following MPH administration. The half-life of MPH is approximately 2 to 3 hours (Volkow, Fowler, Ding, et al., 1998; Volkow, Wang, Fowler, et al., 1998; Wargin, Patrick, Kilts, Gualtieri, Ellington, et al., 1983). This is in contrast to MPH's effects in rats. The length of time plasma levels are maintained at clinically therapeutic levels in rats is significantly shorter (1.4-25 ng/ml at 15 min) compared to humans, and at 30 minutes are almost undetectable (0-4 ng/ml) (Gerasimov, Franceschi, Volkow, Gifford, Gatley, et al., 2000). In the rat a low dose of MPH (1mg/kg) administered orally results in peak plasma concentrations of 40ng/ml at 10 minutes post-administration. This level sharply drops to 15ng/ml within 5 minutes. This dose of MPH is not sufficient to produce significant elevations in DA concentrations within the brain regions known to be affected by MPH treatment.

Determining an appropriate dose level of MPH for the rat that is comparable to what is used clinically for humans is challenging. Depending on the route of administration (i.e. intravenous, intraperitoneal, oral) plasma levels can be significantly

affected. Plasma concentrations of MPH and its metabolites are often used to determine equivalent doses. However, it has been suggested by Gerasimov and colleagues (2000) that using human peak plasma concentrations to determine clinically relevant doses of MPH for the rat may not be appropriate. Differences in plasma concentrations of MPH between rats and humans is dependant upon several factors including the route of administration, volume of drug distribution, drug metabolism, and excretion rates (Wargin et al., 1983; Patrick, Ellington, & Breese, 1984; Mordenti, 1986).

Experiment 1: Chronic MPH

Hypothesis. Chronic pre-injury treatment with MPH will exacerbate cognitive deficits following experimental lateral FP injury. These deficits are related to changes in D2-R functioning or expression resulting from chronic treatment with MPH. The impact of these effects will be most evident in the chronic phase following brain trauma.

Rationale. Based on the findings from Brandon and colleagues (2003), increased release of DA following MPH treatment coincides with the acute elevation of DA levels associated with the excitotoxic phase following experimental TBI. It has also been shown that MPH treatment and experimental TBI are associated with chronic decreases in DAT functioning in the striatum and frontal cortex (Yan et al., 2002). The availability of the DAT for efficient and expeditious removal of DA is essential for cell signaling as well as maintaining dopamine homeostasis. Impaired functioning of the DAT may cause alterations in the availability of presynaptic D2-R's. Based on the hypothesis proposed by Seeman and Madras (1998), the drug induced decrease in impulse triggered release of DA caused by the activation of presynaptic D2-R's may attenuate the responsiveness of

the postsynaptic neuron. The drug induced changes in DA receptors may further impair activity of the D1-R and R2-R leading to further cognitive impairment in the chronic phase following TBI. Therefore, the working hypothesis of the present study is that chronic pre-treatment with MPH will upregulate pre-synaptic D2-R functioning and down-regulate postsynaptic DA functioning thereby exacerbating the hypofunctionality of the DA system following TBI and contribute to poor cognitive outcome.

Dose selection. Oral administration of 5mg/kg MPH is reported by Gerasimov et al., (2000) to be the upper end of clinically relevant doses. In humans, MPH is therapeutic because of its sustained effects in the CNS. Due to the shorter half-life in rats, 1.5 to 2 hours, compared to humans, 2 to 3 hours, MPH was administered via oral gavage twice daily spaced approximately 3 hours apart. Spacing the doses at lease 3 hours apart ensured that the first dose was not exerting any pharmacological effects. This adjusted the daily drug exposure of the rats so it more closely approximates clinical administration (Kuczenski & Segal, 2002).

Experiment 2: Acute MPH

Hypothesis. A bolus injection of MPH 25-30 min prior to experimental lateral FP injury will impair cognitive recovery post-injury. MPH is able to increase levels of DA in brain regions known to be vulnerable to TBI. The increased activity of DA will contribute to and exacerbate the excitotoxic neuronal cascade in the acute phase following injury. This will lead to greater deficits in the chronic phase post-injury.

Rationale. The chronic study was designed to investigate the potential effects of chronic MPH pre-treatment on DA receptors. The rationale for the acute study is to

assess what effects MPH may have in the absence of long-term receptor modulation. For the purposes of determining if an acute treatment is capable of producing a measurable effect, it is necessary to utilize a larger dose to maximize the drug treatment effects. The purpose of this study is to evaluate the effect of a bolus injection of MPH on cognitive outcome following TBI.

Dose selection. Because only one dose level was used in this study, it was necessary to maximize any effects MPH has on TBI outcome. In the rat higher doses of MPH (10mg/kg) have a longer half-life compared to moderate doses of MPH (5mg/kg) (Gerasimov et al., 2000). Based on studies of ADHD, beneficial treatment outcome requires that the drug be active over sustained periods of time in the CNS. Due to the short action of MPH in the rat a large dose with longer activity would fit best with clinical use MPH. The length of time MPH remains pharmacologically active following treatment with 10mg/kg MPH (3-4 hours) is a closer approximation of the pharmacological activity in humans (4-6 hours). The drawback of using the higher dose is the relative difference in clinically observed plasma concentrations in humans (8-10ng/ml) and plasma levels in rats at 3 hours (40ng/ml) (Wargin et al., 1983). Although, as stated in the above section on dose selection, plasma concentrations may not be the best way to determine appropriate doses (Gerasimov et al., 2000).

Methods

General Methodology

The description of the subjects below provides general information. Study specific descriptions of the subjects will be addressed under that experiment's heading. The descriptions of the surgical preparation, FP injury device and injury procedure, neurological assessment, Morris Water Maze (MWM), and statistical analysis are identical for experiment 1 and experiment 2. Any further information related to experiment 1 or 2 will be provided under those headings.

Subjects

Male Sprague-Dawley rats (Hilltop Lab Animals, Inc., Scottsdale, PA) were used in both the chronic and acute studies. The rats were individually housed in a vivarium and their environment was maintained at 22°C in a 12-h dark–light cycle. The animals were allowed free access to food and water in their home cages. All protocols for injury and use of animals followed the guidelines established in the Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services) and were approved by Virginia Commonwealth University's Institutional Animal Care and Use Committee.

Surgical Preparation

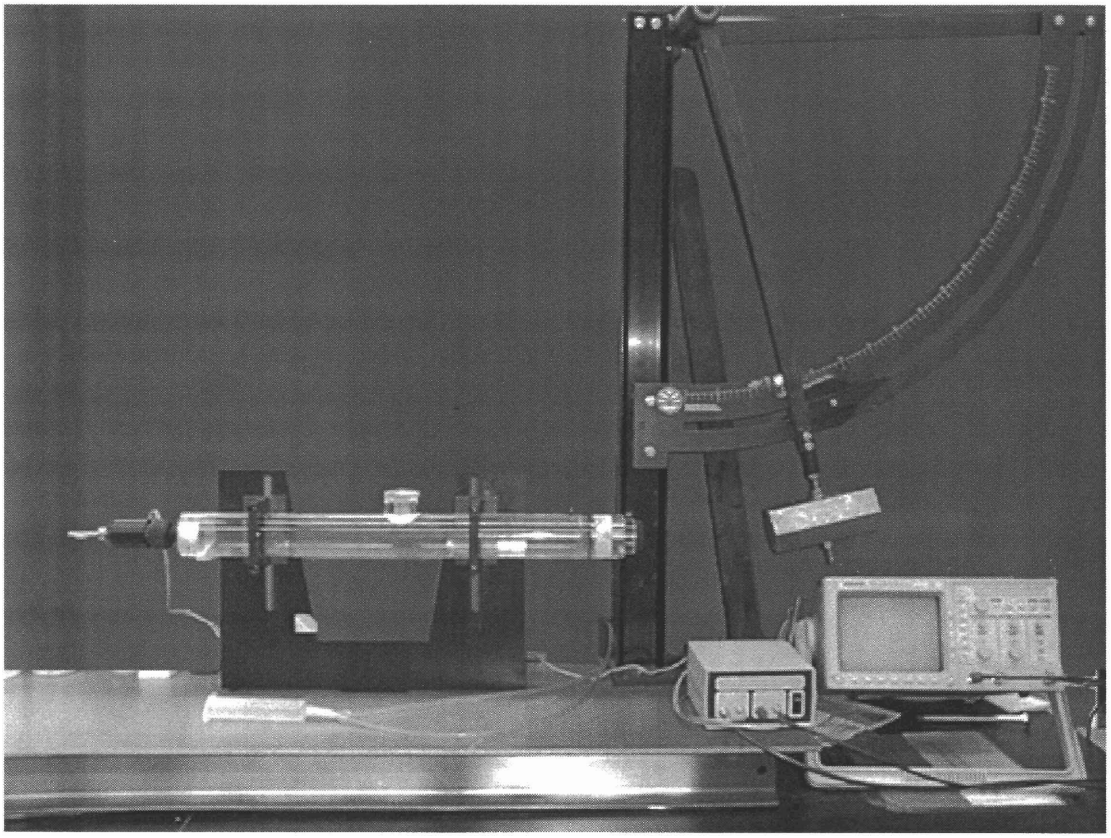
Rats were anesthetized with 4% Isoflourane with 70% N_2O: 30% O_2 mixture for 4 minutes and placed in a stereotaxic frame. The scalp was sagittally incised and a 4.8-mm-diameter lateral craniotomy was made to the right of the sagittal suture between the coronal and lambdoid sutures. Two nickel-plated screws were placed 1 mm rostral to bregma on the ipsilateral side of the craniotomy and 1 mm caudal of the lambdoid suture on the contralateral side of the craniotomy. A Leur-Loc syringe hub was secured on the skull at the site of the craniotomy with cyanoacrylate. This ensured a watertight seal that was necessary for accurate interpretation of the atmospheres of pressure generated by the FP injury device. Dental acrylic was then applied around the syringe hub and the two screws to secure the hub. The scalp was then sutured and Bacitracin was applied to the surgical site. Each subject was monitored for full recovery from anesthesia before they were returned to their home cage.

Fluid Percussion Injury Device

The FP device used to produce experimental TBI was identical to that described in detail by Dixon et al., 1987. Figure 1 shows an image of the injury device. The device consisted of a 60-cm-long and 4.5 cm diameter Plexiglas cylinder with a rubber-covered O ring-fitted Plexiglas piston at one end and, on the opposite end of the cylinder, a 2-cm-long metal housing mounted with an extracranial pressure transducer (Entran Devices, Inc., Model EPN-0300*-100A). This metal housing attaches to a 5-mm tube with a 2.6 mm inner diameter that ends with a male Leur-Loc fitting. This fitting connects with the surgically implanted female Leur-Loc fitting at the time of injury. The entire system is then filled with distilled water. The injury is produced by releasing a metal pendulum

positioned to strike the piston of the injury device. A small volume of distilled water is injected into the closed cranial cavity to produce a brief displacement and deformation of brain tissue. The magnitude of injury is controlled by varying the height from which the pendulum is released.

Figure 1. Fluid percussion injury device used to produce the moderate level lateral injury.



Fluid Percussion Injury

Twenty-four hours after surgical preparation, at the time of injury, the rats were anesthetized by breathing 4.0% isoflurane with 70% N_2O:30% O_2 mixture for 4 min. The surgical site was exposed and the animal was connected to the FP device. The force of the injury administered was between 2.0 to 2.2 atmospheres of pressure (atm), which is equivalent to a moderate-level brain injury. The atm's were recorded by the in-line transducer connected to a storage oscilloscope (Tektronix 5111; Beaverton, OR). Sham-injured controls received the same surgical preparation, anesthesia, and connection to the injury device; however, no injury was delivered. All animals were immediately ventilated with room air until spontaneous breathing was resumed.

Outcome Assessment

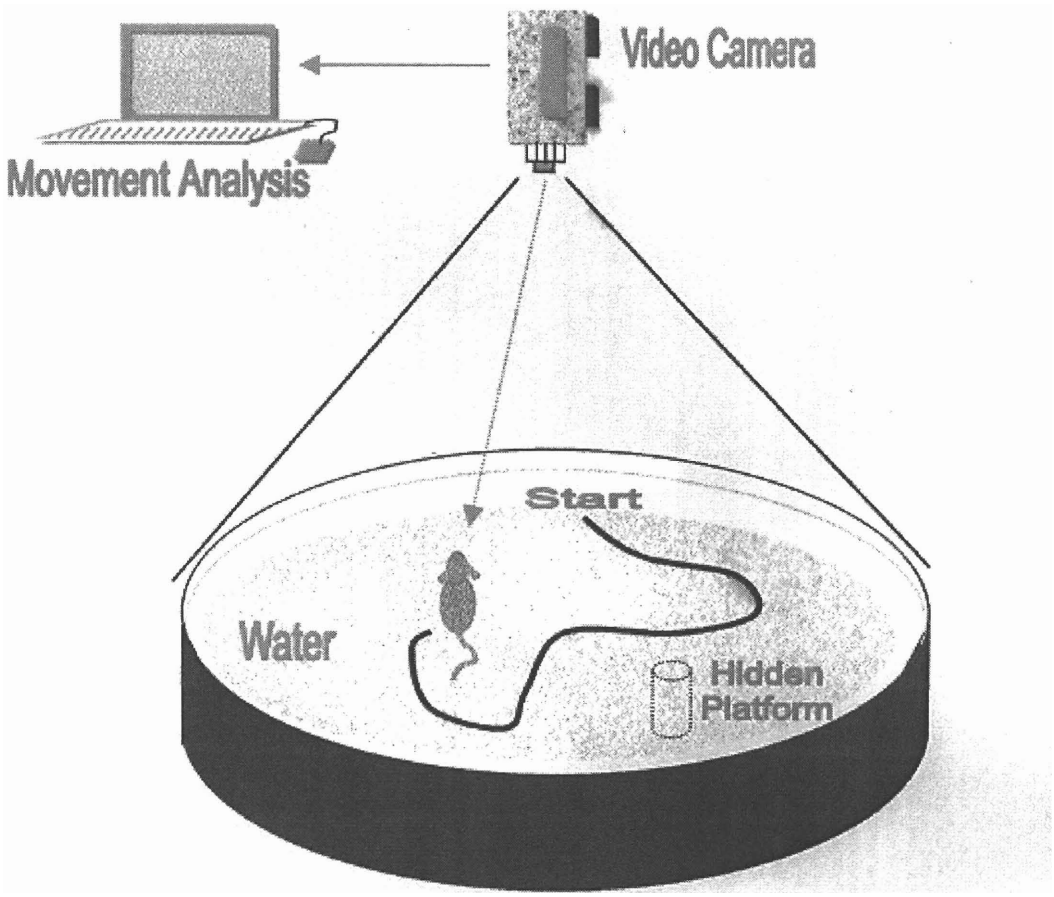
Neurological assessment. Following the injury during the period of unconsciousness, the scalp was sutured closed and neurological assessment was performed based on suppression of the rats' reflexes. The injured rats were tested for suppression of the righting reflex. Once the rat regained consciousness it was monitored for 2–3 h before being returned to its home cage.

Morris water maze. The maze is a large circular tank (180 cm diameter by 45 cm high) filled to a depth of 30 cm with thermostatically controlled warm water maintained between 25° and 28° C. Figure 2 is an image of the Morris water maze and tracking system. For assessment, rats were given four trials per day for 5 consecutive days on post-injury days (PID) 10 to 14 post-injury. For each daily block of four trials the rats were placed in the tank facing the wall at one of the four designated entry points. The order of the entry points was randomized to minimize practice effects. Each rat started

the trials once from each of the four cardinal directions (north, east, south, and west) in random order. The hidden goal platform was positioned 45 cm from the outside wall and was not moved during the experiment. Rats were given a maximum of 120 seconds to find the hidden platform. If the rat failed to find the platform within the allotted time it was placed on the platform by the experimenter. All rats remained on the platform for 30 seconds before being placed in a heated incubator between trials. The inter-trial interval for all the trials was approximately 10 minutes.

The MWM tests the ability of the rat to utilize reference memory for spatial learning and memory (Morris, Garrud, Rawlins, & O'Keefe, 1982). There are several advantages for using the MWM. It is known to be sensitive to hippocampal damage (Morris et al., 1982). The water maze is relatively quick and easy to learn for rats and food does not have to be withheld for the acquisition of this task. Maze performance was assessed on PID 10-14. This timeframe was selected because any motor deficits produced by the injury have subsided and TBI-induced cognitive deficits are larger, thus making any drug effects more apparent (Hamm et al., 1993).

Figure 2. Cartoon of the Morris Water Maze and tracking system.


Video Camera
Movement Analysis
Start
Water
Hidden
Platform

Statistical Analysis

Separate one-way ANOVA's were used to analyze swim speeds and righting times. In both the chronic and acute study the analysis of suppression of the righting reflex includes only the comparison of the two injured groups for each study. Although no formal analysis was performed on the sham groups, all animals in those groups regained the righting reflex in less than 2 min. Righting times of less than 2 minutes was significantly faster compared to the injured groups. A split-plot analysis of variance (ANOVA) was performed on goal latencies in the MWM. The within-subjects variable was the days assessed in the water maze (PID 10-14) and the between-subjects factor was treatment condition (group). A one-way ANOVA was performed for each day of testing in the MWM. The between-subjects factor in the day by day analysis was the treatment condition (group). Post-hoc analyses were made where appropriate using a Student-Newman-Keuls (S-N-K) test. All statistical analyses were performed using SPSS software, alpha = .05.

Experiment 1: Chronic MPH

Methods

Subjects. General descriptions of the rats and their environment are detailed above. Litters of male Sprague-Dawley rats were obtained with their dams on postnatal day 18. Prior to the start day for treatment (postnatal day 28) the rats were weaned and housed 2-3/cage. Animals began treatment with either saline or MPH on postnatal day 28. The age of the rats was selected to better mimic the treatment modality utilized in the clinical treatment of children diagnosed with ADHD (Kuczenski & Segal, 2002). Postnatal day 20-35 in rats developmentally approximates preadolescence in humans

(Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon, 2002a; Prins & Hovda, 1998; Prins, Lee, Cheng, Becker, & Hovda, 1996). Twenty-eight-day-old rats are comparable developmentally to elementary school aged children, which is the average developmental period that a child diagnosed with ADHD would likely begin treatment.

Drug preparation. MPH HCl was obtained through the Pharmacology & Toxicology Department at Virginia Commonwealth University as well as through U.S. Pharmacopeia (Rockville, MD). The MPH HCl was dissolved in 0.9% sterile saline. The volume of drug solution administered to the rats was 2ml/kg. The 5mg/kg dose was calculated for weight in increments of 5 grams. The concentration of the prepared solution was selected to decrease the injection volume in the young rats.

Chronic MPH pre-treatment. Prior to the drug administration the rats were sedated under gas anesthesia (4% Isoflurane) in a mixture of 70% N_2O, and 30% O_2 for 1-2 minutes. This was done to minimize discomfort and prevent injury to the rats caused by contraction of esophageal and abdominal muscles around the gavage needle; this was a particular concern in the younger (smaller) rats. Methylphenidate HCl (5mg/kg) dissolved in 9% saline or equivalent dose of 9% saline was delivered via an 18-guage oral gavage passed down the esophagus into the stomach. Treatment with MPH began on postnatal day 28. Male Sprague-Dawley rats were initially randomly assigned to either vehicle (saline) or drug treated (MPH 5mg/kg) groups. All rats were treated chronically for 30 days twice a day with MPH or saline. A minimum delay of 3 hours and a maximal delay of 6 hours were maintained between the first and second doses each day. The minimum delay protected against compounding effects of the treatments. The maximal delay was established to more closely match clinical treatments.

Surgical preparation. On treatment-day 29 all rats in the chronic study underwent the surgical preparation outlined in the general methodology section above. Treatments administered on this day were scheduled such that there was a 3-hour delay between the first treatment dose and initiation of the pre-injury surgical preparation. Following surgical preparation (average length of surgery was approximately 30min) the rat was allowed to recover for 2 hours before administration of the second treatment dose.

Injury. Upon completion of the 30-day pre-injury dosing regimen rats were randomly assigned to one of 4 treatment groups: MPH + injury, MPH + sham-injury, saline + injury or saline + sham-injury. At the time of injury the rats were 58-days old. Rats assigned to one of the two injury groups received a moderate level lateral FP injury. Rats assigned to either of the sham-injury groups received identical treatment except no injury was delivered. The final dose of MPH was administered at least 3 hours prior to lateral FP or sham injury. The delay between drug administration and time of injury allowed the MPH to be systemically cleared.

Experiment 2: Acute Study

Methods

Subjects. Adult (3-month old) male Sprague-Dawley rats weighing 290-330g were used in this experiment. Information regarding housing and environmental conditions is located in the general methodology section.

Drug preparation. MPH HCl was obtained through the Medical College of Virginia's Pharmacology & Toxicology Department at Virginia Commonwealth University as well as through U.S. Pharmacopeia (Rockville, MD). The MPH HCl was mixed with 0.9% sterile saline in and administered in a volume of 2ml/kg. The same

dosing chart calculated for use in the chronic study (5mg/kg) was also used to calculate the required dose in the acute study (10mg/kg). Drug injection volumes determined by the individual weight of each rat were doubled to attain the 10mg/kg dose. Due to the larger size of the rats and the dose regimen of a single bolus injection, there was no need to make the drug more concentrated as was necessary for the chronic study.

Acute MPH pre-treatment. Prior to the drug administration the rats were sedated under gas anesthesia (4% Isoflurane) in a mixture of 70% N_2O, and 30% O_2 for 1-2 minutes. This was done to minimize discomfort and prevent injury to the rats caused by contraction of esophageal and abdominal muscles around the gavage needle. A bolus injection of MPH HCl (10mg/kg) dissolved in 0.9% sterile saline or equivalent dose of 0.9% sterile saline was delivered via oral gavage passed down the esophagus into the stomach. The rats were randomly assigned to one of 4 treatment conditions: MPH + injury, MPH + sham-injury, saline + injury, or saline + sham-injury. MPH or saline was administered approximately 25-30 minutes prior to sham or lateral FP injury. The selected time interval allowed peak plasma levels to coincide with the time of injury thereby maximizing the effects of MPH treatment on TBI outcome.

Results

Experiment 1: Chronic Study

Outcome Measures

Neurological assessment. Figure 3 shows the mean suppression of the righting reflex (in minutes) following lateral FP injury. A one-way ANOVA was used to determine if there were differences in the suppression of the righting reflex between the two injured groups (MPH + injury and saline + injury). There were no significant differences in the righting times of these groups $F(1,21) = .909$, $p > .05$. This indicates that the injury severity was comparable for both injured groups.

Swim speed analysis. Figure 4 shows the mean swim speeds (cm/sec), averaged across days, for each treatment group. A one-way ANOVA indicated there was no significant effect of treatment group on average swim speed in the MWM, $F(3,45) = .426$, $p > .05$). This indicates that all the groups swam at similar speeds during the assessments in the MWM.

Cognitive assessment. Figure 5 shows the mean latency (in seconds) to reach the goal platform in the MWM on PID 10-14, for all groups. A 4 (Group) X 5 (Day) split-plot analysis of variance (ANOVA) indicated there was a significant effect of treatment condition on MWM performance, $F(3,45) = 8.313$ $p < .001$. A S-N-K post hoc analysis was performed to assess specific group differences. The results indicated that there was a significant difference between the injured and sham-injured groups ($p < .05$). No

significant differences were detected between the MPH + injury and saline + injury groups ($p > .05$). No significant difference was found between the MPH + sham-injury and saline + sham-injury groups ($p > .05$).

Figure 6 shows the day by day comparison of MWM performance by treatment group. Analysis of the mean latency to reach the goal platform for each day was calculated using a one-way ANOVA with the treatment group as the independent variable. The ANOVA revealed a significant difference in the day by day analysis for each treatment group. For each day there was a significant effect of group Day 1, $F(3,45) = 4.855$, $p < .001$; Day 2, $F(3,45) = 7.804$, $p < .001$; Day 3, $F(3,45) = 4.921$, $p < .01$; Day 4, $F(3,45) = 4.834$, $p < .01$; Day 5 $F(3,45) = 3.784$, $p < .05$. A S-N-K analysis confirmed the results from the group by day split-plot ANOVA reported above. The only significant differences were found between the injured and sham-injured groups ($p < .05$).

Figure 3. Chronic Study: Analysis of Injured Groups Righting Reflex. Comparison of chronic pre-treated injured groups showed there was no significant effect of lateral FP injury on suppression of the righting reflex ($p > .05$). The vertical bars represent the two injured groups and the vertical lines represent standard error of the mean.

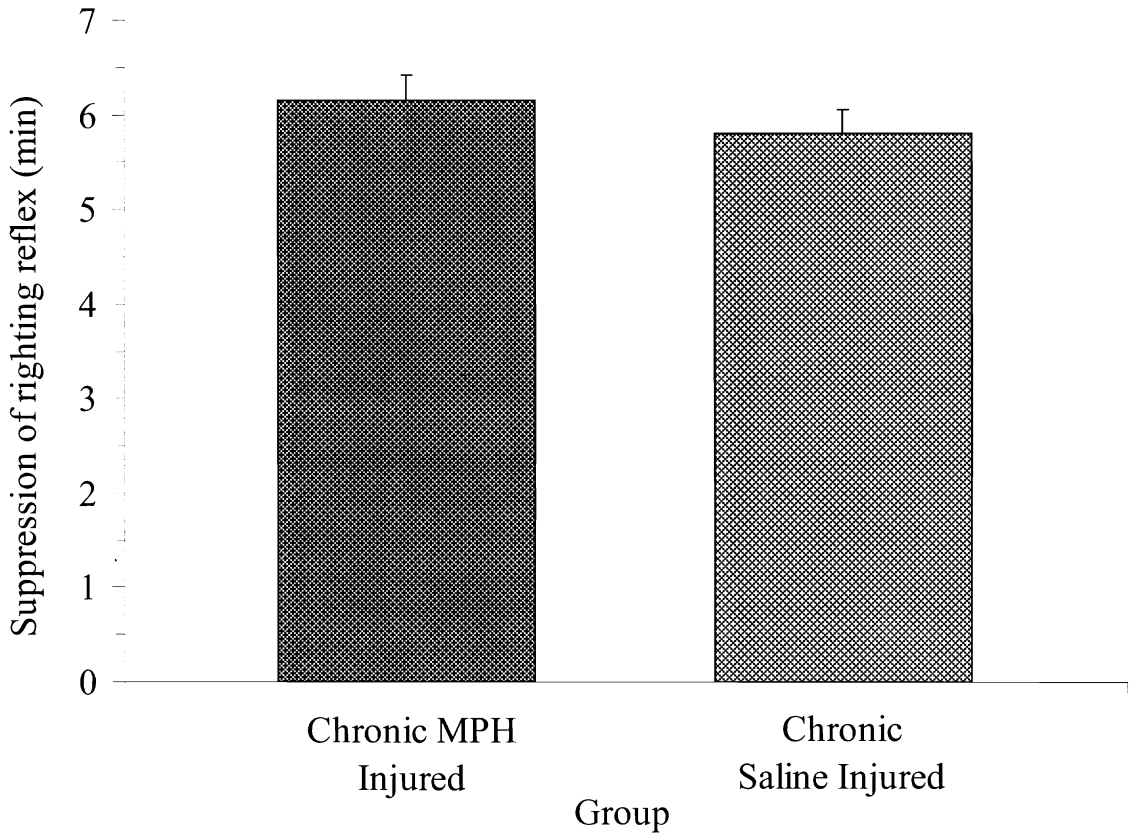

Suppression of righting reflex (min)
7
6
5
4
3
2
1
0
Chronic MPH Injured
Chronic Saline Injured
Group

Figure 4. Chronic Study: Mean Swim Speed Analysis. There was no significant effect of pre-injury treatment on swim speed ($p > .05$). The vertical bars represent the four treatment conditions and the vertical lines represent the standard error of the mean.

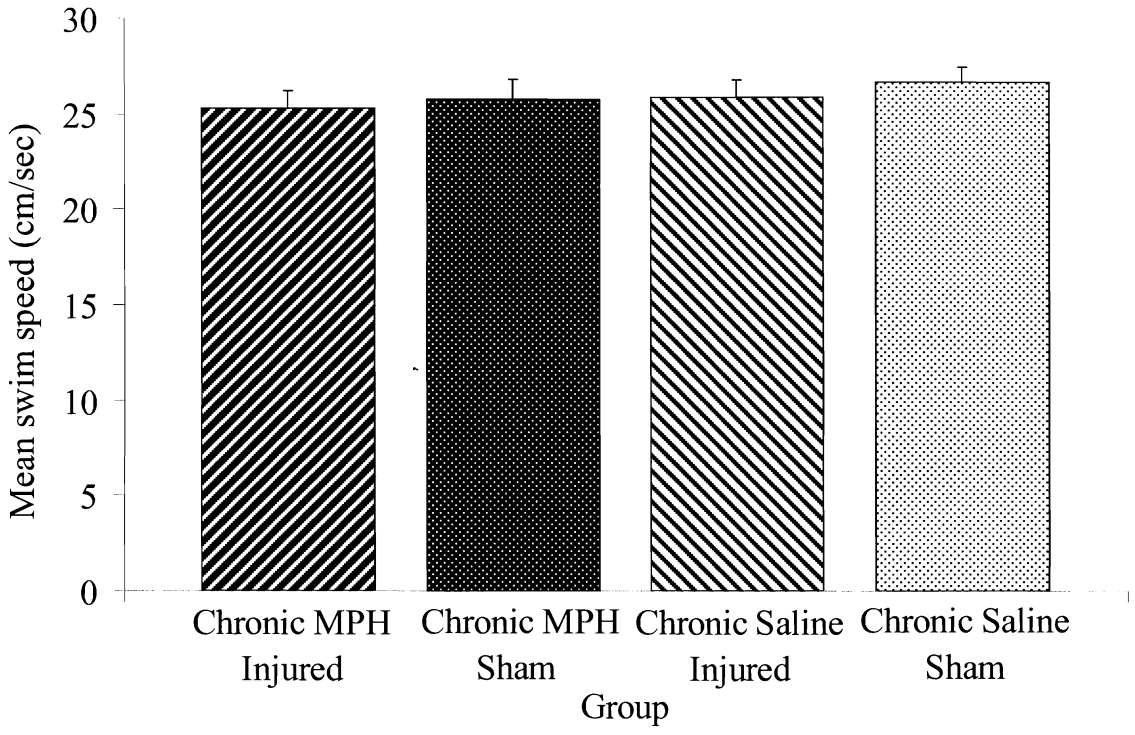

30
25
20
15
10
5
0
Mean swim speed (cm/sec)
Chronic MPH Injured
Chronic MPH Sham
Chronic Saline Injured
Chronic Saline Sham
Group

Figure 5. Chronic Study: MWM Latency. Separate horizontal lines represent the different treatment conditions. There was a significant difference between the latencies of the sham and injured groups ($p < .05$). There was no significant difference between the chronic MPH and chronic saline injured groups ($p < .05$). There was no significant difference between the chronic MPH and chronic saline sham-injured groups ($p > .05$). The vertical lines represent the standard error of the mean.

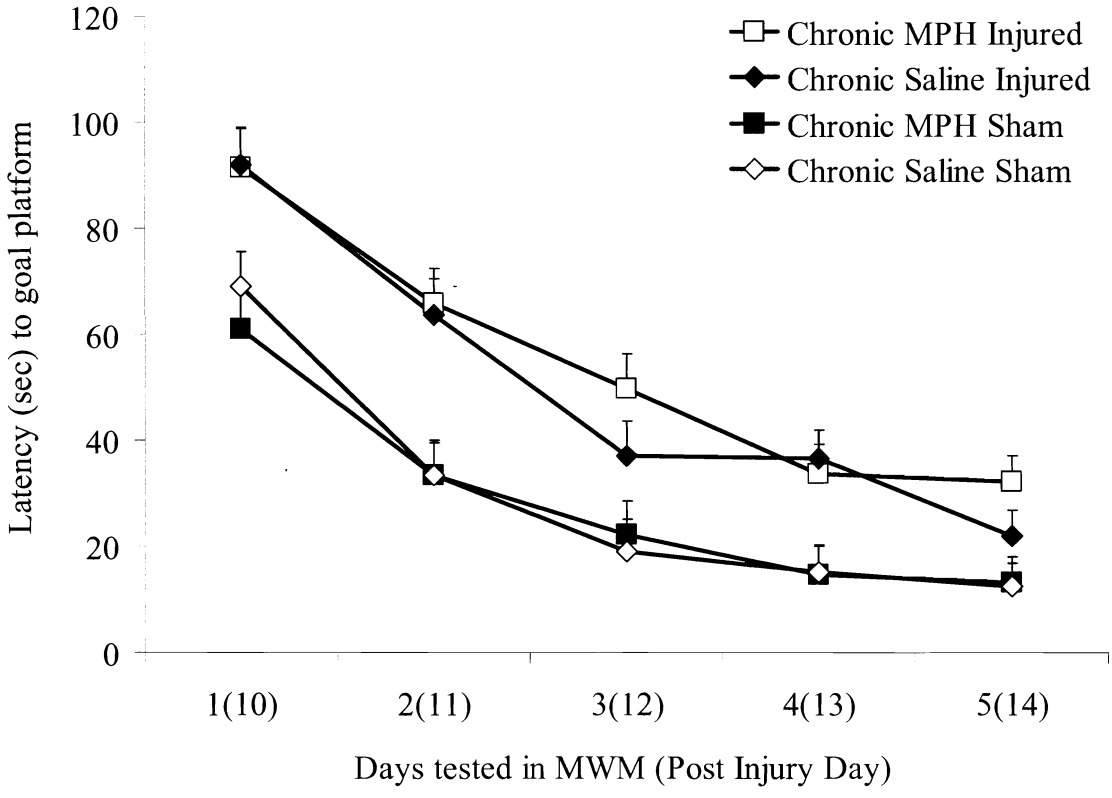
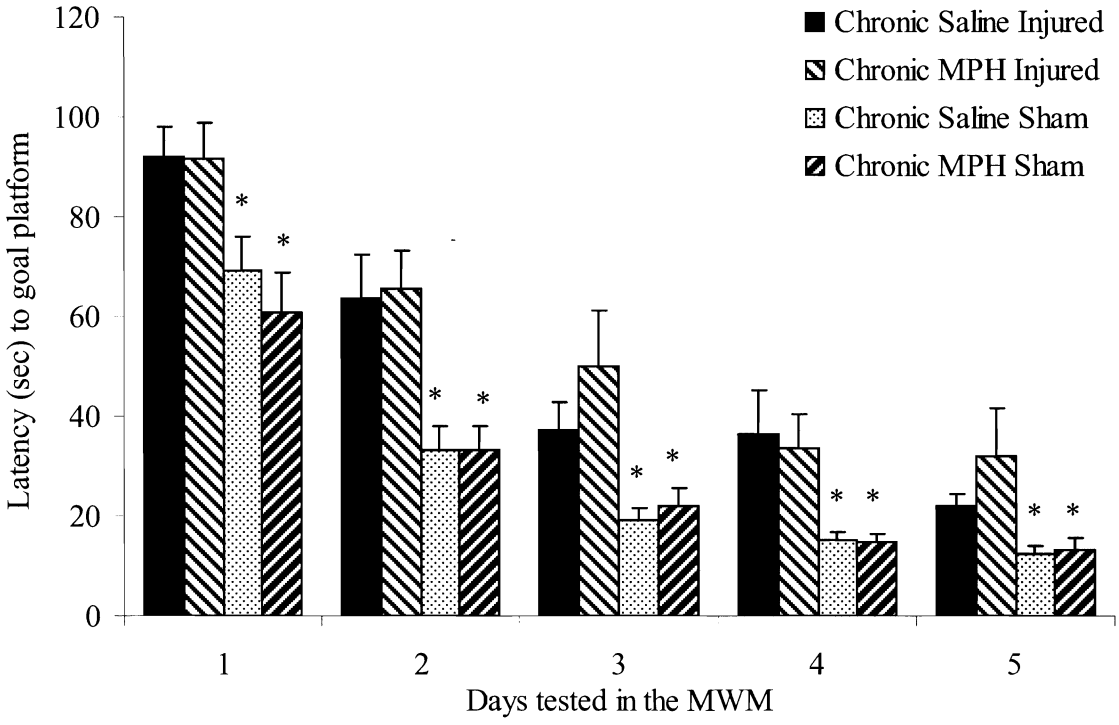

120
100
80
60
40
20
0
Latency (sec) to goal platform
Chronic MPH Injured
Chronic Saline Injured
Chronic MPH Sham
Chronic Saline Sham
1(10)
2(11)
3(12)
4(13)
5(14)
Days tested in MWM (Post Injury Day)

Figure 6. Chronic Study: Day by Day Analysis of Mean Latencies in the MWM. The vertical bars represent the different treatment conditions; vertical lines represent the standard error of the mean. Significant differences between the groups are marked with a star.


Chronic Saline Injured
Chronic MPH Injured
Chronic Saline Sham
Chronic MPH Sham
Latency (sec) to goal platform
120
100
80
60
40
20
0
1
2
3
4
5
Days tested in the MWM

Experiment 2: Acute Study

Outcome Measures

Neurological assessment. Figure 7 shows the mean righting reflex of the injured groups (in minutes) following lateral FP injury. A one-way ANOVA was used to assess differences in suppression of the righting reflex between the two injured groups (MPH + injury and saline + injury) following lateral FP injury. No significant effect was observed between the groups $F(1,21) = .376$, $p > .05$. This indicates there was no effect of acute pre-injury treatment on injury severity.

Swim speed analysis. Figure 8 shows the mean swim speed (cm/sec), calculated for each treatment group, across days in the MWM. A one-way ANOVA indicates there was no significant difference in the swim speeds across groups $F(3,29) = 1.351$, $p > .05$. This indicates that all the groups swam at similar speeds during the assessments in the MWM.

Cognitive assessment. Figure 9 shows the mean latency (in seconds) to reach the goal platform in the MWM on PID 10-14. A 4 (Group) X 5(Day) split-plot ANOVA indicated there was a significant effect for group $F(3,35) = 11.24$, $p < .001$. A S-N-K post hoc analysis was performed to assess specific group differences. There were no significant differences found between the MPH + sham-injury and saline + sham-injury groups ($p > .05$). The MPH + injury group had significantly shorter latencies to the goal platform in the MWM compared to the saline + injury group ($p < .05$). However, the MPH + injury group did not improve to sham levels and had significantly longer latencies to reach the goal platform ($p < .05$). The saline + injury group had the poorest performance in the MWM and was statistically different from all other treatment groups

($p < .05$). This indicates that acute pre-injury treatment with MPH offers some cognitive protection.

Figure 10 shows the day by day analysis of MWM latencies (in seconds) for each treatment group. Analysis of the mean latency to reach the goal platform for each day was calculated using a one-way ANOVA with the treatment group as the independent variable. The ANOVA revealed a significant difference in the day by day analysis for each treatment group. For each day there was a significant effect of group Day 1, $F(3,38) = 4.147$, $p < .05$; Day 2, $F(3,38) = 5.175$, $p < .01$; Day 3, $F(3,38) = 7.169$, $p < .01$; Day 4, $F(3,38) = 5.635$, $p < .01$; Day 5, $F(3,38) = 10.335$, $p < .001$. A S-N-K analysis indicated that on days 4 and 5 in the MWM (PID 13 and 14) that rats in the MPH treated + injured group reached sham levels of performance based on latencies to the goal platform. On days assessment days 1 though 3 in the MWM the only significant differences in latencies to the goal platform were found between the injured and sham-injured groups ($p < .05$).

Figure 7. Acute Study: Suppression of Righting Reflex. This figure shows the effect of lateral fluid percussion injury on suppression of the righting reflex. Pre-treatment with MPH did not affect the righting times of the injured groups ($p > .05$).

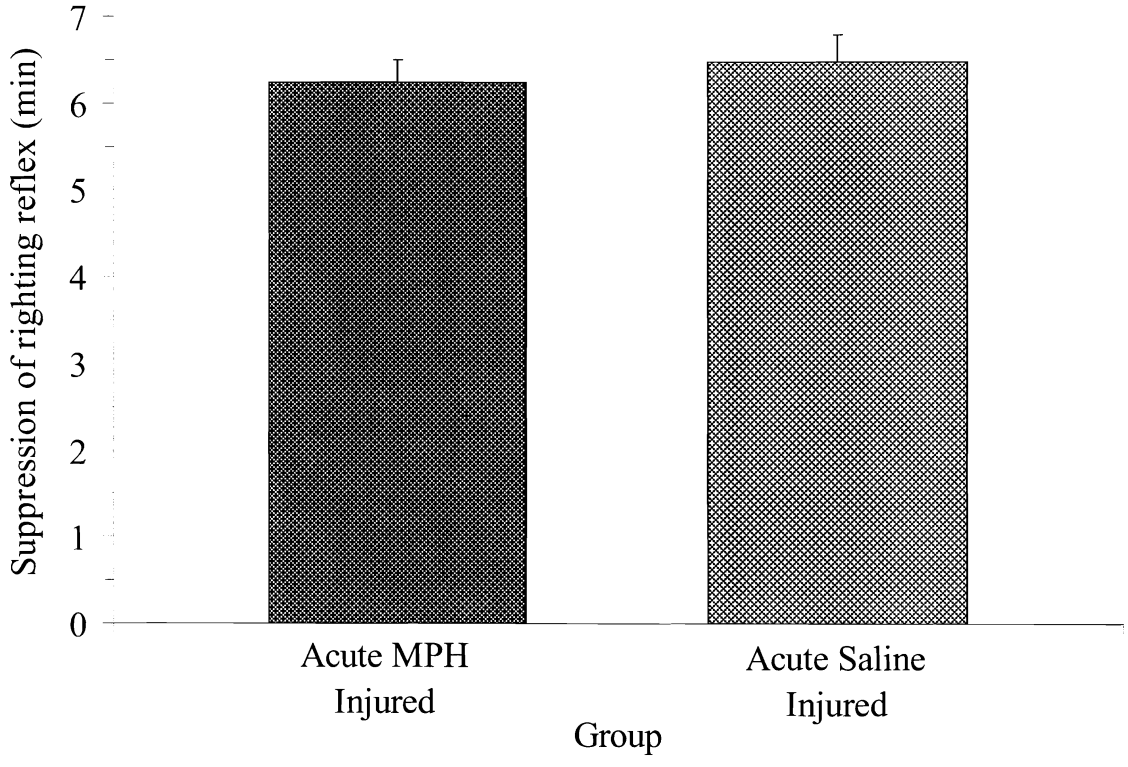

Suppression of righting reflex (min)
7
6
5
4
3
2
1
0
Acute MPH Injured
Acute Saline Injured
Group

Figure 8. Acute Study: Mean Swim Speed Analysis. Pre-injury treatment with MPH had no significant effect on swim speed in the Morris water maze ($p > .05$).

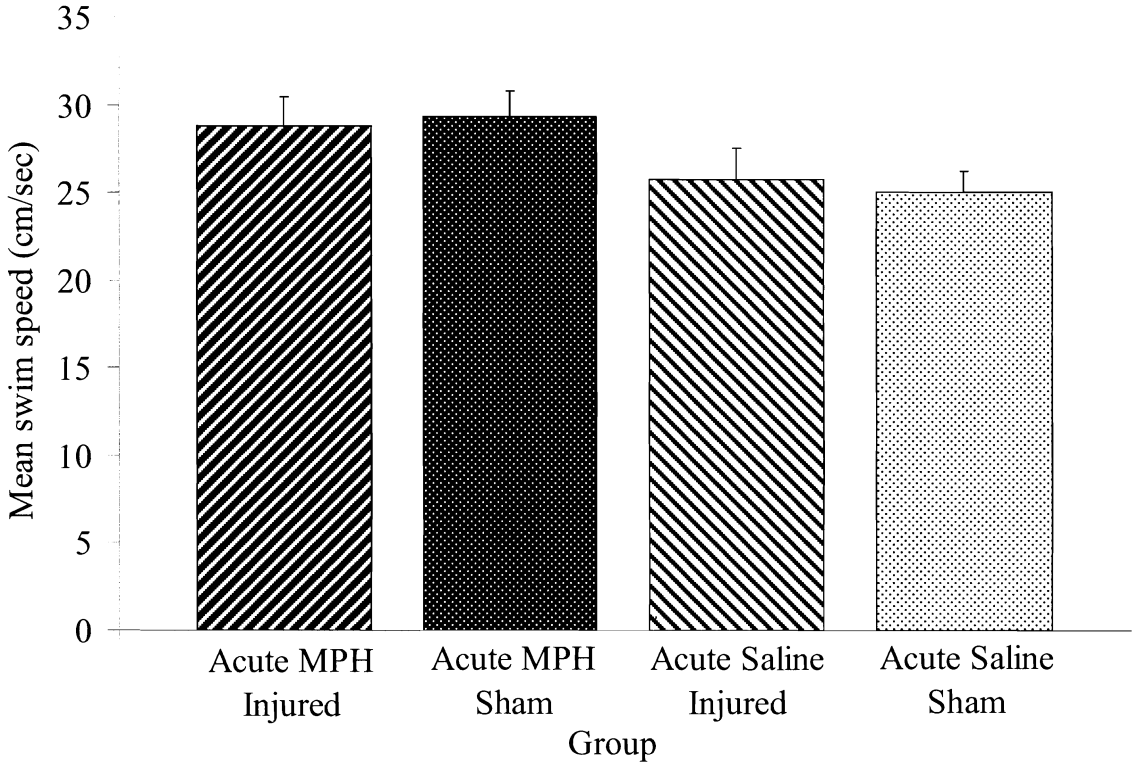

35
30
25
20
15
10
5
0
Mean swim speed (cm/sec)
Acute MPH Injured
Acute MPH Sham
Acute Saline Injured
Acute Saline Sham
Group

Figure 9. Acute Study: MWM Latency. Separate horizontal lines represent the four different treatment conditions. There was a significant difference between the latencies of the sham and injured groups ($p < .05$). There was significant difference between the acute MPH and acute saline injured groups ($p < .05$). No significant difference was observed between the two sham-injured groups ($p > .05$). The vertical lines represent the standard error of the mean.

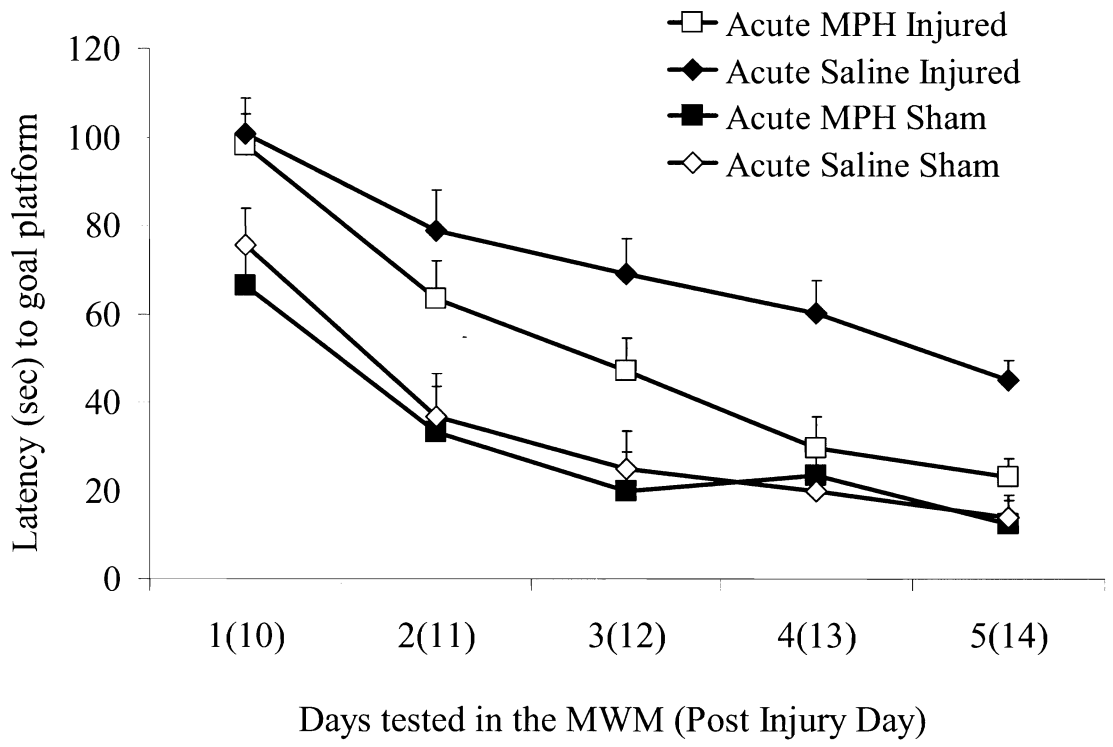
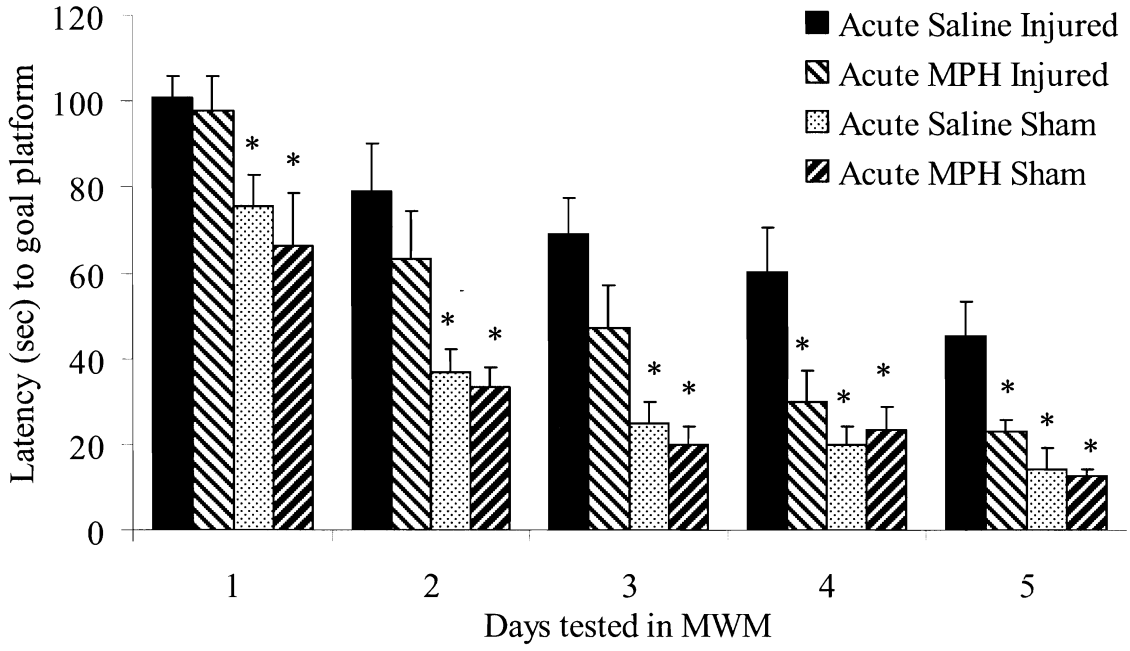

Acute MPH Injured
Acute Saline Injured
Acute MPH Sham
Acute Saline Sham
120
100
80
60
40
20
0
Latency (sec) to goal platform
1(10)
2(11)
3(12)
4(13)
5(14)
Days tested in the MWM (Post Injury Day)

Figure 10. Acute Study: Day by Day Analysis of Mean Latencies in the MWM. The vertical bars represent the different treatment conditions; vertical lines represent the standard error of the mean. Significant differences between the groups are marked with a star.


120
100
80
60
40
20
0
Latency (sec) to goal platform
Acute Saline Injured
Acute MPH Injured
Acute Saline Sham
Acute MPH Sham
1
2
3
4
5
Days tested in MWM

Discussion

Experiment 1: Chronic Study

Effects of Chronic Pre-Injury MPH Treatment

The results of the chronic study indicate that prolonged treatment with MPH had no effect on any of the outcome measures. Analysis of the suppression of the righting reflex indicated that chronic pre-injury treatment with either MPH or saline had no effect on injury severity. Analysis of the swim speeds showed there was no significant difference between any of the groups. This finding is supported by the MWM data. No significant differences were found in MWM performance between the two injured groups (MPH + injury and saline + injury). Similarly, no significant differences were found between the two sham-injured groups (MPH + sham-injury and saline + sham-injury). The only significant difference observed was between the injured and sham-injured groups. This finding was supported by the day by day analysis. These results indicate that the cognitive deficits, assessed by MWM performance, were due to injury effects; not pre-injury treatment with MPH. These findings do not support the main hypothesis that chronic pre-injury treatment with MPH would exacerbate cognitive deficits following TBI.

Clinical Implications

There have been no studies, clinical or pre-clinical, to date that have evaluated the potential interaction between chronic treatment with stimulant medication and cognitive

recovery following TBI. The current study used a rodent model to evaluate any consequences of chronic MPH administration on recovery of cognitive functioning following experimental TBI. It was shown that chronic pre-injury administration of MPH had no impact on cognitive outcome measures. This was found in both the injured and sham-injured groups. These data do indicate that chronic MPH treatment in humans is unlikely to impact cognitive outcome after TBI.

Experiment 2: Acute Study

Effects of Acute Pre-Injury MPH Treatment

There was no significant difference in suppression of the righting reflex between the MPH and saline injured rats; meaning the injury severity was comparable in both groups. This indicates that the improvements in cognitive recovery were not related to differences in the severity of TBI. Analysis of swim speed across days for all the groups did not show any significant differences in the average swim speeds. This indicates that the latency to locate the goal platform was not affected by motor impairments. Similar swim speeds for all the groups also indicates that the amount of time required to locate the goal platform is due to learning rather than accidentally locating the platform due to faster exploration of the maze.

Lateral FP injury, following either 10mg/kg MPH or saline, significantly impaired overall performance in the MWM. However, contrary to what was anticipated, rats that received pre-injury treatment with MPH performed significantly better than rats receiving pre-injury treatment with saline. Despite the improvement over the saline + injury group, the MPH + injury group did not perform as well as either of the treatment + sham-injury groups.

The day by day analysis indicated that rats in the MPH + injury group did not perform at sham levels for the first three days of testing in the MWM. It was not until the fourth and fifth days of testing that the rats in the MPH + injury group reached sham levels of performance. This indicates that the rats in the MPH + injury group did not learn the maze task as quickly as the treatment + sham-injury groups however, by day four in the MWM they are performing at sham levels.

Results from this study did not support the hypothesis that acute pre-injury treatment with MPH would exacerbate cognitive deficits following moderate level FP injury. It was anticipated that the stimulant properties of MPH would facilitate trauma-induced neural excitotoxicity in the acute phase following brain injury. However, cognitive performance was improved in the MPH + injury group as compared to the saline + injury group. This finding indicates that a high dose of MPH administered prior to traumatic brain injury offers some neuroprotection. This is presumed to occur via pathways involved in both MPH treatment and injury. Based on what is known about the action of MPH in the brain and the processes and pathways that are involved in the sequelae of TBI there are several potential explanations for the observed effects.

Potential Mechanisms for Acute Treatment Benefits

One explanation for the improvement in MWM performance by the MPH treated injured group could be attributed to vasopressor effects related to MPH's ability to increase DA levels in the brain. FP injury has been shown to produce transient alterations in cerebral blood flow. It was observed that within seconds of administering a moderate severity level lateral FP injury there was a brief period of hypertension (Muir, Boerschel, & Ellis, 1992). When measurements were taken again at 5 minutes post-

injury there was a significant decrease in blood flow that persisted for at least 60 minutes post-injury (Long, Gordon, Bettencourt, & Bolt, 1996; Muir et al., 1992).

There are varied reports from studies that have examined the effects of MPH on cerebral blood flow (Kim et al., 2001; Mehta et al., 2000; Schweitzer et al., 2004; Szobot et al., 2003). These studies with the exception of Schweitzer et al., (2004) showed that MPH treatment reduced rCBF. In the article by Muir et al., (1992) it is speculated that the reduction in blood flow following TBI may be due to the initial acute hypertensive event. Using a laser-Doppler flowmetry, Muir and colleagues showed that after a moderate-severity FP injury there was a transient 225% increase in parietal cortex blood flow. The change in parietal blood flow occurred seconds after mABP was increased to 294%. It is reported that this may be one of the initial insults on the cerebral vasculature (Muir et al., 1992). It is possible that reducing blood flow immediately prior to the hypertensive period may lessen some of the damage to the cerebral vasculature.

A second potential explanation for the improvement in cognitive performance could be attributed to MPH's effects on glucose metabolism in the brain (Volkow et al., 1998a & (Volkow, Fowler, Ding, et al., 1998); Kim et al., 2001; Lou et al., 1984). Glucose levels are also affected by injury. Clinical reports and experimental models both report elevated cerebral metabolism within the first 30 minutes post-injury (Long et al., 1996; Povlishock & Katz, 2005). The observed hypermetabolism is likely the result of impaired ionic homeostasis and mitochondrial dysfunction and is reported to be indicative of an energy crisis/metabolic dysfunction (Povlishock & Katz, 2005; Vespa, McArthur, Alger, O'Phelan, Hattori et al., 2004). Brain metabolism as measured by

glucose levels are initially elevated after TBI but are then followed by persistent reductions in brain metabolism. This is the basic premise of the biphasic hypothesis.

In the study by Volkow, Fowler, Ding, et al., (1998) a single dose of MPH was administered to determine the effect of MPH on glucose metabolism in the brain. It was found that a single dose of MPH decreased metabolism in the frontal, parietal, and occipital cortices and in the hippocampus (Volkow, Fowler, Ding, et al., 1998). TBI is known to disrupt glucose metabolism in the brain. In the acute phase, immediately following injury, rapid elevation of glucose levels has been linked to metabolic dysfunction that can ultimately lead to cell death (Povlishock & Katz, 2005). The ability of a single dose of MPH to cause short-term decreases in brain metabolism could contribute to improved cognitive outcome.

Another possible explanation for the cognitive improvements could be attributed to similar actions caused by other DA agonists. DA receptor agonists specifically D2-R agonists such as bromocriptine, cabergoline, pergolide, DEHCP, and ropinirole were found to be neuroprotective when administered for seven days prior to hypobaric hypoxic injury (Micale et al., 2006). Additionally, bromocriptine administered 15-minutes prior to injury was also beneficial in reducing cognitive deficits (Kline et al., 2004). It is believed that the beneficial effects of these drugs are due to their antioxidant effects. It is suggested that treatment with dopaminergic drugs is beneficial because these drugs are known to increase the reduction/oxidation ratio within brain tissue following an injury (Micale et al., 2006). It is suggested that the activity of the ergot-derived drugs as well as ropinirole increase the amount of circulating antioxidants in the injured brain via their action at D2-R's (Medico et al., 2002; Micale et al., 2006). Although, MPH does not

solely activate D2-R's, it is possible that MPH-induced increases in DA could increase cerebral anti-oxidative enzymatic activity. The increased levels of antioxidants might be able to prevent damage caused by injury-induced elevations in free radicals.

Future Studies

Further research is needed to determine what mechanism or action of MPH is involved in acute TBI pathology. Based on the findings from the acute study, future studies are needed to determine if therapeutic doses of MPH have similar effects. Although the chronic study did not yield significant results there is room for further study. The current studies employed a single treatment dose. Future research could evaluate other dose levels of MPH in relation to TBI. Another area that warrants further examination is the dosing pattern of the chronic study. In the chronic study the final dose of MPH was administered at least 3-hours prior to injury. Based on the positive results obtained from the acute study, it would be interesting to determine if there is any benefit to administering the final dose of MPH in the chronic study 25-30 minutes prior to the injury. Lastly, other ADHD medications should be evaluated in relation to the effects they may have on TBI recovery.

List of References

References

Acquas, E., & Fibiger, H. C. (1996). Chronic lithium attenuates dopamine D1 receptor increases in acetylcholine release in rat frontal cortex. *Psychopharmachology*, 125, 162-167.

Adams, J. H., Graham, D. I., Murray, L. S., & Scott, G. (1982). Diffuse axonal injury due to nonmissile head injury in humans: an analysis of 45 cases. *Annals of Neurology, 12,* 557-563.

Adams, J. H., Doyle, D., Graham, D. I., Lawrence, A. E., & McLellan, D. R. (1986). Gliding contusions in nonmissile head injury in humans. *Archives of Pathology & Laboratory Medicine, 110,* 485-488.

Amen, D. G. & Carmichael, B. D. (1997). High-resolution brain SPECT imaging in ADHD. *Annals of Clinical Psychiatry, 9,* 81-86.

American Cancer Society. Breast Cancer Facts & Figures 2005-2006. Atlanta: American Cancer Society, Inc. http://www.cancer.org/docroot/stt/stt_0.asp

American Psychiatric Association. (1994). Diagnostic and statistical manual of mental disorders (4th ed.). Washington, DC: Author.

Andersen, S. L., Arvanitogiannis, A., Pliakas, A. M., LeBlanc, C., & Carlezon, W. A., Jr. (2002). Altered responsiveness to cocaine in rats exposed to methylphenidate during development. *Nature Neuroscience, 5,* 13-14.

Aoyama, T., Kotaki, H., & Iga, T. (1990). Dose-dependent kinetics of methylphenidate enantiomers after oral administration of racemic methylphenidate to rats. *Journal of Pharmacobiodynamics, 13,* 647-652.

Beaumont, A., Hayasaki, K., Marmarou, A., Barzo, P., Fatouros, P., & Corwin, F. (2001). Contrasting effects of dopamine therapy in experimental brain injury. *Journal of Neurotrauma, 18,* 1359-1372.

Berlanga, M. L., Simpson, T. K., & Alcantara, A. A. (2005). Dopamine D5 receptor localization on cholinergic neurons of the rat forebrain and diencephalon: a potential neuroanatomical substrate involved in mediating dopaminergic influences on acetylcholine release. *The Journal of comparative neurology, 492(1),* 34-49.

Bolaños, C. A., Barrot, M., Berton, O., Wallace-Black, D., & Nestler, E. J. (2003). Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. *Biological Psychiatry, 54,* 1317-1329.

Bradley. C. (1937). The behavior of children receiving benzedrine. *American Journal of Psychiatry*, 94, 577–585.

Bradley, C. (1950). Benzedrine and dexedrine in the treatment of children's behavior disorders. *Pediatrics, 5,* 24-37.

Brandon, C. L. & Steiner, H. (2003). Repeated methylphenidate treatment in adolescent rats alters gene regulation in the striatum. *The European Journal of Neuroscience,* 18, 1584-1592.

Brandon, C. L., Marinelli, M., & White, F. J. (2003). Adolescent exposure to methylphenidate alters the activity of rat midbrain dopamine neurons. *Biological Psychiatry*, 54, 1338-1344.

Brown, R. W., Gonzalez, C. L., & Kolb, B. (2000). Nicotine improves Morris water task performance in rats given medial frontal cortex lesions. *Pharmacology, Biochemistry and Behavior, 67,* 473-478.

Brown, W. A. (1998). Charles Bradley, M.D., 1902-1979. *American Journal of Psychiatry*, 155, 968.

Brown, W. A. (1977). Psychological and neuroendocrine response to methylphenidate. *Archives of General Psychiatry, 34,* 1103–1108.

Bullock, R., Maxwell, W., Graham, D., Teasdale, G. & Adams, J. (1991). Glial swelling following human cerebral contusion: an ultrastructural study. *Journal of Neurology, Neurosurgery, and Psychiatry, 54,* 427–434.

Castellanos, F. X., Giedd, J. N., Eckburg, P., Marsh, W. L., Vaituzis, A. C., Kaysen, D. et al. (1994). Quantitative morphology of the caudate nucleus in attention deficit hyperactivity disorder. *American Journal of Psychiatry, 151,* 1791-1796.

Center for Disease Control and Prevention. HIV/AIDS. http://www.cdc.gov/hiv/

Center for Disease Control and Prevention. Traumatic Brain Injury. National Center for Injury Prevention and Control. http://www.cdc.gov/ncipc/tbi/TBI.htm

Challman, T. D. & Lipsky, J. J. (2000). Methylphenidate: its pharmacology and uses. *Mayo Clinic Proceedings, 75,* 711-721.

Chase, T. D., Brown, R. E., Carrey, N., & Wilkinson, M. (2003). Daily methylphenidate administration attenuates c-fos expression in the striatum of prepubertal rats. *Neuroreport, 14,* 769-772.

Cheng, C. L. & Povlishock, J. T. (1988). The effect of traumatic brain injury on the visual system: a morphologic characterization of reactive axonal change. *Journal of Neurotrauma, 5,* 47-60.

Cook, E. H., Jr., Stein, M. A., Krasowski, M. D., Cox, N. J., Olkon, D. M., Kieffer, J. E. et al. (1995). Association of attention-deficit disorder and the dopamine transporter gene. *American Journal of Human Genetics, 56,* 993-998.

Cortez, S. C., McIntosh, T. K., & Noble, L. J. (1989). Experimental fluid percussion brain injury: vascular disruption and neuronal and glial alterations. *Brain Research, 482,* 271-282.

Dewitt, D. S., Jenkins, L. W., Wei, E. P., Lutz, H., Becker, D. P., & Kontos, H. A. (1986). Effects of fluid-percussion brain injury on regional cerebral blood flow and pial arteriolar diameter. *Journal of Neurosurgery., 64,* 787-794.

Diller, L. H. (1996). The run on Ritalin. Attention deficit disorder and stimulant treatment in the 1990s. *The Hastings Center Report, 26,* 12-18.

Ding, Y. S., Fowler, J. S., Volkow, N. D., Dewey, S. L., Wang, G. J., Logan, J. et al. (1997). Chiral drugs: comparison of the pharmacokinetics of [11C]d-threo and L-threo-methylphenidate in the human and baboon brain. *Psychopharmacology, 131,* 71-78.

Dixon, C. E., Clifton, G. L., Lighthall, J. W., Yaghmai, A. A., & Hayes, R. L. (1991). A controlled cortical impact model of traumatic brain injury in the rat. *Journal of Neuroscience Methods, 39,* 253-262.

Dixon, C. E., Lighthall, J. W., & Anderson, T. E. (1988). Physiologic, histopathologic, and cineradiographic characterization of a new fluid-percussion model of experimental brain injury in the rat. *Journal of Neurotrauma, 5,* 91-104.

Dixon, C. E., Lyeth, B. G., Povlishock, J. T., Findling, R. L., Hamm, R. J., Marmarou, A. et al. (1987). A fluid percussion model of experimental brain injury in the rat. *Journal of Neurosurgery, 67,* 110-119.

Dougherty, D. D., Bonab, A. A., Spencer, T. J., Rauch, S. L., Madras, B. K., & Fischman, A. J. (1999). Dopamine transporter density in patients with attention deficit hyperactivity disorder. *Lancet, 354,* 2132-2133.

Enomoto, T., Osugi, T., Satoh, H., McIntosh, T. K., & Nabeshima, T. (2005). Pre-injury magnesium treatment prevents traumatic brain injury-induced hippocampal ERK activation, neuronal loss, and cognitive dysfunction in the radial-arm maze test. *Journal of Neurotrauma, 22,* 783-792.

Erb, D. E. & Povlishock, J. T. (1988). Axonal damage in severe traumatic brain injury: an experimental study in cat. *Acta Neuropathologica, 76,* 347-358.

Ernst, M., Zametkin, A. J., Matochik, J. A., Pascualvaca, D., Jons, P. H., & Cohen, R. M. (1999). High midbrain [18F]DOPA accumulation in children with attention deficit hyperactivity disorder. *American Journal of Psychiatry, 156,* 1209-1215.

Faden, A. I., Demediuk, P., Panter, S. S., & Vink, R. (1989). The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science, 244,* 798-800.

Feeney, D. M. (1991). Pharmacological modulation of recovery after brain injury: a reconsideration of *diaschisis. Journal of Neurologic Rehabilitation, 5,* 113-128.

Feldman, R. S., Meyer, J. S., Quenzer, L. F. (1997). *Principles of Neuropsychopharmacology.* Sinauer Associates, Incorporated, Publishers, Sunderland, MA.

Filipek, P. A., Semrud-Clikeman, M., Steingard, R. J., Renshaw, P. F., Kennedy, D. N., & Biederman, J. (1997). Volumetric MRI analysis comparing subjects having attention-deficit hyperactivity disorder with normal controls. *Neurology, 48,* 589-601.

Foda, M. A. & Marmarou, A. (1994). A new model of diffuse brain injury in rats. Part II: Morphological characterization. *Journal of Neurosurgery, 80,* 301-313.

Gaetz, M. (2004). The neurophysiology of brain injury. *Clinical Neurophysiology, 115,* 4-18.

Gatley, S. J., Pan, D., Chen, R., Chaturvedi, G., & Ding, Y. S. (1996). Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Science, 58(12),* 231-239.

Gennarelli, T. A. (1993). Mechanisms of brain injury. *The Journal of Emergency Medicine, 11 Suppl 1,* 5-11.

Gennarelli, T. A. & Graham, D. I. (1998). Neuropathology of the Head Injuries. *Seminars in Clinical Neuropsychiatry, 3,* 160-175.

Gennarelli, T. A., Thibault, L. E., Adams, J. H., Graham, D. I., Thompson, C. J., & Marcincin, R. P. (1982). Diffuse axonal injury and traumatic coma in the primate. *Annals of Neurology, 12,* 564-574.

Gerasimov, M. R., Franceschi, M., Volkow, N. D., Gifford, A., Gatley, S. J., Marsteller, D., et al. (2000). Comparison between intraperitoneal and oral methylphenidate administration: a microdialysis and locomotor activity study. *The Journal of Pharmacology and Experimental Therapeutics, 295(1),* 51-57.

Gill, M., Daly, G., Heron, S., Hawi, Z., & Fitzgerald, M. (1997). Confirmation of association between attention deficit hyperactivity disorder and a dopamine transporter polymorphism. *Molecular Psychiatry, 2,* 311-313.

Grace, A. A. (1995). The tonic/phasic model of dopamine system regulation: its relevance for understanding how stimulant abuse can alter basal ganglia function. *Drug and Alcohol Dependence, 37,* 111-129.

Graham, D. I., McIntosh, T. K., Maxwell, W. L., & Nicoll, J. A. (2000). Recent advances in neurotrauma. *Journal of Neuropathology and Experimental Neurology, 59,* 641-651.

Gualtieri, T., Chandler, M., Coons, T. B., & Brown, L. T. (1989). Amantadine: a new clinical profile for traumatic brain injury. *Clinical Neuropharmacology, 12,* 258-270.

Gurdjian, E. S. (1976). Cerebral contusions: re-evaluation of the mechanism of their development. *The Journal of Trauma, 16,* 35-51.

Haines, D. E. (Ed.). (1997). *Fundamental Neuroscience.* New York: Churchill Livingstone Incorporated.

Hamm, R. J., Dixon, C. E., Gbadebo, D. M., Singha, A. K., Jenkins, L. W., Lyeth, B. G. et al. (1992). Cognitive deficits following traumatic brain injury produced by controlled cortical impact. *Journal of Neurotrauma, 9,* 11-20.

Hamm, R. J., Lyeth, B. G., Jenkins, L. W., O'Dell, D. M., & Pike, B. R. (1993). Selective cognitive impairment following traumatic brain injury in rats. *Behavioural Brain Research, 59,* 169-173.

Hayes, R. L., & Dixon, C. E. (1994). Neurochemical changes in mild head injury. *Seminars in Neurology, 14,* 25-31.

Hayes, R. L., Jenkins, L. W., & Lyeth, B. G. (1992). Neurotransmitter-mediated mechanisms of traumatic brain injury: acetylcholine and excitatory amino acids. *Journal of Neurotrauma, 9(Suppl. 1),* S173-S187.

Hicks, R. R., Smith, D. H., Lowenstein, D. H., Saint, M. R., & McIntosh, T. K. (1993). Mild experimental brain injury in the rat induces cognitive deficits associated with regional neuronal loss in the hippocampus. *Journal of Neurotrauma, 10,* 405-414.

Hoffman, S. W., Fulop, Z., & Stein, D. G. (1994). Bilateral frontal cortical contusion in rats: behavioral and anatomic consequences. *Journal of Neurotrauma, 11,* 417-431.

Hurd, Y. L. & Ungerstedt, U. (1989). In vivo neurochemical profile of dopamine uptake inhibitors and releasers in rat caudate-putamen. *European Journal of Pharmacoogy, 166,* 251-260.

Jenkins, L. W., Lyeth, B. G., Lewelt, W., Moszynski, K., Dewitt, D. S., Balster, R. L., et al. (1988). Combined pretrauma scopolamine and phencyclidine attenuate posttraumatic increased sensitivity to delayed secondary ischemia. *Journal of Neurotrauma, 5,* 275-287.

Jiang, J. Y., Lyeth, B. G., Delahunty, T. M., Phillips, L. L., & Hamm, R. J. (1994). Muscarinic cholinergic receptor binding in rat brain at 15 days following traumatic brain injury. *Brain Research, 651,* 123-128.

Jorge, R., & Robinson, R. G. (2003). Mood disorders following traumatic brain injury. *International Review of Psychiatry, 15,* 317-327.

Joyce, P. R., Donald, R. A., Nicholls, M. G., Livesey, J. H., & Abbott, R. M. (1986). Endocrine and behavioural responses to methylphenidate in normal subjects. *Biological Psychiatry, 21,* 1015–1023.

Kandel, E. R., Schwartz, J. H., & Jessell, T. M. (Eds.). (2000). *Principles of neural science (4th ed.).* New York: McGraw-Hill Companies, Incorporated. Health Professions Division.

Kashihara, K., Ishihara, T., Akiyama, K., & Abe, K. (1999). D1/D2 receptor synergism on CREB DNA-binding activities in the caudate-putamen of rat. *Neurological research, 21(8),* 781-784.

Katayama, Y., Becker, D. P., Tamura, T., & Hovda, D. A. (1990). Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. *Journal of Neurosurgery, 73,* 889-900.

Kim, B. N., Lee, J. S., Cho, S. C., & Lee, D. S. (2001). Methylphenidate increased regional cerebral blood flow in subjects with attention deficit/hyperactivity disorder. *Yonsei Medical Journal, 42(1),* 19-29.

Kline, A. E., Massucci, J. L., Ma, X., Zafonte, R. D., & Dixon, C. E. (2004). Bromocriptine reduces lipid peroxidation and enhances spatial learning and hippocampal neuron survival in a rodent model of focal brain trauma. *Journal of Neurotrauma, 21(12),* 1712-1722.

Kline, A. E., Yan, H. Q., Bao, J., Marion, D. W., & Dixon, C. E. (2000). Chronic methylphenidate treatment enhances water maze performance following traumatic brain injury in rats. *Neuroscience Letters, 280,* 163-166.

Kuczenski, R. & Segal, D. S. (2002). Exposure of adolescent rats to oral methylphenidate: preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. *Journal of Neuroscience, 22,* 7264-7271.

Kuczenski, R., & Segal, D. S. (1997). Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. *Journal of Neurochemistry, 68(5),* 2032-2037.

LaHoste, G. J., Swanson, J. M., Wigal, S. B., Glabe, C., Wigal, T., King, N. et al. (1996). Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. *Molecular Psychiatry, 1,* 121-124.

Langlois, J., Rutland-Brown, W., & Thomas, K. (2004). *Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations, and Deaths* Division of Injury and Disability Outcomes and Programs National Center for Injury Prevention and Control Centers for Disease Control and Prevention Department of Health and Human Services. www.cdc.gov/injury

Lee, J. S., Kim, B. N., Kang, E., Lee, D. S., Kim, Y. K., Chung, J. K., et al. (2005). Regional cerebral blood flow in children with attention deficit hyperactivity disorder: comparison before and after methylphenidate treatment. *Human Brain Mapping, 24,* 157-164.

Leonard, B. E., McCartan, D., White, J., King, D. J. (2004). Methylphenidate: a review of its neuropharmacological, neuropsychological and adverse clinical effects. *Human Psychopharmacology, 19(3),* 151-80.

Lewelt, W., Jenkins, L. W., & Miller, J. D. (1980). Autoregulation of cerebral blood flow after experimental fluid percussion injury of the brain. *Journal of Neurosurgery, 53,* 500-511.

Lighthall, J. W., Dixon, C. E., & Anderson, T. E. (1989). Experimental models of brain injury. *Journal of Neurotrauma, 6,* 83-97.

Long, J. B., Gordon, J., Bettencourt, J. A., & Bolt, S. L. (1996). Laser-Doppler flowmetry measurements of subcortical blood flow changes after fluid percussion brain injury in rats. *Journal of Neurotrauma, 13(3),* 149-162.

Lou, H. C., Henriksen, L., & Bruhn, P. (1984). Focal cerebral hypoperfusion in children with dysphasia and/or attention deficit disorder. *Archives of Neurology, 41,* 825-829.

Lou, H. C., Henriksen, L., Bruhn, P., Borner, H., & Nielsen, J. B. (1989). Striatal dysfunction in attention deficit and hyperkinetic disorder. *Archives of Neurology, 46,* 48-52.

Lyeth, B. G., Jenkins, L. W., Hamm, R. J., Dixon, C. E., Phillips, L. L., Clifton, G. L. et al. (1990). Prolonged memory impairment in the absence of hippocampal cell death following traumatic brain injury in the rat. *Brain Research, 526,* 249-258.

Maltais, S., Côte, S., Drolet, G., & Falardeau, P. (2000). Cellular colocalization of dopamine D1 mRNA and D2 receptor in rat brain using a D2 dopamine receptor specific polyclonal antibody. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 24,* 1127-1149.

Marmarou, A., Foda, M. A., van den Brink, W., Campbell, J., Kita, H., & Demetriadou, K. (1994). A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *Journal of Neurosurgery, 80,* 291-300.

Massucci, J. L., Kline, A. E., Ma, X., Zafonte, R. D., & Dixon, C. E. (2004). Time dependent alterations in dopamine tissue levels and metabolism after experimental traumatic brain injury in rats. *Neuroscience Letters, 372,* 127-131.

Matochik, J. A., Liebenauer, L. L., King, A. C., Szymanski, H. V., Cohen, R. M., & Zametkin, A. J. (1994). Cerebral glucose metabolism in adults with attention deficit hyperactivity disorder after chronic stimulant treatment. *American Journal of Psychiatry, 151(5),* 658-664.

Maxwell, W. L., Irvine, A., Watt, C., Graham, D. I., Adams, J. H., & Gennarelli, T. A. (1991). The microvascular response to stretch injury in the adult guinea pig visual system. *Journal of Neurotrauma, 8,* 271-279.

McIntosh, T. K., Smith, D. H., Meaney, D. F., Kotapka, M. J., Gennarelli, T. A., & Graham, D. I. (1996). Neuropathological sequelae of traumatic brain injury: relationship to neurochemical and biomechanical mechanisms. *Laboratory Investigation, 74,* 315-342.

McIntosh, T. K., Yu, T., & Gennarelli, T. A. (1994). Alterations in regional brain catecholamine concentrations after experimental brain injury in the rat. *Journal of Neurochemistry, 63,* 1426-1433.

Medico, M., De Vivo, S., Tomasello, C., Grech, M., Nicosia, A., Castorina, M., et al. (2002). Behavioral and neurochemical effects of dopaminergic drugs in models of brain injury. *European Neuropsychopharmacology, 12*, 187-194.

Mehta, M. A., Owen, A. M., Sahakian, B. J., Mavaddat, N., Pickard, J. D., & Robbins, T. W. (2000). Methylphenidate enhances working memory by modulating discrete frontal and parietal lobe regions in the human brain. *Journal of Neuroscience, 20,* RC65.

Micale, V., Incognito, T., Ignoto, A., Rampello, L., Sparta, M., & Drago, F. (2006). Dopaminergic drugs may counteract behavioral and biochemical changes induced by models of brain injury. *European Neuropsychopharmacology , 16*, 195-203.

Moll, G. H., Hause, S., Ruther, E., Rothenberger, A., & Huether, G. (2001). Early methylphenidate administration to young rats causes a persistent reduction in the density of striatal dopamine transporters. *Journal of Child and Adolescent Psychopharmacology, 11,* 15-24.

Moll, G. H., Heinrich, H., Trott, G., Wirth, S., & Rothenberger, A. (2000). Deficient intracortical inhibition in drug-naive children with attention-deficit hyperactivity disorder is enhanced by methylphenidate. *Neuroscience Letters, 284,* 121-125.

Mordenti, J. (1986). Man versus beast: pharmacokinetic scaling in mammals. *Journal of Pharmaceutical Sciences*, 75, 1028-1040.

Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297,* 681-683.

Muir, J. K., Boerschel, M., & Ellis, E. F. (1992). Continuous monitoring of posttraumatic cerebral blood flow using laser-Doppler flowmetry. *Journal of Neurotrauma, 9(4),* 355-362.

Murray, C. J. & Lopez, A. D. (1997). Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet, 349,* 1498-1504.

Nilsson, P., Ronne-Engstrom, E., Flink, R., Ungerstedt, U., Carlson, H., & Hillered, L. (1994). Epileptic seizure activity in the acute phase following cortical impact trauma in rat. *Brain Research, 637,* 227-232.

Ommaya, A. K. & Gennarelli, T. A. (1974). Cerebral concussion and traumatic unconsciousness. Correlation of experimental and clinical observations of blunt head injuries. *Brain, 97,* 633-654.

Patrick, K. S., Ellington, K. R., & Breese, G. R. (1984). Distribution of methylphenidate and p-hydroxymethylphenidate in rats. *The Journal of Pharmacology and Experimental Therapeutics, 231,* 61-65.

Pilz, P. (1983). Axonal injury in head injury. *Acta Neurochirurgica. Supplementum, 32,* 119-123.

Povlishock, J. T., & Becker, D. P. (1985). Fate of reactive axonal swellings induced by head injury. *Laboratory Investigation, 52,* 540-552.

Povlishock, J. T., Becker, D. P., Cheng, C. L., & Vaughan, G. W. (1983). Axonal change in minor head injury. *Journal of Neuropathology and Experimental Neurology, 42,* 225-242.

Povlishock, J. T. & Christman, C. W. (1995). The pathobiology of traumatically induced axonal injury in animals and humans: a review of current thoughts. *Journal of Neurotrauma, 12,* 555-564.

Povlishock, J. T., & Christman, C. W. (1994). The pathobiology of traumatic brain injury. In Salzman, S.K., & Faden, A.I. (Eds.) *The Neurobiology of Central Nervous System Trauma*, pp. 109-120. New York: Oxford University Press.

Povlishock, J. T. & Katz, D. I. (2005). Update of neuropathology and neurological recovery after traumatic brain injury. *Journal of Head Trauma Rehabilitation, 20,* 76-94.

Povlishock, J. T. & Kontos, H. A. (1985). Continuing axonal and vascular change following experimental brain trauma. *Central Nervous System Trauma, 2,* 285-298.

Prins, M. L. & Hovda, D. A. (1998). Traumatic brain injury in the developing rat: effects of maturation on Morris water maze acquisition. *Journal of Neurotrauma, 15,* 799-811.

Prins, M. L., Lee, S. M., Cheng, C. L., Becker, D. P., & Hovda, D. A. (1996). Fluid percussion brain injury in the developing and adult rat: a comparative study of mortality, morphology, intracranial pressure and mean arterial blood pressure. *Brain Research. Developmental Brain Research, 95,* 272-282.

Raghupathi, R. (2004). Cell death mechanisms following traumatic brain injury. *Brain Pathology, 14,* 215-222.

Raghupathi, R., Graham, D. I., & McIntosh, T. K. (2000). Apoptosis after traumatic brain injury. *Journal of Neurotrauma, 17,* 927-938.

Rappley, M. D. (2005). Attention deficit-hyperactivity disorder. *The New England Journal of Medicine, 352(2),* 165-173.

Reeves, T.M., Phillips, L.L., Walker, S.A., & Povlishock, J.T. (2004). Unmyelinated axons of the corpus callosum show selective functional and structural changes after injury and FK506 treatment. *Journal of Neuroscience*, 9:1324.

Ribas, G. C. & Jane, J. A. (1992). Traumatic contusions and intracerebral hematomas. *Journal of Neurotrauma, 9 Suppl 1,* S265-S278.

Rothman, S. M. & Olney, J. W. (1986). Glutamate and the pathophysiology of hypoxic--ischemic brain damage. *Annals of Neurology, 19,* 105-111.

Samuels, S. C. & Davis, K. L. (1998). Experimental approaches to cognitive disturbance in Alzheimer's disease. *Harvard Review of Psychiatry, 6,* 11-22.

Schroder, M. L., Muizelaar, J. P., Bullock, M. R., Salvant, J. B., & Povlishock, J. T. (1995). Focal ischemia due to traumatic contusions documented by stable xenon-CT and ultrastructural studies. *Journal of Neurosurgery, 82,* 966-971.

Schweitzer, J. B., Lee, D. O., Hanford, R. B., Tagamets, M. A., Hoffman, J. M., Grafton, S. T., et al. (2003). A positron emission tomography study of methylphenidate in adults with ADHD: alterations in resting blood flow and predicting treatment response. *Neuropsychopharmacology, 28,* 967-973.

Schweitzer, J. B., Lee, D. O., Hanford, R. B., Zink, C. F., Ely, T. D., Tagamets, M. A., et al. (2004). Effect of methylphenidate on executive functioning in adults with attention-deficit/hyperactivity disorder: normalization of behavior but not related brain activity. *Biological Psychiatry, 56,* 597-606.

Seeman, P., & Madras, B. (2002). Methylphenidate elevates resting dopamine which lowers the impulse-triggered release of dopamine: a hypothesis. *Behavioural Brain Research, 130,* 79-83.

Shafritz, K. M., Marchione, K. E., Gore, J. C., Shaywitz, S. E., & Shaywitz, B. A. (2004). The effects of methylphenidate on neural systems of attention in attention deficit hyperactivity disorder. *American Journal of Psychiatry, 161,* 1990-1997.

Sieg, K. G., Gaffney, G. R., Preston, D. F., & Hellings, J. A. (1995). SPECT brain imaging abnormalities in attention deficit hyperactivity disorder. *Clinical Nuclear Medicine, 20,* 55-60.

Silkis, I. (2001). The cortico-basal ganglia-thalamocortical circuit with synaptic plasticity. II. Mechanism of synergistic modulation of thalamic activity via the direct and indirect pathways through the basal ganglia. *Bio Systems, 59(1),* 7-14.

Smith, D. H., Lowenstein, D. H., Gennarelli, T. A., & McIntosh, T. K. (1994). Persistent memory dysfunction is associated with bilateral hippocampal damage following experimental brain injury. *Neuroscience Letters, 168,* 151-154.

Smith, D. H., Okiyama, K., Thomas, M. J., Claussen, B., McIntosh, T. K. (1991). Evaluation of memory dysfunction following experimental brain injury using the Morris water maze. *Journal of Neurotrauma, 8(4),* 259-69.

Solanto, M. V. (1998). Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and integration. *Behavioural Brain Research, 94,* 127-152.

Soloniuk, D., Pitts, L. H., Lovely, M., & Bartkowski, H. (1986). Traumatic intracerebral hematomas: timing of appearance and indications for operative removal. *The Journal of Trauma, 26,* 787-794.

Sosin, D. M., Sniezek, J. E., & Thurman, D. J. (1996). Incidence of mild and moderate brain injury in the United States, 1991. *Brain Injury, 10,* 47-54.

Spalletta, G., Pasini, A., Pau, F., Guido, G., Menghini, L., & Caltagirone, C. (2001). Prefrontal blood flow dysregulation in drug naive ADHD children without structural abnormalities. *Journal of Neural Transmission, 108,* 1203-1216.

Sproson, E. J., Chantrey, J., Hollis, C., Marsden, C. A., & Fonel, K. C. (2001). Effect of repeated methylphenidate administration on presynaptic dopamine and behaviour in young adult rats. *Journal of Psychopharmacology, 15,* 67-75.

Strich, S. J. (1956). Diffuse degeneration of the cerebral white matter in severe dementia following head injury. *Journal of Neurology, Neurosurgery and Psychiatry, 19,* 163-185.

Swanson, J. M., Flodman, P., Kennedy, J., Spence, M. A., Moyzis, R., Schuck, S. et al. (2000). Dopamine genes and ADHD. *Neuroscience and Biobehavioral Reviews, 24,* 21-25.

Swanson, J. M., & Volkow, N. D. (2002). Pharmacokinetic and pharmacodynamic properties of stimulants: implications for the design of new treatments for ADHD. *Behavioural Brain Research, 130(1-2),* 73-78.

Szobot, C. M., Ketzer, C., Cunha, R. D., Parente, M. A., Langleben, D. D., Acton, P. D., et al. (2003). The acute effect of methylphenidate on cerebral blood flow in boys with attention-deficit/hyperactivity disorder. *European Journal of Nuclear Medicine and Molecular Imaging, 3(3),* 423-426.

Tang, Y. P., Noda, Y., & Nabeshima, T. (1997). Involvement of activation of dopaminergic neuronal system in learning and memory deficits associated with experimental mild traumatic brain injury. *European Journal of Neuroscience, 9,* 1720-1727.

Tang, Y. P., Noda, Y., & Nabeshima, T. (1997a). A synergistic interaction between dopamine D1 and D2 receptor subtypes in the memory impairments induced by concussive brain injury (CBI) in mice. *Behavioural Brain Research, 83,* 189-193.

Teo, S. K., Stirling, D. I., Hoberman, A. M., Christian, M. S., Thomas, S. D., & Khetani, V. D. (2003). D-methylphenidate and D,L-methylphenidate are not developmental toxicants in rats and rabbits. *Birth Defects Research. Part B, Developmental and Reproductive Toxicology, 68,* 162-171.

Teo, S. K., Stirling, D. I., Thomas, S. D., & Khetani, V. D. (2003). Neurobehavioral effects of racemic threo-methylphenidate and its D and L enantiomers in rats. *Pharmacology, Biochemistry and Behavior, 74,* 747-754.

Thurman, D., Alverson, C., Browne, D., Dunn, K., Guerrero, J., Johnson, R., Johnson, V., Langlois, J., Pilkey, D., Sniezek, J., Toal, S. (1999). Traumatic Brain Injury in the United States: A Report to Congress. Division of Acute Care, Rehabilitation Research, and Disability Prevention, National Center for Injury Prevention and Control, Centers for Disease Control and Prevention.

Thurman,D., Alverson, C., Dunn, K., Guerrero, J., & Sniezek, J. (1999). Traumatic brain injury in the United States: a public health perspective. *Journal of Head Trauma and Rehabilitation, 14(6),* 602–615.

University of California San Francisco Medical School, Office of Educational Technology. (Internet Souce) iRocket. Dopamine Module. ©2005 University of California Regents. Module content last updated: *Fall 2002/Spring 2003.* http://missinglink.ucsf.edu/lm/IDS_104_dopamine_ILM/Dopamine/Inropage.htm

Vaidya, C. J., Austin, G., Kirkorian, G., Ridlehuber, H. W., Desmond, J. E., Glover, G. H. et al. (1998). Selective effects of methylphenidate in attention deficit hyperactivity disorder: a functional magnetic resonance study. *Proceedings of the National Academy of Sciences of the United States of America, 95,* 14494-14499.

Vallone, D., Picetti, R., & Borrelli, E. (2000). Structure and function of dopamine receptors. *Neuroscience and Biobehavioral Reviews, 24,* 125-132.

Vespa, P., McArthur, D. L., Alger, J., O'Phelan, K., Hattori, N., Wu, C., et al. (2004). Regional heterogeneity of post-traumatic brain metabolism as studied by microdialysis, magnetic resonance spectroscopy and positron emission tomography. *Brain Pathology, 14(2),* 210-214.

Volkow, N. D., Ding, Y. S., Fowler, J. S., Wang, G. J., Logan, J., Gatley, J. S. et al. (1995). Is methylphenidate like cocaine? Studies on their pharmacokinetics and distribution in the human brain. *Archives of General Psychiatry, 52,* 456-463.

Volkow, N. D., Fowler, J. S., Ding, Y. S., Wang, G. J., & Gatley, S. J. (1998). Positron emission tomography radioligands for dopamine transporters and studies in human and nonhuman primates. *Advances in Pharmacology, 42,* 211-214.

Volkow, N. D., Wang, G. J., Fowler, J. S., Gatley, S. J., Logan, J., Ding, Y. S. et al. (1998). Dopamine transporter occupancies in the human brain induced by therapeutic doses of oral methylphenidate. *American Journal of Psychiatry, 155,* 1325-1331.

Volkow, N. D., Wang, G. J., Fowler, J. S., Telang, F., Maynard, L., Logan, J. et al. (2004). Evidence that methylphenidate enhances the saliency of a mathematical task by increasing dopamine in the human brain. *American Journal of Psychiatry, 161,* 1173-1180.

Von Monakow, C. (1969). Diaschisis, the localization in the cerebrum and functional impairment by cortical loci. In K. Pribram (ed.) *Brain and Behavior; Moods, states, and mind,* 1:27-36. Baltimore: Penguine Books (Translated by G. Harris).

Waldman, I. D., Robinson, B. F., & Feigon, S. A. (1997). Linkage disequilibrium between the dopamine transporter gene (DAT1) and bipolar disorder: extending the transmission disequilibrium test (TDT) to examine genetic heterogeneity. *Genetic Epidemiology, 14,* 699-704.

Warburton, D. M., Wesnes, K., Shergold, K., & James, M. (1986). Facilitation of learning and state dependency with nicotine. *Psychopharmacology, 89,* 55-59.

Wargin, W., Patrick, K., Kilts, C., Gualtieri, C. T., Ellington, K., Mueller, R. A. et al. (1983). Pharmacokinetics of methylphenidate in man, rat and monkey. *The Journal of Pharmacology and Experimental Therapeutics, 226,* 382-386.

Wasserstein, J. (2005). Diagnostic issues for adolescents and adults with ADHD. *Journal of Clinical Psychology, 61,* 535-547.

Wax, P. M. (1997). Analeptic use in clinical toxicology: a historical appraisal. *Journal of Toxicology. Clinical Toxicology, 35,* 203-209.

Wehman, P., Targett, P., West, M., & Kregel, J. (2005). Productive work and employment for persons with traumatic brain injury: what have we learned after 20 years? *The Journal of Head Trauma Rehabilitation, 20,* 115-127.

Wei, E. P., Dietrich, W. D., Povlishock, J. T., Navari, R. M., & Kontos, H. A. (1980). Functional, morphological, and metabolic abnormalities of the cerebral microcirculation after concussive brain injury in cats. *Circulation Research, 46,* 37-47.

Whyte, J., Hart, T., Schuster, K., Fleming, M., Polansky, M., & Coslett, H. B. (1997). Effects of methylphenidate on attentional function after traumatic brain injury. A randomized, placebo-controlled trial. *American Journal of Physical Medicine & Rehabilitation / Association of Academic Physiatrists, 76,* 440-450.

Whyte, J., Vaccaro, M., Grieb-Neff, P., & Hart, T. (2002). Psychostimulant use in the rehabilitation of individuals with traumatic brain injury. *The Journal of Head Trauma Rehabilitation, 17,* 284-299.

Yakovlev, A. G. & Faden, A. I. (2004). Mechanisms of neural cell death: implications for development of neuroprotective treatment strategies. *NeuroRx, 1,* 5-16.

Yamakami, I. & McIntosh, T. K. (1989). Effects of traumatic brain injury on regional cerebral blood flow in rats as measured with radiolabeled microspheres. *Journal of Cerebral Blood Flow and Metabolism, 9,* 117-124.

Yan, H. Q., Kline, A. E., Ma, X., Li, Y., & Dixon, C. E. (2002). Traumatic brain injury reduces dopamine transporter protein expression in the rat frontal cortex. *Neuroreport, 13,* 1899-1901.

Zametkin, A. J., Nordahl, T. E., Gross, M., King, A. C., Semple, W. E., Rumsey, J. et al. (1990). Cerebral glucose metabolism in adults with hyperactivity of childhood onset. *The New England Journal of Medicine, 323,* 1361-1366.

Zauner, A. & Bullock, R. (1995). The role of excitatory amino acids in severe brain trauma: opportunities for therapy: a review. *Journal of Neurotrauma, 12,* 547-554.

Zhu, J., Hamm, R. J., Reeves, T. M., Povlishock, J. T., & Phillips, L. L. (2000). Post-injury administration of L-deprenyl improves cognitive function and enhances neuroplasticity after traumatic brain injury. *Experimental Neurology, 166,* 136-152.

Zito, J. M., Safer, D. J., dosReis, S., Gardner, J. F., Boles, M., & Lynch, F. (2000). Trends in the prescribing of psychotropic medications to preschoolers. *JAMA: The Journal of the American Medical Association, 283,* 1025-1030.

Vita

Katharine Coryell Eakin was born on September, 22 1977, in Washington, D.C. and is an American citizen. She graduated from James Madison High School in 1995. She graduated Cum Laude with a Bachelor of Science in Psychology from Virginia Commonwealth University, Richmond Virginia in 1999. She has been a member of Psi Chi, National Honor Society in Psychology, since 1997. In 2001 she matriculated into the Ph.D. program in Biopsychology at Virginia Commonwealth University, Richmond Virginia. While attending Virginia Commonwealth University she was a teaching assistant for two introductory psychology courses from August, 2001 through May, 2003. She taught two undergraduate courses, VCU 101, Introduction to the University, in the fall semesters and VCU 102, Turning Point, Discovering a New MINDSET, in the spring semesters. She taught these courses from January, 2004 through May, 2006. She has presented posters at the 20th Annual National Neurotrauma Society Symposium and 6th Annual International Neurotrauma Symposium, in Tampa, FL in October, 2002 and at the 23rd National Neurotrauma Society Symposium, Washington, D.C. in November, 2005.