2008

THE EFFECTS OF ATOMOXETINE ON COGNITIVE PERFORMACE AND NEUROPLASTICITY AFTER TRAUMATIC BRAIN INJURY

Wendy Reid
Virginia Commonwealth University

School of Medicine
Virginia Commonwealth University

This is to certify that the dissertation prepared by Wendy Murdock Reid entitled THE EFFECTS OF ATOMOXETINE ON COGNITIVE PERFORMACE AND NEUROPLASTICITY AFTER TRAUMATIC BRAIN INJURY has been approved by his or her committee as satisfactory completion of the thesis or dissertation requirement for the degree of Doctor of Philosophy

Robert J. Hamm, Ph.D.,

Linda Phillips, Ph.D., School of Medicine

Thomas Reeves, Ph.D., School of Medicine

John T. Povlishock, Ph.D., School of Medicine

Aron H. Lichtman, Ph.D., School of Medicine

John T. Povlishock, Ph.D., Department Chairman

[Click here and type your School or College Dean's Name]

Dr. F. Douglas Boudinot, Dean of the School of Graduate Studies

[Click here and type the Month, Day and Year this page was signed.]

THE EFFECTS OF ATOMOXETINE ON COGNITIVE PERFORMACE AND NEUROPLASTICITY AFTER TRAUMATIC BRAIN INJURY

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

WENDY MURDOCK REID
Bachelor of Science, North Carolina State University, 1999

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

Co-Director: Linda Phillips, Ph.D.
Professor, Anatomy and Neurobiology

Virginia Commonwealth University
Richmond, Virginia
May 2008

Acknowledgements

I would like to thank my graduate mentor, Dr. Robert Hamm, for taking a chance me on as a PhD. Candidate, especially since I was an anatomy student with an interest in behavior. I have learned so much from you over the past few years, and I appreciate your guidance, patience and insight. I would also like to especially thank Dr. Linda Phillips for working with me and supporting my research and ideas. Thanks to the Department of Anatomy and Neurobiology and Dr. John Povlishock for supporting and training me. Also, I would like to thank my other distinguished committee members Thomas Reeves, and Aron Lichtman. It has been a great pleasure to know and work with all of them, and I have learned much from watching and interacting with them.

I would like to thank my fellow graduate students and friends, Janna Harris, Niki Kokiko, Mark Whiting, Katharine Eakin and Kelly Warren, who have leant me their support and advice throughout this endeavor. I have enjoyed our camaraderie and look forward to future collaborations. In addition, I would like to thank three technicians who have been a tremendous help in the experiments presented, as well as am excellent source of technical knowledge: Nancy Nixon-Lee, Raiford Black, and Lesley Harris. Finally, I am very grateful to my husband, Timothy Reid, for his constant encouragement and support both intellectually and personally, and for his patience with me during comprehensives and the writing of this dissertation. Thanks to my parents for encouraging my curiosities over the years and for giving me strong ethical principles and a desire to contribute to society. I hope that I will always make my family proud. Finally, I would like to thank my dog, Mr. Magoo, who never asked "When are you going to be done with your dissertation?" and Steven Pinker for writing a book that first ignited my fascination with cognitive neuroscience.

Partial funding for this dissertation was supported by Eli Lilly and Company.

Table of Contents

Page

List of Figures

Page

List of Abbreviations

ABC	Avidin-biotin-peroxidase complex
Ach	Acetylcholine
AD	Alzheimer's disease
ADHD	Attention Deficit Hyperactivity Disorder
atm	Atmosphere
BDNF	Brain-derived Neurotrophic factor
CCI	Controlled cortical impact
CNS	Central nervous system
DA	Dopamine
FPI	Fluid percussion injury
ICP	Intracranial pressure
GABA	Gamma aminobutyric acid
GAP-43	Growth-Associated Protein- 43
h	hour(s)
IML	Inner molecular layer
i.p.	intraperitoneal
IR	Immunoreactivity
LTP	Long-term potentiation
MAG	Myelin Associated Glycoproteins

mRNA	Messenger ribonucleic acid
MWM	Morris Water Maze
NE	Norepinephrine
NET	Norepinephrine Transporter
NGF	Nerve growth factor
NT	Neurotransmitter
PID	Post-injury day
RFD	Remote functional Depression
SEM	Standard error of the mean
SLM	Stratum lacunosum moleculare
SO	Stratum oriens
SR	Stratum radiatum
TBI	Traumatic brain injury
VTA	Ventral Tegmental Area

Abstract

THE EFFECTS OF ATOMOXETINE ON COGNITIVE PERFORMACE AND NEUROPLASTICITY AFTER TRAUMATIC BRAIN INJURY

By Wendy Murdock Reid

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

Virginia Commonwealth University, 2008

Major Director: Robert J Hamm, PhD., Department of Psychology
Co-Director: Linda Phillips, Ph.D., Department of Anatomy and Neurobiology

Catecholaminergic neurotransmission is regionally altered following injury, and drugs aimed at these systems offer promising avenues for post-TBI pharmacotherapies. Atomoxetine is a selective norepinephrine transporter (NET) inhibitor currently indicated for treatment of attention-deficit hyperactivity disorder (ADHD). The studies in this dissertation were designed to test the efficacy of atomoxetine for treating cognitive deficits following experimental TBI and the potential mechanism for any beneficial effect. The first part of the study focused on behavioral recovery following atomoxetine treatment.

Several important questions of dose, therapeutic window, and duration of treatment were addressed in these studies. Sprague-Dawley rats were subjected to lateral fluid-percussion injury (L-FPI) of moderate severity (2.08 atm +/- .05). Four experiments were performed. In the first study, atomoxetine (.3 mg/kg, 1mg/kg, 3 mg/kg, or 9 mg/kg) or vehicle was administered daily on post injury days (PID) 1-15. Cognitive assessment was performed using the Morris water maze on PID 11-15. L-FPI resulted in significant cognitive impairment when compared to Sham-Injury. Treatment with lower doses of atomoxetine (.3mg/kg, 1mg/kg, and 3mg/kg) significantly attenuated the cognitive deficits in injured animals. Treatment with the higher dosage (9mg/kg) of atomoxetine resulted in animals that were not significantly different than injured-vehicle treated animals. The optimal response was achieved using 1 mg/kg atomoxetine. In the second study, treatment with atomoxetine (1mg/kg) or vehicle was delayed for 11 days post-injury. Rats were administered atomoxetine daily for 15 days and cognitive assessment was performed on PID 25-29. In this study, treatment with atomoxetine (1 mg/kg) did not result in improved cognitive performance. In the next study atomoxetine was given daily on PID 1-7 and then treatment was terminated. The animals were tested in the MWM on PID 11-15. We found that atomoxetine treatment for 7 days post-injury provides an enhancement of cognitive deficits that is not significantly different from sham animals. We then investigated whether a single treatment of atomoxetine 24 h after brain injury could influence behavioral outcome on days 11-15. From this study, we found a single dose of atomoxetine is not as effective as chronic treatment. Finally, we investigated changes in the protein expression of brain-derived neurotrophic factor, growth-associated protein-43, and

synaptophysin on day 7 PID to investigate what effect atomoxetine may have on brain plasticity and regeneration. We found that atomoxetine can enhance both GAP-43 and BDNF, but not synaptophysin at this time point. In conclusion, this is the first study to show that low doses of atomoxetine initiated early after experimental traumatic brain injury results in improved cognition. Furthermore, we show that enhancement of catecholamines via atomoxetine treatment during periods of postinjury-induced plasticity can provide long-term functional and structural benefits.

CHAPTER 1 INTRODUCTION

Epidemiology of TBI

Traumatic brain injury is one of the leading causes of death and disability, and a serious health concern in the United States (NIH conference 1999, Rutland-Brown et al., 2006). In 2002 alone, TBI accounted for approximately 6.5% of all deaths occurring in the United States (CDC, 2002). Globally, TBI is the leading cause of death of people under 45 and accounts for 1% of all deaths. It is reported that by 2020 TBI will be the 3rd leading cause of death and disability in the world (Murray and Lopez, 1997). In the Unites States, the NHIS and CDC report that approximately 1.4-1.5 million brain injuries occur annually (Langois et al., 2003, 2006). Of these, approximately 1.1 million people will receive care from an emergency room, 235,000 people will require hospitalization and 50,000 will die. Acute management of TBI and various prevention measures have led to fewer fatalities and an overall increase in the number of survivors. The increased survival rates have led to increased numbers of patients facing the long term consequences of TBI. The National Hospital Discharge Survey estimates 70,000 to 90,000 people will endure long-term loss of function and disability, including physical, neuropsychological, emotional, and behavioral impairments. This has led to an estimated 5.3 million Americans, or 2% of the nation's population, living with TBI related disabilities (Thurman et al, 1999; Langlois et al., 2006).

The leading causes of traumatic brain injury include falls, motor vehicle or traffic related crashes, and assault (Langlois et al., 2006). TBI occurs most often in young adults, with a peak incidence at age 15-19. Smaller peaks occur in younger children age 0-4 and in the elderly, 75 and up. Males show twice the incidence rate and a death rate 3.3 times higher than females (Thurman et al., 1999). In the U.S higher TBI rates are seen in minorities and lower socioeconomic groups, likely because of exposure to firearms and higher homicide rates.

TBI can affect all major areas of functioning, which makes the economic consequences of TBI astonishing. TBI-related disabilities have been linked to poor outcome in vocational functioning and often prevent the successful reentrance of patients into society (Cicerone et al., 2004). Direct and indirect TBI-related costs are estimated at nearly 60 billion dollars a year (Finkelstein et al., 2006). The individual health care costs for the patient and the patient's family are also enormous. Hospitalization charges alone can range from $32,000 to $100,000 (Davis et al., 2007). The lifetime costs for one person surviving a severe TBI can be as high as $4 million (National Institute of Neurological Disorders and Stroke 1989). This takes into account costs associated with post-injury rehabilitation and treatment, unemployment, and family care. The high costs associated with TBI often leave individuals and families at an increased risk for filing for bankruptcy (Hollingworth et al., 2007). The statistics clearly highlight the need for research that can elucidate the underlying pathology of TBI and find effective treatments for these patients.

Clinicians most often distinguish brain injury as mild, moderate, or severe. Although studies vary, approximately 80% of brain injuries are classified as mild, 10% as moderate, and 10% as severe (Bruns and Hauser, 2003). The Glasgow Coma Scale (GCS) is the most widely used method to classify the severity of injury and is based upon patient assessment of eye opening, verbal ability, and motor ability. Other factors such as duration of unconsciousness, skull fractures, ischemia, seizures, and computerized tomography (CT) scan reports are taken into account when determining GCS score. A patient who is able to follow commands, is fully oriented, and has spontaneous eye opening would receive a GCS score of 15. A patient with no verbal or motor response and no eye opening would receive a GCS score of 3. Mild injuries then correspond to a score of 13-15, moderate injuries to a score of 9-12, and severe injuries to a score of 3-8. The scores have prognostic value regarding eventual neurological recovery and rehabilitation, however, because the score is left open to doctor's interpretation the objectivity of the GCS often comes into question. Still mild, moderate, and severe injuries are characterized by very different pathologies and result in varying degrees of motor and cognitive deficits.

A TBI may be classified as mild if loss of consciousness or the period of disorientation and confusion is less than 30 minutes. Mild injuries are the most prevalent type of TBI, but they are often missed at the initial time of injury or not reported because patients may not present any clear signs or symptoms. Cognitive sequelae that usually develop within the first month following mild injuries include problems with attention, information processing and memory. Although studies vary considerably, most suggest

there is a progressive diminution of cognitive deficits over the first year post-injury (Katz, and DeLuca, 1992).

Moderate brain injury is defined as an injury resulting in loss of consciousness of 30 minutes to 6 h and a GCS of 9-12. There is great variation in the outcome of moderate severity and it very difficult to study because of variations in the definition of what constitutes a moderate injury. There is a low mortality rate of approximately 7-9%. The degree of deficits as well as the recovery period from moderate injury is significantly longer than what is seen with mild injury. Moderate injury results in increased unemployment and increased cognitive deficits compared to mild injury (Rimel et al., 1982). Although memory problems are the most common complaint, patients also suffer from problems with attention, concentration, information processing, "executive functions", impulsiveness, perseveration, and language complications. Studies show that at one year post-injury, patients still have memory impairments and behavioral problems that prevent full recovery (Tabaddor et al., 1984).

Severe TBI is defined by an injury that results in a loss of consciousness of more than 6 h and a GCS of 3-8. The incidence of mortality and disability are highest in this group. The lifelong costs for one person surviving a severe TBI can be as high as 4 million, and few people are able to return to work (National Institute of Neurological Disorders and Stroke 1989; Wehman et al., 1993). Significant deficits in memory, attention, and information processing, as well as a number of motor, behavioral, and emotional problems often compromise a patient's independence and quality of life and

require them to have assisted living situations (Colantonio et al., 2007). Survivors of severe TBI have the highest risk for suffering persistent, life-long deficits following injury.

Functional Deficits

TBI results in a myriad of functional impairments including physical, neurological, psychological, and cognitive deficits. Although, treatment strategies have focused on treating the symptoms of these disorders individually, it is very common for patients to experience them simultaneously. The extent and type of deficits reflects a number of factors including the severity of diffuse axonal injury and the location, depth, and volume of focal injury (Katz and Alenander, 1994). Although focal lesions may produce neurobehavioral syndromes depending on the location of damage, it is generally believed that diffuse injury leads to a more global dysfunction of circuits. Other factors such as age, gender, preexisting morbidities, injury severity and extracranial or systemic injury can also affect outcome. Therefore, the recovery times are variable, and may never be reached for some patients.

From a clinical perspective, the etiology can be very difficult to classify which results in variable clinical management (Ghajar et al., 1995). A comprehensive integrated neurological assessment which takes into account biological disturbances in brain function, a patient's emotional and psychological reactions to impairments in cognition and behavior, including awareness and acceptance of these impairments, and finally disruptions of relationships and work capacities is needed to develop an effective treatment approach that focuses on the patient's problems. However, many patients have

multiple injuries and life-threatening situations which prevent immediate neurological assessment and treatment. In addition, it can be difficult to interpret which aspects of cognition are impaired using standard behavioral paradigms. Various imaging tools have allowed for more accurate clinical assessment of TBI. Structural imaging using computed tomography (CT) and magnetic resonance (MR) allows for early detection of abnormalities such as hemorrhage, contusion, edema, midline shift, and herniation that may require surgery and special treatment (Furlow, 2006). In addition, the neuropsychological sequelae of TBI can be evaluated using functional imaging techniques such as single-photon emission CT (SPECT), positron emission tomography (PET) and functional-MRI (fMRI), which captures brain activity or "function" as reflected through cerebral metabolic rate (CMR) or cerebral blood flow (CBF). These tools have allowed more accurate diagnoses and better treatment protocols.

The initial and transient sequelae of TBI, during the period of posttraumatic amnesia, can include periods of delirium, agitation, confusion, disorientation, and amnesia. Patients often initially present posttraumatic headaches, vertigo, anxiety, autonomic dysfunction, as well as sensory and motor deficits. Delayed and persistent movement impairments, epilepsy, chronic pain, sleep disruption/chronic fatigue and/or some sensory impairment commonly develop following injury. There is also a significant amount of evidence to suggest TBI leads to a number of affective psychiatric disorders including, aggressive, and anxiety disorders and depression (for a review, see Fleminger, 2008). These often lead to mood disorders and dramatic personality changes (NIH Consensus Conference, 1999), which may be a direct result of TBI related damage or indirectly

caused by the social consequences of TBI. TBI patients are at a very high risk of divorce, suicide, unemployment, and substance abuse, which further exaggerates the already stressful situation (NIH Consensus Conference, 1999; Bushnik et al., 2003).

Cognitive Deficits

Initial and persistent cognitive deficits are often the most prominent features following TBI of any severity (for a review, see McAllister, 2008). Although, the cognitive deficits observed vary from individual to individual and severity of injury, there is a degree of consistency as to the nature and frequency of deficits observed. The most common complaints include attentional impairments, frontal executive impairments (e.g. problem solving, mental flexibility, initiation and inhibition), language impairments, and memory and learning dysfunction. The neurobiology and neurochemistry of attention, executive function, and memory and the TBI-induced changes in these processes will be discussed in more detail below. Important to this discussion is the evidence that suggests the neural circuitry and neurochemistry of different components of attention and memory overlap significantly. For example, the hippocampus, long recognized as a crucial structure in declarative memory processes, has recently been shown to have extensive connections to areas of the prefrontal cortex that are involved in working memory and appears to play a role in aspects of attention (Wall et al., 2001). Likewise strategic encoding and retrieval processes, thought to be centered in temporal lobe structures and the hippocampus, also engage areas of the frontal cortex (Kapur et al., 1995; Nyberg et al., 1996). Therefore, it must be remembered that these cognitive processes are served by a

distributed network of interacting brain regions, rather than being localized to a single region.

Attention and TBI

Impairments in attention appear to be a universal effect of TBI at all levels of injury severity (Gronwall, 1987; van Zomeren and Brouwer, 1994; Raskin et al., 2000). Attention is not a unitary concept and is broadly divided into subcomponents that include general arousal, selective, sustained and divided attention, as well as information processing and executive aspects (van Zomeren and Brouwer, 1994). These components of cognition involve a network of brain regions including the parietal cortex, dorsal frontal cortex, and cingulated regions, working with the basal ganglia, thalamus, and hippocampus. The brain stem is responsible for the overall attentional "tone" or degree of arousal (Mesulam, 2000). Because attention is important at many levels of cognition, these elements reflect widely dispersed neural networks that can be disrupted by both focal and diffuse injury during TBI. One of the most frequent complaints following injury is reduced information processing speed or a reduction in cognitive efficiency, which is thought to result from diffuse white matter dysfunction. TBI patients consistently demonstrate reduced reaction time (RT) proportional to task severity (Gronwall, 1987). Although improvements are seen over time, persistent deficits in these tasks are common (Lezak, 1995).

In addition to slowed information processing, other aspects of attention are also affected, especially in moderate to severe TBI patients (Schmitter-Edgecombe and Kibby, 1998). Disorders in selective attention (the ability to inhibit irrelevant processes or

distractions), sustained attention (the ability to sustain attention over a period of time, or vigilance), and divided attention (the ability to divide attention between two or more sources, multi-tasking) are all frequent complaints following brain injury (Park et al., 1999; Whyte et al., 2000; Brouwer et al., 2001). In addition, patients often perform worse in tasks that require components of working memory and executive functions, thus once again it is important to remember that a number of the domains of attention are dependent on other cognitive domains, as well as emotional functioning (Rios et al., 2004). The evidence taken together suggests attentional deficits are common following both mild TBI, in which the deficits may resolve in 1-3 months (Gronwall, 1991), as well as following more moderate to severe injuries, in which persistent long-term deficits are more common (Levine et al., 2000).

Executive Functions & Frontal Lobe Syndrome

Executive function is a broad term that encompasses many aspects of higher-order behavioral processes such as reasoning, planning, inhibiting inappropriate behaviors, organizing, and sequencing, as well as the executive memory processes and executive attentional processes (Eslinger et al., 1996). Studies in patients with moderate to severe TBI reveal deficits in conceptual reasoning and flexibility (Millis et al., 2001), strategic memory (Levin and Goldstein, 1986), planning (Leon-Carrion et al., 1998) and attentional executive processes (Levin et al., 1991). These processes are considered to be the domain of the frontal lobes and its complex projections and feedback loops, and thus often referred to as frontal lobe syndromes. Many of these cognitive processes are commonly affected by focal and diffuse damage to cortical tissue (Crepeau and Scherzer 1993; Levin et al.,

1992; Leon-Carrion et al., 1998). The anterior regions of the brain are particularly vulnerable to contusional damage (Levin et al., 1992), but also may experience some degree of diffuse axonal injury (Gentry et al., 1988).

An understanding of frontal lobe functions has been advanced by an understanding of the frontally guided networks involving the prefrontal cortex, posterior cortex, basal ganglia, ventral striatum, and thalamus (Schultz, 2001). Neuroimaging studies in humans and non-human primates have found the dorsolateral and ventrolateral prefrontal cortex and parietal cortex are particularly important areas for supporting working memory and the components of executive functioning (e.g., switching between tasks and inhibition). Anticipation and reward behavior, which are important for many aspects of executive function including the motivational aspects, involve the cortex, thalamus, and ventral striatum, with complex cortico-striatal-thalamo-cortical loops. Therefore focal or diffuse damage to any of these brain regions can have dramatic effects on executive function, as well as memory and attention.

Memory & The temporal lobe syndromes

Memory impairments are among the most common cognitive complaints and a cardinal feature of TBI. In the acute stages following injury intervals of retrograde amnesia and posttraumatic amnesia are dramatic. As time progresses, many aspects of memory impairment become evident and often persist though life (King et al., 1995). Memory is often subdivided into: 1.) declarative memory (explicit), which includes both episodic memory for events, and semantic memory for general facts and 2.) implicit

memory, which includes procedural learning, priming, and conditioning (Markowitsch, 2000).

Disruption to episodic memory processes is a hallmark feature of TBI, whereas implicit, or procedural memory (e.g. motor skills or puzzle solving), is generally left unimpaired (Shum et al., 1996; Richardson, 2000). Explicit memory is under the domain of the medial temporal lobes, thalami, and other structures and is the result of at least three different types of processing referred to as: encoding, consolidation, and retrieval. Encoding refers to the process by which information is acquired and processed when first encountered. This includes associating new information with previous knowledge so that one can integrate knowledge with what one already knows. Consolidation refers to the processes by which new information is converted to long-term storage, and is accompanied by changes in gene and protein expression and subsequent structural changes. Retrieval refers to the processes that recall the stored information and bring information from many locations together. Some studies have found dysfunction at all stages of memory processing (Curtiss et al., 2001), however , recent clinical studies suggest TBI patients suffer from deficits in consolidation, retention, and retrieval processes rather than problems with encoding (Vanderploeg and Crowell, 2001, Curtiss and Vanderploeg, 2001).

The Hippocampus and Learning & Memory

The hippocampus is one of the most intensively researched structures in the central nervous system. The hippocampus is an important region involved in acquisition, consolidation of explicit memories, which can be easily investigated in animals using

paradigms that look at spatial learning, or contextual learning (Anagnostaras et al., 2001; Morris et al., 2003). The hippocampus is also involved in retrieval of memories, although evidence suggests memories become independent of the hippocampus over time (Squire et al., 1995).

The rodent hippocampus extends caudally between the neocortex and diencephalon before curving ventrally toward the temporal lobe. It receives polymodal sensory information via the entorhinal cortex and has extensive connections with subcortical and frontal cortical structures (Witter et al., 2004). The hippocampal formation is divided into subfields based on morphological and cytoarchitectonic criteria, and includes the entrohinal cortex, dentate gyrus, the hippocampus proper (CA1, CA2, and CA3) and the subiculum. Early studies supported the idea of hippocampal place cells (O'Keefe and Nadel, 1978), or an allocentric map of space that is based on relationships of external spatial cues with no internal reference frame. Numerous studies have confirmed the hippocampus plays an integral role in spatial memory navigation and other aspects of memory (Morris et al., 1982; Sutherland et al., 1983; McGregor et al., 2004), but the specific nature still remains elusive. In addition, considerable evidence suggests functional heterogeneity along the axis of the hippocampus. The more dorsal (septal) regions of the hippocampus, which receive most of the structures visuospatial input, appear to be more important for spatial memory, and the ventral (temporal) portions are more important for non-spatial memory or innate information processing, such as fear-related behavior and anxiety. The hippocampus is noted for the prevalence of a type of synaptic plasticity known as long-term potentiation (LTP, Bliss and Collingridge, 1993). LTP that is

dependent on NMDA receptors has been strongly linked to memory (Martin et al., 2000), and to the memory-associated firing properties of hippocampal neurons (Shapiro and Eichenbaum, 1999).

The anatomy and role of distinct hippocampal subregions

The Dentate Gyrus: The dentate gyrus is composed of three distinct layers: a molecular layer, a granule layer, and a polymorphic layer, or hilus. The principle cell is the granule cell, which consists of an elliptical cell body located within the granule cell layer, apical dendrites of which extend into the molecular layer, and axons collateralizing in the polymorphic area and extending into CA3. The molecular layer contains very few cell bodies, except for some basket cells and interneurons. The granule cells receive direct input from the entorhinal cortex via the perforant path and then give rise to mossy fiber axons that project through the hilus to area CA3.

The dentate gyrus is unique within the brain in being one of only two areas of the adult brain that has continued neurogenesis (Gross, 2000). New granule cell neurons are generated from progenitor cells that reside in the subgranular zone, which borders the hilus and the granule cell layer within the DG (Martin and Clark, 2007). Within one week they migrate a short distance to the granule cell layer and begin expressing neuronal markers. This formation of new granule cells as been implicated in the creation of distinct memory traces for very similar items or events occurring at different times, a process often referred to as pattern separation (Becker, 2005).

Hippocampus Proper (CA1 and CA3): The hippocampus proper consists of CA1, CA2, and CA3 subregions. The CA2 region is less characterized than CA3 and CA1,

however, the border between CA2 and CA3 is marked by the termination of the stratum lucidum. The major cell type is the pyramidal cell, which forms the pyramidal cell layer, as well as interneurons. The cell bodies are located within the pyramidal cell layer, and basal dendrites extend into the stratum oriens (SO) layer. Apical dendrites extend through the stratum radiatum (SR) and stratum lacunosum-moleculare (SLM) layers. The SR contains Schaffer collaterals, and the SLM contains perforant pathway fibers. Schaffer collaterals extend from CA3 to CA1, mostly to the SR and to a lesser extent SO.

CA3 connections within this region as well as to CA1 are extensive and divergent. On average, one granule cell neuron innervates 12 CA3 neurons; in turn, each CA3 neuron innervates 50 other CA3 neurons via axon collaterals, as well as 25 inhibitory cells via other axon collaterals (Martin and Clark, 2007). The net result is a 600- fold amplification of excitation, and approximately 300-fold amplification of inhibition. Finally, a single CA1 neuron may be innervated by as many as 6000 CA3 neurons. CA1 also receives input from the EC perforant path fibers, of which terminate in the SLM. Although theoretical, the extensive recurrent connectivity of CA3 appears suited for pattern completion, or the reconstruction of an output pattern from a degraded input, and it has been suggested that CA1 acts as a 'novelty detector', integrating and detecting cortical information concerning attention and the current situation, with stored information from the hippocampus (Lisman et al., 2000; 2005; Grunwald 2006). This is supported by evidence that specific synapses within CA3 and CA1 undergo specific structural changes, which may underlie the mechanisms of LTP and LTD (Lauri et al., 2007; Massey and Bashir, 2007). However, it

is far from certain what role each region of the hippocampus plays in the process of encoding, consolidation, and retrieval of memories.

The Hippocampus and TBI

Bilateral temporal lobe lesions around the anterior region of the hippocampus are invariably associated with severe memory impairments, and the hippocampus is known to be a vulnerable region to ischemia, seizures and TBI (Schmidt-Kastner, 1996; Lyeth et al., 1990). Therefore the hippocampus has been the focus of many animal studies investigating the effects of TBI. Among diffuse injuries, the hippocampus is known to be particularly susceptible in humans, with neuronal loss occurring in greater than 80% of fatal TBIs (Kptapka et al., 1994). Several studies have demonstrated a loss of cells in the hilus, CA3 and CA1 regions of the rodent hippocampus at various time points following experimental TBI (Baldwin et al., 1997, Grady et al., 2003). Axonal injury is also common throughout the hippocampus following injury (Povlishock et al., 1996). Studies have demonstrated alterations in granule cell mossy fiber projections (Santhakumar et al., 2001) and subsequent synaptogenesis in CA1 following TBI (Scheff et al., 2005). However, cognitive deficits can exist in the absence of any overt hippocampal cell death or axonal injury, and therefore may reflect subtle neurochemical changes that disrupt circuitry (Lyeth et al., 1990). Furthermore, long- term potentiation (LTP), a major synaptic event implicated in learning and memory, undergoes significant changes following experimental brain injury (Reeves et al., 1995). Reports have noted significant changes in LTP as long as 15 days after injury (Reeves et al., 1995; Sanders, 2000). The Morris water maze (MWM), radial arm maze, and other similar memory tasks that require spatial

discrimination are sensitive to damage in the hippocampus (Morris et al., 1982). Hippocampal lesions as well as experimental brain injury models have consistently demonstrated deficits in performance of the spatial navigation tasks of the MWM (Eichenbaum et al., 1990; Hamm et al., 1993). Because TBI consistently produces hippocampal-dependent cognitive damage, as well as reproducible histopathology, the hippocampus is a unique model system for studying the neuropathology and biology of TBI and the effects of therapeutic treatments.

Neuropathology & Mechanisms of Brain injury

Classification of Brain Injury: Primary, Secondary, Focal, and Diffuse

Classification of brain damage after trauma should take into account the full spectrum of clinical presentation and outcome. Early classifications of brain injury helped identify potentially preventable complications in patients, in which TBI was considered to be either primary (induced by mechanical forces), which occurs at the moment of injury, or secondary (non-mechanical forces), which are delayed events that occur independently or as a direct result of primary injury. Primary injury that occurs as a result of the initial insult is difficult to treat therapeutically; therefore, treatment interventions have focused on attenuating extensive secondary injury that does not manifest clinically until a delayed period of time (Werner and Engelhead, 2007). Another classification has been derived based on structural brain damage after trauma using the terms focal and diffuse injury. Primary injury includes physical damage from contact as well as angular movement that can be both focal and diffuse. Secondary injury can also be classified as focal and diffuse,

but is more variable than primary injury and generally involves cascades of events resulting from combined structural, biochemical and molecular changes.

Focal injury typically includes lesions of the scalp, the dura and skull fractures, as well as contusions, intracranial hematomas, edema, alterations in cerebral blood flow, increased intracranial pressure, cerebral ischemia, regional hypometabolism and vascular changes. One explanation for the hemorrhaging and the formation of hematomas following injury is the rupturing of cerebral arterioles caused by the shearing and tensile forces generated by the injury (Povlishock and Christman, 1995). In most cases contusions, which are the hallmark of head injury, are the byproduct of hemorrhagic lesions within the gray matter or at the gray-white interface and contribute to neuronal damage and ischemia (for review, Povlishock and Katz, 2005). Contusions are generally located on the frontal and temporal poles, the lateral and inferior surfaces of the frontal and temporal lobes, and above the Sylvian fissure (Povlishock and Christman, 1995; Gennarelli and Grahan, 1998; Gaetz, 2004). Diffuse brain injury refers to a number of pathologies that are widely distributed including the consequences of acceleration and deceleration forces on white matter, often referred to as diffuse axonal injury (DAI), diffuse vascular injury, diffuse hypoxic-ischemic injury, and diffuse hypometabolism. Although the terms focal and diffuse refer to specific pathologies following injury, the majority of evidence suggests they do coexist and have common secondary and metabolic consequences. Therefore, experimental injury models that combine both focal and diffuse injury may better replicate the pathology of human head injury.

Understanding the pathophysiology following injury is important for understanding the functional deficits observed following injury and for determining an optimal treatment strategy. One of the devastating consequences of injury is the loss of neural, glial and endothelial cells. Necrosis and apoptosis are the two major types of cell death observed. Apoptosis, or programmed cell death is different from necrosis in that it requires ATP and protein synthesis for its execution and forms a characteristic fragmentation that is thought to be the result of activation of various proteases, such as caspases and calpains (Porter et al., 1999). Neurons, oligodendrocytes, and astrocytes have all been shown to die from apoptosis following TBI (Newcomb et al., 1999). Necrotic and apoptotic cell death are common secondary injury consequences that are seen both in the contusional and pericontusional sites, but also in diffuse areas of the brain. In the rat, the volume of cortical contusion and the size of the ventricles increase as time passes post-injury (Smith et al., 2000). Neuronal loss also occurs in diffuse areas such as the hippocampus, and apoptotic neurons have been observed in the human hippocampus up to 12 months after injury (Williams et al., 2001). Traumatic axonal injury (TAI- known as DAI in humans) results from the acceleration/deceleration forces observed in trauma, in which focal alterations in the axolemma lead to progressive changes in axonal transport, swelling and eventually delayed or "secondary" axotomy (Grady et al., 1993; Kelly et al., 2006). Within 3- to 6-h post-injury, axonal swelling increases to form a retraction bulb and ultimately results in axonal separation, depending on the fiber type and size (Povlishock and Kontos, 1985; Farkas and Povlishock, 2007). Therefore, axonal injury can not be attributed solely to shearing of axons that occurs at the moment of injury but rather is the

result of delayed injury-induced changes within the axon. One consequence of which is downstream deafferntation, or Wallerian degeneration, which may provide an explanation not only for the morbidity associated with DAI, but also set the stage for subsequent adaptive (or maladaptive plasticity), such as sprouting of adjacent intact nerve fibers and functional recovery of neuronal disruption. The widespread metabolic and neurochemical changes that may lead to the pathology observed following TBI will be discussed in more detail below.

Metabolic & Neurochemical Changes

The metabolic and neurochemical consequences of TBI involve regional changes as well as global abnormalities that change over time. Identifying specific changes and the compounds that are involved allows targeted therapeutic strategies to be developed. The biphasic model of injury, based on work by von Monakow (1969) and Feeney (1991) describes the consequences of injury in terms of acute and chronic events (for a review, see Whiting and Hamm, 2006). The biphasic hypothesis in animals describes an acute excitation phase lasting approximately 24 h, followed by a chronic depressed phase, lasting for weeks or months. Some of the more important changes and mechanisms are reviewed below.

The acute stage is characterized by a phase of neuroexcitation, and several studies have demonstrated that neuroexcitation following TBI significantly contributes to the pathophysiology observed following injury in both animals and humans (Faden et al., 1989; Hayes et al., 1992; Bullock et al., 1992). In addition, there is a widespread hyperglycoylcis that lasts for a relatively brief period of time post-injury and may be a

compensatory response to satisfy the heightened cellular energy demand following TBI (Yoshino et al., 1991; Jiang et al., 2000; Thomas et al., 2000). When glutamate, the primary excitatory NT in the CNS, activates NMDA receptors, it opens ion channels that permit the inflow of calcium and facilitates neuronal depolarization and signal transduction (Hayes and Dixon, 1994). Experimental and clinical evidence suggests extracellular glutamate and aspartate are excessively elevated post-injury, which leads to a cascade of metabolic disturbances, including the influx of Na^{+}, efflux of K^{2+}, and subsequent Ca^{2+} influx into the cell (Faden et al., 1989; Katayama et al., 1990; Globus et al., 1995). The changes in Ca^{2+} homeostasis are believed to play a pivotal role in neuronal cell death. Increased intracellular Ca^{2+} is thought to lead to mitochondrial impairment and the induction of apoptotic cell death cascades involving caspaces (Knoblach et al., 2002). Likewise, increases in extracellular K^{2+} have negative effects and may contribute to disruption of energy homeostasis, vasoconstriction, and changes in glycolysis (Siesjo and Wieloch, 1985). Excessive glutamate release can lead to the generation of free radicals and proteolytic enzymes, and further release of excitatory amino acids, which eventually contributes to cellular edema and the cytotoxic destruction of cells through both direct and indirect pathways. The hippocampus is particularly vulnerable to excitotoxic events, and thus, excitotoxicity has been suggested to be one potential explanation for the observed cognitive deficits following injury (Temple et al., 2001). Electrophysiological studies have shown that both mild and moderate FPI can suppress LTP in the hippocampal CA1 field (Reeves et al., 1995). In animals, this rise in extracellular glutamate is short-lived, lasting less than 24h, however, in humans glutamate concentrations may be elevated for several

days or perhaps weeks (Baker et al., 1993; Palmer et al., 1994). Therapeutic strategies used in the acute phase have focused on glutamate antagonism to prevent excitotoxicity and preserve neurons and axons; however this strategy has met with limited success (Tolias and Bullock, 2004). Other strategies have focused on inhibition of calcium channels; however, considerable uncertainty remains over the effects of these drugs (Langham et al., 2003). Acute surges in epinephrine, norepinephrine (NE), dopamine (DA) and serotonin (5HT) have also been reported in proportion to injury severity, as well as regional changes in the tissue concentration of these neurotransmitters (Prasad et al., 1992; McIntosh et al., 1994). Acute elevation of catecholamines is predictive of poor recovery following injury.

Although much research has been dedicated to understanding the mechanisms of acute pathology, much less is known about the chronic phase of injury. In contrast to the acute stage, the chronic phase of injury is characterized by widespread neuronal depression which includes decreased cellular activity and efficacy (Yoshino et al., 1991). This period of chronic neuronal hypofunction lasts for days to weeks and depends on severity of injury. It has been shown that the early hyperglycolytic phase changes to reduced glucose metabolism, which lasts until the patient recovers (Bergsneider et al., 2001; Wu et al., 2004). Acute surges in neurotransmitters abate with time, and decreases in cerebral metabolism are seen in the chronic period post-injury. Chronic reductions in cholinergic function are also reported in both animals (Dixon et al., 1994; Jiang et al., 1994) and humans (Arciniegas, 2003). Catecholamine levels are similarly affected and will be discussed in more detail in a subsequent section.

The origins of chronic neuronal hypofunction following TBI are uncertain. However, it is possible that the dramatic cell loss, axonal damage, and deafferntation from both focal and diffuse damage lead to global metabolic effects by disconnecting neurons and altering hundreds of circuits. Post-traumatic neural depression was first described as diaschisis, which refers to a state of functional depression in areas remote from the lesion (von Monakow, 1914). Feeney has recently revised this theory and termed it Remote Functional Depression (RFD) (Feeney, 1991). The theory of RFD states that a lack of neural input to remote brain regions results in depression of neuronal activity. Although controversial at first, the theory of RFD has been confirmed by measures of blood flow, metabolism, electrical activity, and neurotransmitter levels (Meyer et al., 1970; Hovda et al., 1987; Yoshino et al., 1991; Baron et al., 1992). Prolonged depression of neural input may result in structural changes in remote brain regions, and may lead to the functional impairments seen following brain injury. Both PET and SPECT assessments of neural depression correlate with specific dysfunction in cognitive tasks, in which greater depression is directly proportional to the amount of dysfunction (Goldenberg et al., 1992; Ricker et al., 2001). Taken together, the findings from both basic and clinical studies support the theory of RFD.

Other Mechanisms involved in TBI

Oxidative damage, manifested primarily as lipid peroxidation, has been implicated in many of the pathological changes following TBI (Ercan et al., 2001). Following injury, alterations in blood flow without adequate restoration of metabolic substrates, as well as other mechanisms such as the initiation of the arachidonic acid (AA)

cascade, excessive glutamate release and the increase in intracellular calcium all lead to the production of reactive oxygen species (ROS) and AA cascade metabolites (Ikeda and Long, 1990). ROS cause peroxidative destruction to the lipid bilayer of cell membranes and the microvasculature, which may affect the BBB integrity (Povlishock and Kontos, 1992). They also affect regulatory mechanisms, including activation of cytokines and regulation of calmodulin-regulated gene transcription (Yao et al., 1996). ROS overwhelm any endogenous antioxidants and are a key contributor to necrotic cell death and a promoter of apoptosis (Kannan and Jain, 2000). The final products of the AA cascade produce pro-inflammatory elements and have been associated with neuronal death and poor outcome in experimental TBI (Hall, 1985). There are also dramatic changes in axons, in which it is thought that Ca^{2+} permeability leads to activated calpains, caspases and the formation of ROS which in turn disrupt axonal transport and may lead to axonal collapse and detachment (Buki et al., 1999, 2000). A number of studies have investigated therapies that block specific steps in the AA cascade and nitric oxide (Slavik et al., 1999) or use antioxidants and free radical scavengers to prevent free radical damage (Shohami et al., 1997; Marklund et al., 2001; Nakamura et al., 2003).

Profound changes in the BBB have also been observed in both animals (Povlishock et al, 1978) and humans (Soares et al., 1992) following TBI. The permeability of the BBB is usually altered following injury (Povlishock et al., 1978; Cortez et al., 1989), allowing a number of foreign proteins and immunocompetent cells found in the plasma, such as leukocytes, circulating antibodies, and pro-inflammatory cytokines to enter the brain and cause damage through numerous secondary cascades. The accumulation of leukocytes has

been correlated with the onset of lowered CBF, brain swelling, and elevated ICP (Schoettle et al., 1990; Zhuang et al., 1993). There is also an accumulation of macrophages, both from glial production and the periphery, which produce soluble factors, including cytokines. Inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukins may contribute to the physiological cascades of brain damage following injury, including apoptosis and necrosis (Fan et al., 1995; Friedlander et al., 1996). Several therapeutic approaches have focused on blocking these factors with some success. Cyclosporine A, a immunosuppressive compound that inhibits activation of T-lymphocytes, showed some success as a neuroprotective agent and improved cognition in experimental studies (Alessandri et al., 2002). More recent strategies have begun to investigate the neuroprotective effects of cerebral hypothermia which may protect the vasculature and prevent secondary cascades, however, a controlled clinical trial did not find any evidence of benefit and thus many questions remain (Suehiro and Povlishock, 2001; Clifton et al., 2001).

Mechanisms of Neural Recovery

A large and increasing number of studies have demonstrated the ability of the brain to adapt to the dramatic changes of injury, with changes in functional and structural reorganization including dendritic and axonal sprouting, synaptogenesis, neurogenesis, altered synaptic activity, and recruitment of parallel pathways (Schallert et al., 2000; Scheff et al., 2005; Richardson et al., 2007). These changes, referred to as plasticity, may serve an adaptive role in recovery or maladaptive role, depending on the type and severity of injury. The scope of these compensatory mechanisms, their time course of presentation,

and their relationship to injury severity and recovery of function are not well understood, however, several studies are beginning to investigate these mechanisms and search for therapeutic strategies to enhance these endogenous responses. Three major physiological responses that are involved in the recovery of function are compensatory neuronal sprouting to restore lost synapses, the proliferation and differentiation of new cells, and biological modifications to modify the efficacy of existing synapses and reverse diaschsis.

Glia and the extracellular matrix both play an important role in the modulation of neuronal activity and synaptic plasticity (Horner and Palmer, 2003; Dityatev and Schachner, 2003). Glia, by releasing NTs and other extracellular signals can affect neuronal excitability and synaptic transmission. Glia, essentially communicate with other glial cells through gap junctions, allowing for a glial network that interacts with neurons (Haydon, 2001). Following injury, the blood brain barrier (BBB) is disrupted and the microglial response is initiated, stimulating the proliferation of astrocytes. Reactive astrocytes help build a glial scar, which may serve to repair the BBB and limit cellular degeneration. However, despite its protective effect, the formation of a glial scar, which consists mainly of reactive astrocytes and proteoglycans, specifically chondroitin sulphate proteoglycans (CSPGs), is one of the major barriers to successful regeneration. Although the potential for axonal sprouting may be limited by the post-injury cellular environment, lesion studies have demonstrated axons from nearby neurons are able to sprout to nearby synapses and reinnervate denervated zones, and thus strengthen or reorganize previously existing connections (Selzer, 2003; Dancause et al., 2005). For example, following entorhinal cortex lesions (ECL) sprouting fibers have been found arising from contralateral

entorhinal cortex (Deller, 1996), commissural/associational projections, mossy fibers (Steward, 1992), septo-dentate projections, and central noradrenergic fibers in the dentate gyrus (Peterson et al., 1994). In addition, lesioned corticospinal tract axons can sprout into the intact grey matter and establish novel circuits with long spinal neurons that bypass the lesion site (Bareyre *et al*., 2004). Therapeutic approaches aimed at these processes have attempted to enhance the endogenous plasticity response by counteracting CNS growth-inhibitory components, specifically CNS myelin proteins (e.g. NOGO-A, MAG) and extracellular matrix components (Bushli and Schwab, 2005; Falo et al., 2006).

Following brain damage, there is a release of various growth-promoting factors and growth inhibitory factors which create a unique cellular milieu. Growth factors such as brain derived growth factor (BDNF), nerve growth factor (NGF), and fibroblast growth factor (FGF) are all affected at various time points post-injury (DeKosky et al., 1994; Hicks et al., 1999). These growth factors all function in the normal brain to support neuronal survival, increase sprouting of neurites, and facilitate the growth of neurons to their appropriate targets (Huang and Reichardt, 2001); therefore, increases in these growth factors may be important for regeneration and neurogenesis following TBI. Neural depression following brain injury may affect the levels of these neurotrophins as is seen in other disorders (Schmidt and Duman et al., 2007). Studies have suggested that neurotrophin expression is regulated by neuronal activity (Zafra et al., 1990; Thoenen, 1995), and vagus nerve stimulation therapies, as well as drugs that increase norepinephrine have been shown to increase levels of neurotrophins (Follesa et al., 2007; Calabrese et al., 2007). Neurotrophic factors interact with a number of effectors, such as cAMP, which

may help to overcome inhibitory actions of growth-inhibitory proteins (Cui, 2006). Numerous studies have demonstrated that neurotrophin treatments following injury have positive effects on axonal regeneration and cell survival (Saatman et al., 1997; Yoshimura et al., 2003). One growth factor treatment hat has gained recent support is erythropoietin (EPO). EPO has been shown to be neuroprotective and to stimulate angiogenesis, neurogenesis, and increase the levels of other growth factors (Shein et al., 2008). Thus it appears that growth factors and treatments that influence their expression have the potential to influence outcome at multiple levels.

The adult brain also contains neural stem cells that have the potential to differentiate into neurons, astrocytes, and oligodendrocytes (Song, et al, 2002; Galli et al., 2008). Neurogenesis, or the process of functional integration of new neurons, has been demonstrated in the adult brain, specifically in the subventricular zone (SVZ) and the subgranular zone of the dentate gyrus (Eriksson et al., 1998). Following experimental injury in LFP and CCI models, cell proliferation becomes dramatically increased in the hippocampus as early as 2 days post-injury and gradually returns to baseline by day 35 (Sun et al., 2005; Urrea et al., 2007). Furthermore, it appears that most of the proliferating cells in the DG differentiate and become neurons, some of which receive synaptic input (Kernie et al., 2001; Sun et al., 2007). Moreover, mRNA levels of several cell cycle genes and markers for neuronal progenitor cells were up-regulated in the cortex and hippocampus following experimental injury (Rall et al., 2003). It is tempting to speculate that these changes may represent an attempt by the brain to replace lost neurons and create new functional circuits, however, it is well known that there are many blockades including the

glial scar formation and inflammatory processes that may limit neural recovery. Still, therapeutic treatments aimed at manipulating the environment to create a more favorable milieu for neurogenesis offers hope as a therapeutic treatment following injury.

Finally, synaptic reorganization also occurs through strengthening of existing connections and an attempt to increase neuronal activity. The CNS has many redundant pathways, and thus when a primary pathway is destroyed or lost to injury, a secondary pathway may acquire increased importance (Stroemer et al., 1995). There also may be an unmasking of silent synapses, which is to say if a primary afferent is lost, other "silent", or secondary, afferents may become more important (Blitz et al., 2004). Changes in receptors following injury may be another mechanism used to compensate for the loss of inputs in order to increase synaptic efficacy. Therapies aimed at these processes include environmental enrichment and physical therapy to enhance experience-driven relearning (Hoffman et al., 2008), and various stimulant and neurotransmitter therapies to increase brain activation and reverse RFD. Norepinephrine, in particular, has been suggested to play a major role in the development of diaschisis and if resolution of metabolic depression is a form of brain plasticity, it appears to require an optimal catecholamine environment (Feeney et al., 1988).

Pharmacological Approaches to Treatment of TBI

This chapter has reviewed some of the mechanisms of brain injury and neural recovery, highlighting various therapeutic treatments being investigated following brain injury. The acute stages have focused on neuroprotective agents to protect cells and axons by preventing extensive secondary damage. Treatment of chronic cognitive impairments

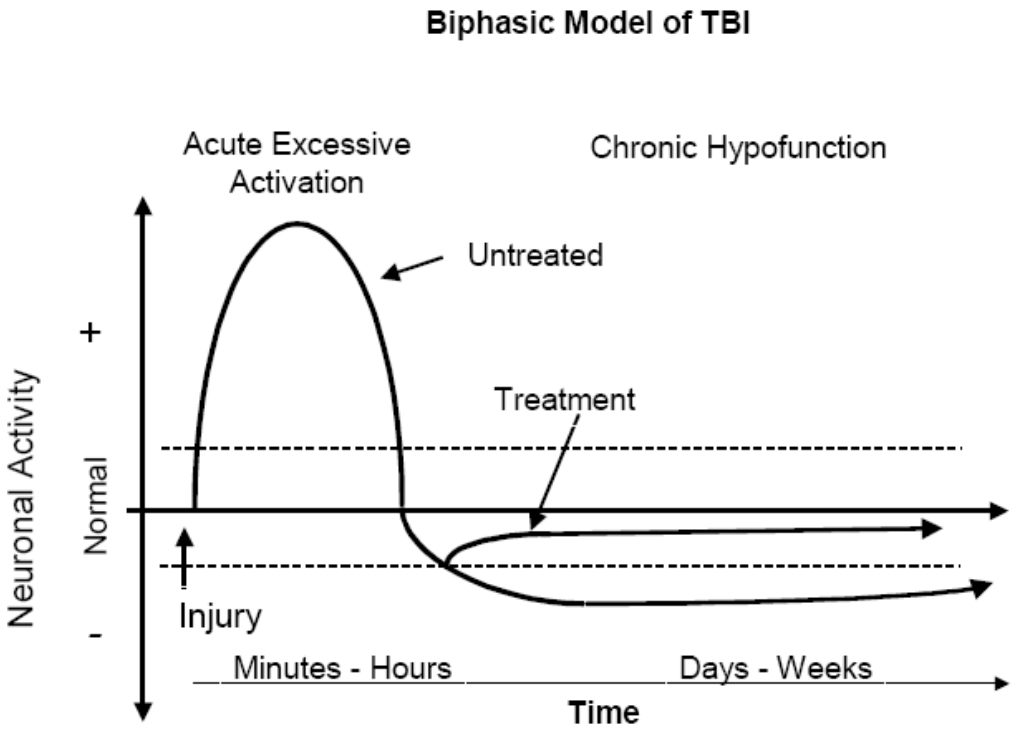
following brain injury has focused on neurorehabilitation, including a combination of restorative or compensatory approaches to enhance plasticity and neural reorganization and restore functional outcome. The complexity of TBI pathology may require a combination of many therapies that are given at different time points. In addition, outcome is highly dependent on both the type and severity of injury. Therefore it is important to have many options for therapeutic intervention.

Pharmacological agents that target neurotransmitter dysfunction have commonly attempted to compensate for the TBI-induced changes in these systems. Careful consideration of the time course of these TBI- induced changes and the timing of treatment are important to avoid adverse side effects. The differences in activity and metabolism between the acute and chronic stages are crucial to understand when developing potential pharmacological treatments to facilitate functional recovery (Figure 1. The Biphasic model). According to the biphasic model, interventions targeting the chronic phase of TBI should be designed to increase neuronal activity to previously normal levels. Treatments that are effective in the acute phase may be detrimental when administered in the chronic phase (Hamm et al., 1993). For example glutamate antagonists administered in the acute phase to reduce neuronal activity and prevent secondary damage, are detrimental if administered in the chronic stage, when glutamate agonists or drugs that increase neuronal activity are favored (Phillips et al., 1999). Drugs that exacerbate neural depression following injury, such as haloperidol or GABA antagonists, also delay recovery (O'Dell et al., 2000; Wilson et al., 2003). Targeting neural depression for therapeutic treatments also has advantages in that it affords an extended window for initiating treatment, allowing

physicians to initially focus on stabilization of patients. It is also measurable with imaging techniques, and able to be correlated with behavioral deficits.

Pharmacological treatment with neurotransmitters in the chronic stages has focused on cholinergic and catecholaminergic treatments. The rationale for treatment is based on evidence that multiple NT pathways are disturbed following injury and therefore, treatments that have known effects on these systems may have an important role in facilitating recovery. In addition, many of the neurobehavioral symptoms that are present after TBI show considerable resemblances to symptoms in other neuropsychiatric conditions, such as attention-deficit/hyperactivity disorder (ADHD), Alzheimer's disease, alertness in narcolepsy, and mental speed of processing seen in many conditions. Therefore, treatments that are normally used to treat these conditions may have some benefit in the TBI population. The catecholamines have also been suggested to be beneficial for promotion of recovery from coma and minimally responsive states, although there has been little research in this area (Giacino and Trott, 2004). Although there appears to be some benefit from drug treatment, many clinical questions remain such as: Which patients will respond favorably to treatment with these agents? Which cognitive functions may be facilitated? What is the optimal dose? When should treatment be initiated and for how long? What is the duration of effect? Answering these questions helps to elucidate the potential mechanisms of these drugs, and leads to a better understanding of when these drugs should be used.

Figure 1. A diagram of the biphasic model for treatment following TBI. Neuronal activity is increased during the acute stage, followed by a sustained chronic neuronal depression lasting for weeks or months post-injury. Acute interventions should decrease neuronal activity, whereas chronic or subacute interventions should aim for increasing neuronal activity. Successful treatments in the chronic stage should return neuronal activity to near baseline or uninjured levels.


Biphasic Model of TBI
Acute Excessive Activation
Chronic Hypofunction
Untreated
Treatment
Neuronal Activity
+
Normal
-
Injury
Minutes - Hours
Days - Weeks
Time

The Catecholamines: Norepinephrine and Dopamine

Norepinephrine

Norepinephrine, Receptors & The Norepinephrine Transporter

Norepinephrine (NE) is a neurotransmitter found in the central nervous system and biosynthesized from the amino acid tyrosine, which is decarboxylated to produce dopamine, and hydroxylated to form NE (Axelrod, 1974). NE plays an important role as a neuromodulator in the adult CNS and has been implicated in attention/arousal, mood regulation, sleep regulation, depression, anxiety and aspects of memory. The central noradrenergic system has two distinct projection sites: those originating from the lateral tegmental noradrenergic cells, which are implicated in sexual and feeding behaviors, and those originating from the locus ceruleus (LC), which are implicated in cognition. The LC resides in the pons and fibers from these cell bodies project to wide areas of the brain including the cortex, hippocampus, thalamus, limbic system, and cerebellum. NE innervation appears to be nonjunctional, that is to say NE receptors are far more widely distributed than their nerve terminals, and the NE receptor distribution does not resemble the pattern of NE fiber distribution. Two types of LC-derived axonal terminals have been described: conventional synaptic structures and nonsynaptic varicosities. The later are believed to release NE extrasynaptically allowing for its diffusion over some distance in the microenvironment before it may act on surrounding neurons, glial cells, and blood vessels (Marien et al., 2004).

NE interacts with three families of adrenergic receptors: the α1, the α2 and the β adrenergic receptors. Because of their mechanism of action, these receptors are referred to

as G-protein-coupled receptors. The binding of NE induces a conformational change that allows the receptor to interact with and activate a G protein, which in turn leads to the dissociation of the α and βγ subunits and downstream stimulation or inhibition of various effectors. α1 receptors are generally coupled to G_q proteins that activate phospholipase C and phosphotidyl inositol intracellular signaling, resulting in activation of protein kinase C (PKC) and the release of intracellular calcium via inositol 1, 4, 5-triphosphate (Duman & Nestler, 1995; Birnbaum et al., 2004). Activation of α1-adrenergic receptors leads to up-regulation of CREB-mediated gene transcription. α2 autoreceptors are generally found presynaptically and are coupled to G_i proteins (Duman & Nestler, 1995; Ramos et al., 2006), which can reduce intracellular cAMP production by inhibiting adenylyl cyclase. Both α1, α2 receptors are found throughout the cortex, however, only certain subtypes of these receptors are found within the hippocampus (Winzer-Serhan and Leslie, 1997).

β -adrenergic receptors are primarily located in the cortex, nucleus accumbens and striatum, with lower, yet significant densities in the amygdala and hippocampus. These receptors are found in postsynaptic sites on DG granule cells and interneurons as well as in glial processes in the hippocampus (Guo and Li, 2007). They are also found primarily postsynaptically on dendritic spines, GABAergic neurons, and glia in the frontal cortex (Aoki et al., 1998). There are three β-adrenergic subtypes (β1, β2, and β3), and all are positively coupled to adenyl cyclase via activation of Gs (Pupo and Minneman, 2001). β -adrenergic receptor activation of cAMP results in stimulation of PKA. PKA can phosphorylate and activate CREB and other transcription factors, leading to altered regulation of gene expression. CREB is expressed throughout the brain and is involved in

synaptic plasticity and other cellular functions in many brain regions. Studies suggest that memory consolidation and memory retrieval by the hippocampus may be dependent on beta-adrenergic activity and cAMP-PKA signaling (Arnsten et al., 2005; Korz, 2007). This evidence taken together suggests central β-adrenoceptors are involved in neuronal and behavioral plasticity (Berridge et al., 1996).

The norepinephrine transporter (NET) is located on the plasma membrane of noradrenergic neurons where it functions to reuptake NE from the synapse into the presynaptic terminal, therefore terminating and limiting the action of this neurotransmitter. The NET not only regulates the longevity of NE in the synapse but also plays a very important role in presynaptic and postsynaptic homeostasis (Zhou, 2004). Studies using NET-deficient mice, have found profound changes in NE homeostasis and synaptic plasticity, suggesting a role for the NET in these processes (Xu et al., 2000). NETs also transport other catecholamines, including DA and epinephrine. Strong NET expression is found globally throughout the brain including such areas as the adult cortex, hippocampus, thalamus, and amygdala, correlating with areas of dense NE innervation (Sanders et al., 2005). There are also high levels of NET found in the LC and DA-rich areas such as the ventral tegmental area (VTA) and PFC, in which the NET also contributes to the clearance of DA in these brain regions. The NET has also been found on astrocytes and endothelium, suggesting a role for these cells in regulation of NE (Inazu et al., 2003). Given that NET's control the spatial and temporal aspects of NE action, modulation of NET activity would be expected to influence NE neurotransmission. Selective or mixed monoamine transporter inhibitors have been developed to treat a variety of disorders

including mood disorders, personality disorders, depression, Alzheimer's disease, chronic pain, migraine, stroke and trauma (Van Moffaret et al., 1999; Olivier et al., 2000; Wong et al., 2000; Zhou et al., 2003).

NE and Cognition

The noradrenergic system is a well-known modulator of neuronal activity and has been implicated in many aspects of brain function, including vigilance, arousal, attention and aspects of learning and memory (Aston-Jones et al., 1991; Cole and Robbins 1992; Berridge 1993). NE has been termed both a neurotransmitter and a neuromodulator. NE projections from the LC innervate the entire neuroaxis, positioning this NT to enact global state-change functions and influence the outcome characteristics of the brain when NE is elevated. The cellular effects of NE are modulatory in nature, altering the "signal-to – noise" activity in circuits and allowing synaptic transmission to be more effective, and thus presumably enhancing cognition and behavioral responses in brain areas innervated by NE (Woodward et al., 1991).

The NE projections to the frontal cortex are important for mechanisms of attention and arousal, as well as working memory. Elevating central NE activity, through α_2-adrenergic autoreceptor antagonists or NET inhibitors, improved performance in tasks assessing attention and working memory (Bunsey and Strupp 1995; Sagvolden and Zu 2008), whereas reduced NE transmission had deleterious effects on attention (Smith and Nutt 1996). Depletion of forebrain NE in rats leads to distractibility and attentional deficits in a variety of paradigms (Carli et al., 1983; Cole and Robbins, 1992). Furthermore, NE receptor blockade induced a state of sedation and behavioral inactivity.

The hippocampus has one of the denser regions of adrenergic terminals, supporting the role of NE in learning and memory in this region (Korz, 2007). NE innervation is particularily dense in areas receiving mossy fiber inputs, including the dentate gyrus and stratum lucidum (Moudy et al., 1993). Several studies have demonstrated the importance of NE signaling for consolidation of emotionally laden events, especially for aversively trained animals, which may involve the amygdala and hippocampus (Ferry and McGaugh, 2000). Other studies have begun to investigate a role of NE in spatial navigation tasks and have found that NE is important for consolidation and retrieval of spatial memories (Murchison et al., 2004; Korz, 2007). Animals with reduced cortical NE levels performed significantly worse in the spatial memory task of the MWM compared to matched controls (Collier et al., 2004). Enhancing NE tone prior to retention testing showed significantly less "forgetting" in a spatial memory task of the MWM (Sara et al., 1989).

NE mechanisms may also play an important role in neuroplasticity in the hippocampus. Considerable evidence indicates NE increases the signal- to- noise ratio by inhibiting neuronal background firing and enhancing responses to significant stimuli. In vitro studies in cortical and hippocampal slices show that NE depletion reduces or eliminates LTP (Harley, 1998), whereas NE agonists increase LTP (Izumi et al., 1999). There is considerable evidence to suggest NE manipulations can modulate early LTP in several hippocampal pathways (Munro et al., 2001; Harley, 2008). These mechanisms have focused on beta-adrenergic receptors. For example, NE enhances LTP in the mossy fiber-CA3 synapse via beta-adrenergic mechanism, and pharmacological activation of beta- adrenergic receptors can rescue CA1 LTP in genetic strains with impaired LTP

(Schimanski et al., 2007). NE in the dentate gyrus permits long-term perfornat path potentiation, and phasic LC activation produces delayed protein synthesis-dependant LTP of synaptic plasticity (Dahl and Sarvey, 1989). Activation of the LC causes a slow onset LTP in the dentate gyrus, and adrenergic agonists result in the conversion of early LTP to protein-dependent late LTP in CA1 and the dentate gyrus (Harley, 2007). Finally, depletion of NE and destruction of NE terminals results in a dramatic acceleration in the rate of seizure development with electrical stimulation of limbic or cortical structures (Lewis and Westerburg, 1987). Therefore, NE plays an important role in maintaining excitatory/inhibitory homeostasis and influences synaptic plasticity.

Finally, a number of disease states show alterations in NE neurotransmission. In aged animals, even a small degree of LC cell loss correlates strongly with impaired memory (Leslie et al., 1985). Furthermore, electrical stimulation or pharmacological activation of the LC protects from age-related memory loss (Birthelmer et al., 2003). NE has been theorized to play a role in the progression of Alzheimer's disease (for a review, see Marien et al., 2004). This theory may be related to the interactions between NE and acetylcholine in the brain. There are many overlapping terminal fields, where these neurotransmitters may influence each other, including the basal forebrain (Zaborszky et al., 1993). The interactions between these NTs may also explain why drugs that increase extracellular levels of NE also tend to increase acetylcholine levels (Tzavara et al., 2006). Even more interesting, are the studies that have found the appearance of ingrowth of NE fibers and increases in NE concentrations in the hippocampus following cholinergic lesions (Harrell et al., 2005). This study suggests peripheral adrenergic sprouting and

hippocampal ingrowth can be neuroprotective and may be able to replace the central cholinergic system in terms of function.

NE has also been thoroughly investigated in the context of ADHD. Several recent studies, suggest that NE may be even more important that DA, particularly in regards to treatment (Biederman et al., 2000). In addition, NE has also been hypothesized to play an important role in stress and depression, and has been implicated in the effects of antidepressant drugs (Laifenfeld et al., 2005). Depression and stress have been associated with impairments of structural plasticity (Fuchs et al., 2004), and studies have found antidepressant treatments, which increase NE and 5-HT, induce increases in protein kinase C (Morishita and Aoki, 2002), calcium-linked protein kinases (Consogno et al., 2001) protein kinase A (Nestler et al., 1989). It is hypothesized that these changes lead to increases in CREB and changes in gene expression, including an increase in neurotrophic factors. These changes may account for the clinical success of antidepressants and explain why there is often a delay between initiation of antidepressants and observed clinical effects. In contrast, too much NE has been linked to the symptoms of mania, schizophrenia, and PTSD. The evidence taken together highlights the delicate balance between catecholamine levels and disease states, where too much or too little can cause various levels of dysfunction.

Norepinephrine and Mechanisms Important to TBI

Apart from the role of NE as a neurotransmitter, NE may influence the survival, maintenance and plasticity of CNS neurons (including the regulation of neurotrophins), glial functions, and inflammatory responses. NE has been hypothesized to play a role in

neurogenesis in both the subventicular zone and the hippocampus. Following, dexefaroxan treatment (which increases NE release) cell proliferation, assessed by BrdU labeling, was significantly increased relative to saline treated controls in the olfactory bulb, while apoptotic cell death was decreased (Veyrac et al., 2005). In the hippocampus, NE depletion was found to affect the proliferation of adult hippocampal granule cell progenitors (Kulkarni et al., 2002), while, treatment with dexefaroxan, which increases NE activity, increased the survival and differentiation of new hippocampal granule cells, but had relatively no effects on proliferation (Rizk et al., 2006). NE also been suggested to have effects on neurotrophins such as brain-derived gowth factor (BDNF) and nerve growth factor (NGF), which could influence these processes (Fawcett et al., 1998; Garcia et al., 2003; Ivy et al., 2003). For example, NE increases production of BDNF in cultured astrocytes (Juric and Carman-Krzan, 2006). Desipramine treatment, as well as other antidepressants that affect the levels of NE, have been shown to increase expression of CREB mRNA and protein in the hippocampus (Nibuya et al., 1996). Other studies have found desipramine also increases the levels of both BDNF and its receptor TrkB (Nibuya et al., 1995; Conti et al., 2002). Furthermore, selective NE reuptake inhibitors have been shown to increase adult neurogenesis and phosphorylated CREB (Duman et al., 2000). While the mechanisms underlying these effects are not completely understood, it does suggest NE pharmacology may influence neural recovery through enhancing post-injury-induced plasticity.

Dopamine

Dopamine, and Dopamine Receptors

Dopamine is the other catecholaminergic neurotransmitter that serves a neuromodulatory role in the CNS. Dopamine is synthesized in the cytoplasm of dopaminergic neurons from the precursor L-DOPA. Tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of both DA and NE. The DA transporter (DAT), located in the presynaptic membrane serves to reuptake DA into the membrane to be re-used or metabolized by monoamine oxidase (MAO). DA neurons originate from the ventral tegmental area (VTA) and substantia nigra pars compacta (SNC) and play a critical role in behavioral functions ranging from motor planning to cognition (Bjorklund and Lindvall, 1984). There are three main DA pathways-the nigrostriatal projections from the SNC to the basal ganglia, the mesolimbic pathway between VTA and limbic striatum, and the mesocortical pathway from the VTA to the to the frontal, cingulate, and hippocampal cortices (Bjorklund et al., 1984). The frontal cortex receives the majority of VTA DA projections in the rat, and in turn, receives excitatory projections from the PFC (Van Edyn et al., 1987). The nucleus accumbens and amygdala receive dopaminergic innervation from the VTA, which are thought to facilitate reward-seeking and emotional behavior (Groenewegen and Uylings, 2000). Cortical DA projections to the frontal cortex play a role in cognitive processes including working memory and motor planning (Sawaguchi and Goldman-Rakic, 1994). The dorsolateral prefrontal cortex receives a large amount of DA projections that are thought to be involved in is involved in attention, initiative, motivation, planning, decision making, working memory, and other higher order cognitive functions.

The VTA also send DA projections to the CA1/subiculum area of the hippocampus, which are thought to have a role in suppressing hippocampal excitability and/or novelty detection (Gasbarri et al., 1997).

Dopamine interacts with five receptor subtypes (D1-D5). All five are slow metabotrophic receptors that modulate other ion channels and receptor systems. In both rats and humans, DA receptors are found in the caudate-putamen, nucleus accumbens, olfactory tubercle, substantia nigra, periventricular nucleus of the hypothalamus, dentate gyrus, and the endopiriform cortex. D1 and D5 receptors are members of the D1-like receptor family, and the others are members of the D2-like receptor family. D1-like and D2-like receptors play opposing regulatory roles. D1 receptor activation activates cAMP or IP3 second messenger pathways and leads to intracellular signaling cascades involving PKA, phosphorylation of DARP32, and inhibition of protein phosphatase-1, and ultimately leads to activation of CREB, and transcription of immediate early genes (IEG) and late response genes (LRG) that mediate long term changes in ionic channel strength. D2 receptor mediated regulation is more complex, and there are many opposing opinions between researchers about the role of these receptors. D2 receptors suppress cAMP and activate platelet derived growth factor (PDGF) initiation of intracellular Ca^{2+} release, which ultimately inactivates NMDA receptors (Surmeier et al., 1995). In the rat, D1 and D2 receptors are found on both pyramidal and non-pyramidal neurons in the frontal cortex, however, a larger population of D1 receptors is found in this region (Benes and Beretta, 2001). In the hippocampus studies have shown a prominent labeling of D1-like receptors dorsally in granular cells of the dentate gyrus and the subiculum, but only a few labeled

cells in the stratum oriens and radiatum of CA1-CA3 (Fremeau et al., 1991). Recent studies suggest that D5 receptors are the predominant D1-like receptor in this region. D5 immunoreactive neurons are localized in the hilus and granular cells of the dentate gyrus, in pyramidal cells of the subiculum, but also in CA1 and CA3 (Ciliax et al., 2000; Khan et al., 2000). The distribution of D1 receptors at the subcellular level in the hippocampus appears in both pyramidal cells and dendrites (Bergson et al., 1995). In contrast, low levels of D2 receptor mRNA has been detected in the hippocampus (Levey et al., 1993). Although it is uncertain exactly how receptor distribution affects cognition, the brain clearly has multiple receptors for neurotransmitters. The types and amount of each receptor expressed in different brain regions appears to play an important role in the regulation of synaptic plasticity and memory processes.

Dopamine and Cognition:

DA is an important neurotransmitter in many frontal lobe processes such as executive functions and working memory. Lesions of the mesocortical DA projections impair working memory in monkeys (Brozoski et al., 1979) and rats (Simon, 1981). Behavioral studies have shown that an optimal level of dopamine and D1 receptor activation is crucial for delay dependent tasks requiring working memory functions subserved by the frontal cortex (Goldman Rakic, 1995). Experimental studies in rats, monkeys, and humans all highlight the inverted U-shaped relationship between DA levels and cognition.

In the hippocampus, the CA1 domain receives DA projections from the VTA that terminate predominantly in the SLM, and firing of these DA neurons in the VTA has been

linked with the prediction of motivationally significant events and reward, as well as novel events and stimuli (Gasbarri et al., 1997; for review, see Schultz, 2006). In the hippocampus, blockade or knockout of D1/D5 receptors impairs LTP in CA1 (O'Carroll and Morris, 2004), and dopamine agonists enhance the magnitude and persistence of CA1 LTP (Otmakhova and Lisman, 1996). DA agonists have been shown to enhance memory in both spatial and non-spatial tasks (Bach et al., 1999; Bernabeu et al., 1997). Conversely, treatments that decrease hippocampal DA activity impair spatial memory (Gasbarri et al., 1996). It is tempting to speculate, that DA activity in response to the reward and novelty of locating an escape platform may lead to subsequent stabilization of synaptic changes, however, this hypothesis requires significantly more investigation.

The Catecholamines and TBI

Several studies looking at neurochemical changes following TBI suggest that alterations in neurotransmitters, including changes in NT production, delivery or both, occur within networks both acutely and chronically and may differ depending on the brain region examined (Hayes et al., 1992; McIntosh et al., 1994). Experimental models have shown NE and DA levels rise in the acute phase, but are depressed in the sub- acute or chronic phase (Dunn-Meynell et al., 1994;Levin and Kraus, 1994). Plasma NE levels are elevated in the acute phase after TBI, and this elevation correlates with injury severity (Hamill et al., 1987; McIntosh et al., 1994; Schwarz et al., 1997). Some studies have suggested the early increase in NE may be protective and help stabilize the blood-brain barrier (Dunn-Meynell et al., 1998), whereas others have suggested NE may facilitate the damaging effects of glutamate. The chronic post-injury state, however, is characterized by

a relative decline in NE and DA metabolism (Goldstein, 1999; Donnemiller et al., 2000). One study found NE metabolism begins to decrease in the brain between 6h and 24 h after injury (Dunn-Meynell et al., 1998). Another found a 27% decrease in the size of LC neurons in the chronic phase following impact acceleration injury that gradually returns to normal over one month (Fujinaka et al., 2003). The same study confirmed that NE neurotransmission is affected beginning around 24-48 h after experimental TBI and remains low until eight weeks post-injury. Therefore, even after cell bodies recover, NE turnover may not. Norepinephrine fiber loss has been reported in the hippocampus following central-fluid percussion and the combined FPI+ entorhinal lesion model at 15 days post injury (Zhu et al., 2000). Similarly, DA tissue levels have been reported to decrease at 1 h. post-injury and remain depressed for up to two weeks following fluid-percussion injury (FPI) (McIntosh et al., 1994). Subsequent studies using controlled cortical impact (CCI) found cortical DA metabolism was depressed for up to four weeks post-injury (Massucci et al., 2004). DA kinetics, including turnover and release of DA, are also affected in the striatum, suggesting a hypodopaminergic state following trauma (Wagner et al., 2005). In addition, DA projections to the medial septal area (MSA) can modulate hippocampal acetylcholine release, and TBI has been shown to decrease extracellular DA levels. In humans, tyrosine, the precursor molecule for catecholamine production, was significantly lower than normal in TBI patients up to 2 months post-injury (Aquilani et al., 2003). Further studies using fMRI, suggest posttraumatic working memory impairments are specifically attributable to noradrenergic dysfunction (McAllister et al., 2004). The results taken together suggest chronic catecholamine hypofunction may

be one result of TBI. Because the catecholamines have a neuromodulatory role in the CNS and exercise a facilitating effect on many networks, damage to these neurons and their ascending axons could have diffuse effects on a wide range of cognitive functions (Dunn-Meynell et al., 1994).

In contrast, some studies have suggested that catecholamine synthesis and tissue levels are not depressed in the chronic stage, but actually elevated at one and two weeks post-injury (Tang et al., 1997; Kobori et al., 2006; Yan et al., 2001). Neurochemical disturbances clearly reflect the nature of the injury, as well as the affected brain regions (McIntosh, 1996). Therefore, differences between injury models are likely responsible for the conflicting results. The neuromodulatory neurotransmitters, NE and DA, are functionally interrelated and changes in one NT system may be felt downstream in another system by influencing neuronal firing rates or the sensitivity of receptors, which is often what happens in the development of mood disorders (Stokes et al., 1987). Therefore, NE and DA have been suggested to play a major role in the formation of diaschisis, or RFD (Feeney and Sutton 1998). Still many questions remain, and it is unknown to what extent alterations in catecholamine metabolism following TBI represent compensatory mechanisms versus changes due to loss of input or other secondary damage which may directly contribute to TBI-associated deficits. While the contributions of catecholamine dysfunction to cognitive deficits and the changes in catecholamine metabolism require further investigation, the findings from experimental studies do suggest these neurotransmitters are reliable targets for pharmacological interventions to improve functional outcome.

Catecholamine theory of recovery

Treatments that act on central neurotransmitter systems have been shown to alter recovery following brain injury. Considerable attention has been given to drugs that affect cholinergic and catecholaminergic systems. Several studies in animals and humans have investigated the effects of catecholaminergic agents following experimental and human brain injury. Some of the more pertinent treatments are reviewed below.

Amphetamine: Recovery of function after cortical injury, and the maintenance of that recovery in both animals and human may depend on the integrity of the noradrenergic system. The beneficial effects of NE following brain injury have been well documented for several years. Considerable evidence indicates pharmacologically enhancing brain NE concentrations following injury is beneficial, both at acute time points and at more delayed time points. (Feeney et al., 1997; Gladstone and Black, 2000; Goldstein, 2003; McIntosh, 1993). Amphetamine (AMP) activates both the NE and DA systems, and there is evidence that a single dose of dextroamphetamine (D-AMP) increases the rate of motor recovery in experimentally injured rats (Feeney et al., 1982). Although the exact mechanism of amphetamine is unknown, it is postulated to release pools of DA and NE from the locus ceruleus (LC) as well as block catecholamine reuptake into the presynaptic terminal (Boyeson et al., 1990). More recent studies using the fluid-percussion model on injury found AMP treatment can attenuate increases in lactate and free fatty acids seen after injury (Dhillion et al., 1998). Many subsequent studies have confirmed the role of NE in cortically injured rats by showing AMP administered 24h after cortical ablation injury accelerates motor recovery, as long as it is coupled with beam-walking (Dhillon et al.,

1998). It appears that D-AMP induced-recovery in both animals and humans requires co-training (Gladstone and Black, 2000), suggesting catecholaminergic stimulant treatment may serve as a facilitator of the learning experience. For example, when patients were treated with D-AMP paired with physical therapy, they found AMP enhanced the speed of recovery and extent of recovery from hemiplegia, which persisted for 1 year after discontinuation of the drug (Walker et al., 1995). Interestingly, studies that failed to couple drug regimen and physical therapy did not produce beneficial results (Feeney et al., 1997). It also appears therapeutic window is an important factor in D-AMP treatment. When treatment with D-AMP was delayed one month, no significant functional benefit was found, suggesting a critical window early after injury for treatment (Goldstein et al., 2003). These studies in animals provide a convincing role for AMP in motor recovery; however, relatively few studies have investigated AMP and cognition following injury. Some studies found cognitive function was improved if AMP was administered at 10 min, but not at 24h following L-FPI (Dose et al., 1997), suggesting the time period for treatment with this drug is limited to time points very early after injury. D-AMP is sometimes used to treat impairments in arousal, speed of processing, attention, and memory in the TBI population although the evidence to support its use is sparse (Hornstein et al., 1996). AMP is also a controlled substance with both abuse and addictive potential, which further limits the use of this drug in this population.

Methylphenidate: Methylphenidate is another stimulant that increases the release of both NE and DA, and at higher doses, blocks the reuptake of these NTs. Clinically it is indicated for attention deficit disorder to improve attention tasks and mental

processing (Malone et al., 1994). Early studies suggested that methylphenidate may improve posttraumatic impairments in arousal, speed of processing, attention, memory, mood, and aspects of behavior (Gualtieri et al., 1988; Mooney et al., 1993; Plegner et al., 1996). In experimental studies, Kline and coworkers (2000) found chronic methylphenidate treatment initiated 24h after Lateral-CCI and continued for 18 days resulted in significantly reduced latencies to find the platform in the spatial navigation task of the Morris water maze. Interestingly, this study found no improvement in motor function. These studies suggested methylphenidate may be an effective treatment for a range of cognitive dysfunction following injury. Clinically, however, Whyte and coworkers (1997, 2004) demonstrated that the primary benefit of methylphenidate is on post-traumatic impairments of processing speed, and to a lesser extent on subjective ratings of behavior and mood. Other clinical studies suggest that although methylphenidate might facilitate the rate of recovery, it may not necessarily improve long-term outcome (Plenger et al., 1996). Still, recent guidelines for pharmacologic treatment following TBI suggest methylphenidate is recommended to enhance attentional function, and the speed of cognitive processing, as well as an option to enhance learning and memory (Warden et al., 2006).

L-Deprenyl: L-Deprenyl is a selective and irreversible monoamine oxidase-B (MAO-B) inhibitor used clinically in the treatment of Parkinson's disease (Kieburtz et al., 1994). L-Deprenyl has been shown to protect hippocampal pyramidal cells from ischemic damage and improve cognitive performance of aged rats (Knoll, 1993). Following experimental brain injury of moderate severity, chronic L-Deprenyl treatment for 7 days

post-injury significantly reduced cognitive deficits in the spatial navigation task of the MWM on days 11-15 and decreased catecholaminergic fiber loss in the hippocampus (Zhu et al., 2000). By terminating drug treatment before MWM testing, this study suggests augmenting NE and DA levels may have long-term effects on functional outcome. However, the mechanism is uncertain and may be related to L-Deprenyl's effects on oxidative free radicals or enhanced synthesis of growth factors, rather than augmenting catecholaminergic neural transmission. A recent case study in 4 patients shows L-Deprenyl has potential for the management of apathy following TBI (Newburn and Newburn, 2005).

Amantadine and Memantine: Amantadine and memantine are non-competitive NMDA receptor antagonists; however, there effects following injury appear to be more related to their ability to increase DA release, stimulate dopamine receptors and/or enhance postsynaptic dopamine receptor sensitivity (Page et al., 2000; Peeters et al., 2003, 2004). Amantadine treatment, initiated 24h after injury and continued for 18 days, has been shown to improve performance in the MWM spatial task (Dixon et al., 1999). Clinically, Nickels et al., found amantadine improved attention, concentration, arousal, speed of processing, agitation and anxiety in patients (1994). Meythaler et al. (2002), in a double-blind, placebo controlled randomized trial, found significant improvements in functional outcome after six weeks of treatment. Other studies in the brain injured population have found significant improvements in executive functioning, agitation, and subjective cognitive improvements (Kraus et al., 1997; Zafonte et al., 1998). Memantine has gathered some interest from the TBI community; however, at present there are no studies

regarding the use of this treatment for posttraumatic cognitive deficits. Preliminary studies in patients with dementia suggest memantine may have some benefit for treating dementia and may contribute to behavioral modification (Orgogozo et al., 2002). Future studies are needed to examine its efficacy and safety in brain- injured patients.

Other NE and DA Augmentation Strategies

Many DA agonist therapies have been shown to attenuate the deficits associated with frontal lobe syndrome (McDowell et al., 1998). Chronic bromocriptine, a D_2 Receptor agonist, initiated 24h after CCI injury improved both working memory and spatial acquisition in the MWM (Kline et al., 2002). Bromocriptine also improved histological outcome in CA3 of the hippocampus. Some antidepressants, which inhibit NE reuptake, have been shown to improve the recovery process (Boyeson and Harmon, 1993). In humans, amitriptyline, desipramine, and protiptyline have all been suggested to improve recovery in some TBI patients (Reinhard et al., 1996; Boyeson and Harmon, 1993). In addition, intrarventricular infusion of NE or the NE and DA precursor L-DOPA, co-administered with carbidopa, facilitates recovery (Kikuchi et al., 2000). Conversely, drugs that antagonize catecholaminergic transmission, such as haloperidol were found to slow functional recovery (Feeney et al., 1982; Wilson et al., 2003).

The mechanisms through which noradrenergic pharmacotherapy improve functional recovery are unknown. Resolution of diaschisis and enhancement of brain plasticity have been proposed, including unmasking of latent connections, axonal sprouting, and recruitment of alternative pathways (Gladstone and Black, 2000; Feeney, 1997). Norepinephrine depletion may affect many aspects of brain plasticity following

TBI including sprouting of axons and dendrites, new receptor generation, and enhancements in connections due to stimuli. Amphetamine and drugs that increase NE have been shown to facilitate plasticity, neurogenesis, and neurotrophin increases (Stroemer et al., 1998; Malberg et al., 2000; Butefisch et al., 2002), whereas decreasing NE pharmacologically decreases BDNF and plasticity associated with exercise (Garcia et al., 2003). The plasticity-promoting effects of NE, may be due to the cooperative actions of NE with other neurotransmitters, neurotrophins or immediate early genes (p-CREB c-fos) (Marien et al., 2004; Laifenfeld et al., 2002). Two important considerations appear to be the dose dependence and timing of drug administration, as well as task-relevant experience.

The therapeutic benefits observed with DA agonists following TBI also implies a role for DA in post-injury cognitive deficits. The potential mechanism of DA-enhanced recovery following trauma may be attributed to the role of DA in maintaining both excitatory and inhibitory homeostasis. DA receptor signal transduction pathways are complex, but both NMDA and AMPA glutamate receptors can be modulated by DA (Sutton and Schuman, 2005). In cultured hippocampal cells, Sutton and colleagues (2005) demonstrate that D1-like receptor activation leads to increases in protein synthesis, specifically AMPA subunit GluR1. DA acting through D1-like receptors, increases intracellular cAMP and activates PKA, which leads to numerous downstream targets including voltage-and ligand-gated ion channels, CREB, and a number of proteins involved in signal transduction and gene regulation (Neve et al., 2004). Other studies have suggested DA agonist therapy reduces oxidative stress, and has antioxidant like

characteristics (Kline et al., 2004). The different clinical effects of DA agonists in post-injury treatment may be explained on the basis of their differential actions on specific classes of DA receptors (e.g., D1-like or D2-like, presynaptic versus postsynaptic, and excitatory versus inhibitory) and may have different effects in various brain regions. Although pharmacological enhancement of NE and DA following TBI has yielded positive results in animals and humans, the precise mode of impairment on these NT systems is not fully understood and more experimental studies are needed to access the effects of these agents following TBI. Many questions about drug safety, the most efficacious dose, when treatment should be initiated to be effective and discontinuation still remain to be investigated more thoroughly.

Atomoxetine

Atomoxetine [(-)-N-methyl-3-phenyl-3-(o-tolyloxy)-propylamine hydrochloride] is a selective norepinephrine transporter (NET) inhibitor approved by the FDA for the treatment of symptoms of Attention Deficit/Hyperactivity Disorder and co-morbid symptoms of depression and anxiety (Pataki et al., 2004). Atomoxetine shows a high selectivity for the presynaptic NET, with a low affinity for other monoamine transporters (Gehlert et al., 1993). The in vitro affinity of atomoxetine for NET is 5nM (K_i value), and the affinity is 15 and 290-fold lower for 5-HT and DA transporters (Bymaster et al., 2002). Atomoxetine also has a minimal affinity for other neurotransmitter transporters and neuronal receptors (Gehlert et al., 1995). PET studies demonstrated that atomoxetine occupies the NET in a dose-dependent and saturable fashion (Seneca et al., 2006). The

primary mechanism of action of atomoxetine is thought to be inhibition of the reuptake of NE into the presynaptic terminal. The clinical efficacy is thought to result, at least in part, from increased synaptic concentrations of NE and the postsynaptic adaptations that ensue. In a rodent model, atomoxetine also increased extracellular levels of DA levels in the frontal cortex (Bymaster et al., 2002). DA reuptake by the NET occurs in select regions of the brain, such as the frontal cortex, where low densities of the DA transporter are found (Moron et al., 2002). The increase in DA is not observed in the striatum or nucleus accumbens. Therefore, unlike psychostimulants that are associated with addictive potential due to effects on the midbrain dopamine system, atomoxetine appears to lack these effects and to have low addictive potential. Other clinical advantages are emerging, such as benefits for sleep quality over psychostimulants and possible efficacy in the treatment of comorbid tics.

In humans, atomoxetine has a half-life of approximately 5 h, and it is primarily eliminated by oxidative metabolism. Maximal concentrations of atomoxetine are reached in the plasma between 1 and 2 h. Although atomoxetine has been well characterized in humans, relatively few studies have looked at the pharmacokinetics and pharmacodynamics in animals. One study in Fisher rats found the distribution and elimination half-lives of atomoxetine following intravenous administration were .09 and 1.4 h, respectively, with a longer residual phase lasting between 12-24 h (Mattiuz et al., 2003). The same study found there was low bioavailability following oral administration in the rat, suggesting this form of drug delivery should not be used in rodent studies. The

primary circulating plasma metabolite is 4-hydroxyatomoxetine-*O*-glucuronide, and no reactive intermediates or electrophilic species have been found.

Atomoxetine has positive effects on cognition using various behavioral paradigms in animals. Using a 5-choice serial reaction time task, low doses of atomoxetine treatment significantly reduced impulsivity and improved overall attention (Navarra et al., 2008). In animals, atomoxetine is effective at suppressing motor hyperactivity and improving attention in a model of ADHD (Moran-Gates et al., 2005). Atomoxetine also improves performance in two animal models of memory, the object recognition test and the radial arm-maze test (Tzavara et al., 2006). Recent studies confirm that atomoxetine increases cholinergic neurotransmission and enhances memory in both animals and humans (Foster et al., 2006; Tzavara et al., 2006). Atomoxetine has also been shown to reduce the effects of nicotine withdrawal, confirming its involvement with acetylcholine (Davis and Gould, 2007). Several studies have shown drugs that positively modulate acetylcholine, also improve cognitive outcome following TBI (Dixon et al., 1997; for a review, see Warden et al., 2003).

Clinical trials with atomoxetine in children and adults have shown that it is effective in maintaining control of ADHD, as well as preventing relapses of ADHD symptoms without increases in adverse effects (Christman et al., 2004). Atomoxetine has also proved to be mildly successful in treating the symptoms of major depression, with clinical evidence of remission of depression after only 7 days of treatment (Kaplan et al., 2007). More recently, atomoxetine has demonstrated clinical effectiveness when used in combination with other selective serotonin reuptake inhibitors. Finally, atomoxetine has

been investigated as a rehabilitative therapy for stroke patients, and a single oral dose of atomoxetine significantly improved performance in stroke patients using a paradigm that measures motor memory as a short-term model of use-dependent plasticity (Foster et al., 2006).

Atomoxetine has gathered considerable attention from the clinical TBI community for treating TBI- related attentional problems, hypoarousal, depression, and initiation disorders (Ripley et al., 2006). However, no experimental or clinical studies have been undertaken to date, and the potential of atomoxetine remains undetermined. Based on previous studies, we posit that drugs that are positive modulators of the catecholamines will improve recovery of cognitive function following TBI. This dissertation addresses dose response, therapeutic window and duration of treatment, all of which are important considerations in the treatment of TBI patients. Therefore, the goal of this dissertation was to define an optimum therapeutic strategy for atomoxetine treatment following injury, and investigate a potential mechanism for the observed effects.

Outcome Measures

GAP-43

Growth-associated- protein-43 (GAP-43) is a nervous-system specific, acidic membrane protein present in high levels in motile growth cones of elongating axons. GAP-43 has been implicated in a number of nervous system processes such as axon guidance, synaptic plasticity and neuroregeneration. The rat protein contains 226 amino acids, and N-terminus membrane binding region, a calmodulin domain that is calcium

independent, and several phorphorylation sites, including protein kinase C (PKC) phosphorylation site serine 41 (Ser41) (Chapman et al., 1991; Benowitz and Routtenberg, 1997). GAP-43 is believed to play an important role in the guidance of axons to their correct targets and in modulating the formation of new connections between neurons (Benowitz and Routtenberg, 1997; Irwin and Madsen 1997), but GAP-43 is down-regulated following synaptic rearrangement (Skene et al., 1986). Depletion of GAP-43 alters neurite outgrowth and growth cone morphology. In contrast, overexpression promotes neurite sprouting, which is dependent on its PKC phosphorylation sites (Aigner et al., 1995). The exact mechanism is unknown. However, GAP-43 is implicated in regulating interactions between cytoskeletal components and the plasma membrane. GAP-43 is phosphorylated at serine 41 by protein kinase C-beta (PKC-β), which regulates calmodulin binding and activates GAP-43, and dephosphorylated by phosphates. GAP-43 expression in the nervous system has been well characterized both in developmental states and in the adult rat brain. In the adult rat, GAP-43 expression is relatively high in the mature hippocampus and olfactory bulb, areas associated with continuing structural plasticity (de la Monte et al., 1989).

Factors that Modulate GAP-43 Expression

Numerous factors including growth factors, hormones and neurotransmitter agonists and antagonists have been shown to affect GAP-43 expression both *in vitro* and *in vivo.* Fibroblast growth factor (FGF-2), epidermal growth factor (EGF), insulin growth like factor-1 (IGF-1 and -2), ciliary neurotrophic factor (CNFT) and nerve growth factor (NGF) have all been shown to increase both mRNA and protein expression of GAP-43 *in*

vitro (Costello et al., 1990; McNamara and Routtenberg, 1995; Piehl et al., 1998). *In vivo*, brain-derived growth factor (BDNF), NGF, and FGF-2, increase the expression of GAP-43 (Kobayashi et al., 1997; Fournier et al., 1997; Mearow, 1998; Schicho et al., 1999). Drugs that affect neurotransmitter levels and neuronal activity have been shown to affect GAP-43 also. *In vitro,* following transaction of the Schaffer collateral pathway, activation of non-NMDA receptors appears to be necessary for GAP-43 expression, reactive sprouting, and the recovery of neural transmission (McKinney et al., 1999). Exposure to the NMDA antagonist MK-801 blocks induction of GAP-43 mRNA, suggesting some neuroprotective therapies used in trauma may prevent successful regenerative responses (Cantallops and Routtenberg, 1999). Although atomoxetine itself has not been shown to influence GAP-43, similar drugs that affect the NE transporter have been found to increase expression of GAP-43. For example, chronic treatment with the antidepressants desipramine, a NE and 5HT reuptake inhibitor, or tranylcypromine, a monoamine oxidase inhibitor, have found GAP- 43 mRNA and protein expression is increased (Chen et al., 2003; Laifenfeld et al., 2005). In situ hybridization, indicated that following desipramine treatment, GAP-43 levels are specifically increased in the dentate gyrus (Chen et al., 2003), correlating with dense NE projections from the locus ceruleus to this region of the hippocampus. Dopamine (D1) agonists also increase GAP-43 expression (Williams et al., 2006). Neuronal GAP-43 expression has also been shown to be increased *in vitro* by administration of NE (Laifenfeld et al., 2002), concurrent with increases in two neurite-out growth promoting genes, neural cell adhesion molecule L1 and laminin.

Gap-43 Following Brain injury

Numerous studies using a myriad of brain injury models have found alterations in GAP-43 expression are common following injury. The first studies demonstrated increased GAP-43 following seizures in conjunction with mossy fiber sprouting (Meberg et al., 1993). Kindling, which involves repeated electrical stimulation, also increases GAP-43 expression. Perforant path kindling leads to increased expression of GAP-43 in the CA1 stratum lacunosum moleculare (SLM) and the inner molecular layer (IML) of the DG (Dalby et al., 1995). Numerous lesion studies have also demonstrated increases in GAP-43. Following lesions to CA3 pyramidal cells, GAP-43 is prominently found in new axon collaterals (McKinney et al., 1997). Increased expression of GAP-43 is found in commissural fibers following fimbria/fornix and perforant path lesions (Patanow et al., 1997), and transgenic mice overexpressing GAP-43 can induce partial regeneration of Purkinje cells following axotomy (Buffo et al., 1997). GAP-43 is also expressed in response to deafferentation during reactive synaptogenesis (Schauwecker et al., 1995, 2000). Following entorhinal cortex lesions, significant increases in GAP-43 in the contralateral "sprouting" hippocampus are found (Lin et al., 1992). The timing of these increases appears to be transient. Reports suggest increases in GAP-43 occur between 2-15 days following injury (Masliah et al., 1991; Benowitz et al., 1990). These results suggest GAP-43 plays a role in the synaptic remodeling that occurs following lesions of the hippocampus.

Transient changes in GAP-43 expression have also been found following experimental spinal cord injury, ischemia, and traumatic brain injury models. Increases in

GAP-43 were observed following compression injury in the adult rat spinal cord beginning around PID 4 and lasting until PID 9 (Li et al., 1996). Transgenic mice over expressing GAP-43 produce long axonal projections (Bomze et al., 2001). GAP-43 is elevated in the infarct penumbra at 4 days following cerebral ischemia and at 3 days in the boundary, suggesting the role of for GAP-43 in cortical sprouting (Furuya et al., 1997). GAP-43 has also been reported in the hippocampus following global ischemia (Schmidt-Kastner et al., 1997). Interestingly, NMDA receptor blockade prevents the induction of GAP-43 following ischemia, suggesting some level of neuronal activity is necessary for its expression (Luque et al., 2001). Finally, GAP-43 expression is increased in the hippocampus and cortex following C-FPI of moderate severity (Hulsebosch et al., 1998). In cats, GAP-43 expression has been found up to 28 days in injured axons, suggesting a prolonged regenerative attempt (Christman et al., 1997). Using L-FPI, increases in GAP-43 expression were reported in the hippocampus at 48 h following injury but decreasesd from sham groups at later time points (Emery et al., 2000), suggesting there may be a limited time for enhancing or influencing post injury plasticity. Although expression of GAP-43 is transiently increased after moderate injury in many models, a recent study documented no changes in GAP-43 expression in the hippocampus or cortex following severe-CCI (Thompson et al., 2006). Therefore, it appears the ability for axonal and synaptic plasticity that occurs following moderate injury is diminished by more severe injuries.

Several studies have investigated the effects of treatments on GAP-43 expression following different injuries. Increases in both GAP-43 and synaptophysin were found in

the ipsilateral and contralateral cerebral hemispheres of unilateral sensorimotor cortex injured rats following amphetamine treatment (Storemer et al., 1998). Inhibition of Nogo-A, a protein present in myelin which may prevent successful axonal regeneration following injury, prevented attenuated post-injury downregulation of hippocampal GAP-43 which correlated with cognitive improvement (Marklund et al., 2007). GAP-43 axonal sprouting may be initiated by brain-derived neurotrophic factor-trkB signaling (BDNF will be discussed in more detail in a later section, Dinocourt et al., 2006). Several studies suggest neurotrophic factors increase GAP-43 expression and this correlates with behavioral and functional improvements following injury (Dixon et al., 1997; McDermott et al., 1997; Fernandez et al., 1999). Although the results differ depending on injury paradigms, as well as time course, the evidence of elevated GAP-43 expression during fiber outgrowth and synapse formation following CNS lesions and experimental TBI support the use of GAP-43 as a marker for synaptic plasticity in the adult CNS (Benowitz and Routtenberg, 1997).

Synaptophysin

Synaptophysin (SYN) is a 38 kDa glycoprotein found in the membrane of NT containing presynaptic vesicles and is involved in vesicular trafficking, docking and fusion of the synaptic plasma membrane (Wiedenmann and Franke, 1985; McMahon et al., 1996). SYN provides a molecular marker for axonal nerve terminals and synapses, but it is also used to estimate changes in synaptic number after cell loss or during neuronal remodeling and rearrangement (Masliah et al., 1990). For example, SYN has been shown to be downregulated in cortical areas of aged animals and human Alzheimer's patients, and the numbers correlated with disease progression (Ingelsson et al., 2004; Reddy et al., 2005).

The decrease in SYN in Alzheimer's disease has been explained as a consequence of synaptic loss preceding neuronal death.

Different experimental injury models have investigated changes in synaptophysin following brain injury. Although a number of these studies have demonstrated changes in SYN expression, the time course has not been fully determined for each model and thus some questions still remain. Following C-FPI, there was a slight reduction in SYN density, which may be attributed to cell death and the loss of synapses. However, when FPI and entorhinal cortical lesion were combined there was a change in the distribution of SYN, suggesting synaptic reorganization, or reactive synaptogenesis (Prins et al., 2003). Following moderate CCI, synaptophysin levels were elevated in the ipsilateral hippocampus beginning at 24h after injury and lasting for 21 days, although the 7 day time point was elevated but not significant (Thompson et al., 2006). Following L-FPI, increased innumostaining of SYN was found in the cortex and subcortical white matter as time progressed post-injury (Shojo and Kibayashi, 2006). Although, most previous studies have explained increased immunoreactivity for synaptophysin in terms of synaptogenesis and neuronal regeneration, this study found increased synaptophysin in degenerated neuronal cell bodies and axons, suggesting that the increased SYP in the cortex following injury may represent inhibition of axonal transport and dysfunction of the synapse. Interestingly, there were no changes found in SYN levels at 2, 15 or 30 days after moderate fluid-percussion brain injury when western blot analysis was used (Shojo and Kibayashi, 2006). Clinically, Murdoch et al. (1998) reported a significant decrease in SYN levels, measured by western blot, in the cingulate gyrus of patients with head injury at 3h and 12.5

days after trauma. Clearly there are obvious differences in injury models that are seen across a variety of outcome measures, however, these studies prove that both the model, and the time course for SYN induced changes must be considered when interpreting SYN levels. Still, the results observed following TBI and other disorders support the use of SYN as a molecular marker for changes in synaptic number in this experiment.

Brain-derived growth factor

Brain-derived growth factor (BDNF) belongs to the family of growth related neurotrophins that includes nerve growth factor (NGF), neurotrophin-3, and neurotrophin 4/5. BDNF is the most abundant neurotrophic factor, and is found in high levels in the hippocampus and cerebral cortex (Phillips et al., 1990; Murer et al., 2001). BDNF binds to two types of receptors, but shows the highest affinity for tropomyosin-related kinase B (TrkB, Kaplan and Miller, 2000). BDNF has been implicated in playing an important role in the development of immature neurons, neural regeneration, synaptic transmission, synaptic plasticity, and neurogenesis (Patterson et al., 1996). At the synapse, BDNF is important for both presynaptic and postsynaptic plasticity mechanisms, as well as long-term potentiation (Pang et al., 2004; Messaoudi, 2005). BDNF also plays a role in increasing presynaptic NT release and increasing synapse formation (Tyler and Pozzo-Miller, 2001). Enhanced expression of BDNF also improves cognition, and thus BDNF has been suggested to play a role in learning and memory (Messaoudi, 2005). BDNF has been shown to mediate neuronal protection and enhancement of neurite outgrowth/axonal regeneration through its association with TrKB and its downstream effects on signaling

pathways (Cui et al., 2006). Moreover BDNF has been shown to increase the expression of GAP-43 (Klocker et al., 2001)

Alterations in BDNF expression have been hypothesized to play a role in depression and neurodegenerative diseases, and may have a role in the effects of antidepressant treatments. Chronic antidepressant treatment has been shown to increase BDNF expression and induce hippocampal neurogenesis (Tardito et al., 2006; Sairanen et al., 2005). Specifically, duloxetine (a 5-HT and NE reuptake inhibitor) has been shown to up-regulate BDNF mRNA and protein expression in the cortex, as well as affect the subcellular distribution of BDNF (Calabrese et al., 2007). Other antidepressants that affect NE levels, such as desipramine and reboxetine, enhance BDNF expression and increase cell proliferation and neurogenesis in the dentate gyrus after chronic administration (Malberg et al., 2000; Czeh et al, 2001). Several lines of evidence suggest that acute treatments (single-dose) fail to induce significant increases in BDNF, whereas more chronic treatments with antidepressants upregulate BDNF. BDNF regulation changes have also been observed in the brains of Alzheimer's (AD) patients. Decreased concentrations of BDNF have been found in the hippocampus and temporal cortex of AD patients, which may interfere with neuronal integrity and plasticity (Ferrer et al., 1999; Connor et al., 1997).

Neurotrophins have been hypothesized to be an important neural substrate in TBI because of their roll in cell survival and neural plasticity (Gillespie et al., 2003). Some studies have suggested that BDNF may be neuroprotective when given early, through a mechanism that involves caspase-3 (Kim and Zhao, 2005). BDNF has also gained

attention for being a major modulator of MAG and Nogo-A inhibition. *In vitro* studies demonstrate that neurons stimulated with BDNF have an enhanced ability to counteract the effects of inhibitory proteins through a mechanism involving the action of PKA (Gao et al., 2003). Thus, BDNF may be particularly beneficial in the post-TBI environment in overcoming the resistance to regeneration. The recovery of neuronal cells and non-neuronal cells may depend on the presense of growth factors and their receptors which act to support neuronal survival and sprouting of neuritis, and over come barriers to regeneration. For these reasons, BDNF has been used as an outcome measure following injury and for investigating the effects of therapeutic treatments. Several therapeutic agents, including exercise, vagus nerve stimulation and simvastatin, increase BDNF, which was correlated with increased functional outcome following TBI (Griesbach et al., 2004; Wu et al., 2008; Chytrova et al., 2008).

Important to this experiment are the studies that show increased NE and DA neurotransmission can induce neuronal BDNF expression within the cortex and hippocampus (Fawcett et al., 1998; Ivy et al., 2003). Therefore these neurottansmitters may be important signals in the brain for the mechanisms of neuronal survival, neuroplasticity, and neurogenesis (for a review, see Marien et al., 2004). Catecholamine activity can also positively regulate astrocytic neurotrophic support. *IN vitro* studies have shown that cultured cortical astrocytes, when exposed to DA, NE, or в-adrenergic agonists, exhibit marked increases in BDNF mRNA expression (Schwartz et al., 1994; Inoue et al., 1997; Miklic et al., 2004; Juric et al., 2006). Although the exact relationship between NE

and neurotrophins is unclear, the evidence taken together suggests there is a relationship between catecholamine levels and BDNF.

Summary

Human TBI is a serious health and social concern both in the U.S. and globally. The vast majority of brain injuries are mild to moderate in severity, and often leave individuals with persistent cognitive and behavioral complaints. Initial and persistent cognitive deficits, including problems with attention, information processing speed, and memory are among the most common complaints. Therefore, it is extremely important to find therapeutic treatments that improve cognitive outcome following TBI.

Significant evidence suggests the catecholaminergic systems are altered following injury, and several studies have found improved outcome following treatments with positive modulators of the catecholamines. Therefore, we posit that treatments that enhance catecholaminergic tone will positively influence structural and functional outcome following injury. Atomoxetine is a selective NET inhibitor that is indicated for ADHD and symptoms of depression and anxiety. The purpose of this study was to first examine the effects of atomoxetine on behavioral outcome in the MWM following a moderate level of experimental injury. We will also address important issues that are clinically relevant including treatment dose, therapeutic window, and duration of treatment. Finally, we will examine the expression of GAP-43, synaptophysin, and BDNF IR in the hippocampus to establish neuroplasticity correlates of the effects of atomoxetine following TBI. Based on

the literature, there is evidence that NET inhibitors and dugs that positively modulate NE/DA may influence neurotrophins, and synaptic plasticity.

CHAPTER 2 METHODS

Subjects

Adult (3month-old) male Sprague-Dawley rats (Hilltop Lab Animals, Inc., Scottsdale, PA) weighing 300-360g were used in the study. Animals were individually housed with free access to food and water in a 12-h dark-light cycle at 22° C. All procedures 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 the Institutional Animal Care and Use Committee.

Drug Preparation

Atomoxetine was provided by Eli Lilly and Co. (Indianapolis, IN). Atomoxetine was dissolved in isotonic saline solution to achieve desired doses. All drug injections were administered intraperitoneally (i.p) based on previous literature (Swanson et al., 2006).

Experimental models of TBI: Rationale to use L-FPI

The understanding of TBI has been greatly enhanced by validating research in experimental models of injury. Various models of experimental TBI are currently available, which are designed to replicate certain aspects of human brain injury. Such models have used rodents, cats, rabbits, pigs and non-human primates to investigate the specific mechanisms leading to the various sequelae of brain injury (Dixon et al., 1987;

Gennarelli et al., 1982; Povlishock, et al., 1994). No one animal model can replicate all aspects of human TBI, thus there are several preclinical models of TBI to properly characterize its underlying pathology. Each of the models offers valuable information about certain aspects of the course of TBI. A number of variables such as location of injury, the severity of trauma, and type of pathology are all taken into consideration when characterizing animal models. For example, some of the models replicate the pathology of focal injury (e.g., cortical contusion injury), whereas other models focus more on diffuse injury (e.g., impact- acceleration, weight drop model by Marmarou et al., 1994). Other injury models have combined the effects of TBI with secondary effects such as hypoxia, hypotension, ischemia, or entorhinal deafferntation (Cernak, 2005; Prins et al., 2003; Zhu et al., 2000). In human TBI there is extensive overlap of focal and diffuse injury, therefore, experimental injury models that mimic both the focal and diffuse nature of TBI are particularly beneficial in studying the underlying mechanisms of brain injury. It is important that these models can produce results that are reproducible and quantifiable, clinically relevant, and produce a continuum of injury severities.

Fluid-percussion injury (FPI) is the most commonly used rodent model of TBI. In this model injuries can be generated from either a central or lateral location. Central fluid-percussion injury delivers the fluid pulse along the central suture midway between bregma and lambda. Lateral fluid-percussion injury (L-FPI) delivers the injury to the parietal lobe midway between the coronal and lambdoid sutures. L-FPI produces a combination of both focal and diffuse injury and has become one of the most extensively utilized models of injury (for review, Thompson et al., 2005). The immediate physiological responses

include changes in blood pressure, brief respiratory arrest, decreased cerebral perfusion pressure, and reduced cerebral blood flow (Lighthall et al., 1989; Pfenninger et al., 1989). The predominant histological feature of L-FPI is a focal cortical contusion with accompanying intraparenchymal hemorrhage (McIntosh et al., 1989). There is considerable neurodegeneration in and around the contused tissue that can last up to one year post-injury (Graham et al., 1997; Pierce et al., 1998). Over the course of days to months, progressive degenerative cascades persist in additional brain regions such as the hippocampus (Cortez et al., 1989; Hicks et al., 1993), thalamus (Pierce et al., 1998; Rodriguez-Paez et al., 2005), striatum (Hallam et al., 2004; Hicks et al., 1996), and amygdala (Hallam et al., 2004; Colicos et al., 1996). The injury generally affects the ipsilateral side, and few reports have demonstrated significant cell loss in either the brain stem or the contralateral side, which can be used as a qualitative comparison for some outcome measures (Pierce et al., 1998; Grady et al., 2003). In addition, FPI show a direct relationship between the majority of pathological alterations and injury severity. There are also dramatic subcellular and molecular responses that have been documented in L-FPI, such as changes in an array of cell-death, housekeeping, neurotrophic, cytokine, cell adhesion, and immediate early genes, as well as proteins involved in cell signaling, cytoskeleton, and synaptic transmission (Hayes et al., 1995; Hicks et al., 1997; Natale et al., 2003; O'Dell 2000). Finally, FPI creates consistent and reproducible cognitive dysfunction using widely accepted behavioral paradigms (Hamm et al., 1993). Cognitive deficits in memory have been observed between 48h and two weeks after FPI of moderate severity, which gradually improves towards sham levels over a year. More severe injury

leads to enduring memory and behavioral dysfunction. The clinical relevance, validity, and reproducibility of L-FPI support the use of this model to investigate both the pathology of TBI as well as the potential of pharmacological therapies to affect functional outcome. Therefore, L-FPI is well suited for the examination of our research questions. To demonstrate the validity of our model, we have included cresyl violet photomicrographs from Sham, Injured, and Injured-Atomoxetine Treated groups (Figure 3).

Surgical Preparation

All animals were anesthetized with 4% Isoflurane with 70% N_2O: 30% O_2 mixture for 4 minutes and placed in a stereotaxic frame. The scalp was sagitally incised and a 4.8 mm lateral craniotomy was trephined into the skull to the right of the sagittal suture midway between bregma and lambda. A Leur-Loc syringe hub with a 2.6 mm diameter was secured on the skull at the site of the craniotomy with cyanoacrylate adhesive. Two nickel-plated screws were placed 1mm rostra to bregma on the ipsilateral side and 1 mm caudal of the lambda on the contra lateral side. Dental acrylic was applied around the hub. The scalp was then sutured and animals were allowed to recover.

Fluid-Percussion Brain Injury

The fluid percussion device (Figure 2) used to produce experimental brain injury was identical to that used previously on rodents and was described in detail by Dixon et al., (1987). Briefly, the device consists of a 60 cm long and 4.5 cm diameter Plexiglas cylinder with a rubber-covered O ring-fitted Plexiglas piston at one end. The opposite end of the cylinder has a 2 cm long metal housing that contains a pressure transducer. At the end of the metal housing is a 5mm tube with a 2.6-mm inside diameter which connects

with the surgically implanted Leur-Loc fitting implanted over the rat skull. The device was filled with isotonic saline. The injury was produced by a pendulum that strikes the piston of the injury device and injects a small volume of saline into the cranial cavity to produce a brief displacement and deformation of brain tissue. The extra cranial pressure pulse was expressed in atmospheres (atm).

Twenty-four h after the surgical preparation animals were anesthetized with 4% isoflurane with 70% N_2O: 30% O_2 mixture for 4 minutes. Animals in the injured condition were then injured at 2.08 $^{+}$/- .05 atm, equivalent to a moderate-level brain injury. Righting reflexes were recorded in all injured groups. Rats assigned to sham-injury groups were anesthetized and connected to the injury device but received no injury.

Figure 2 A picture of the fluid-percussion device.

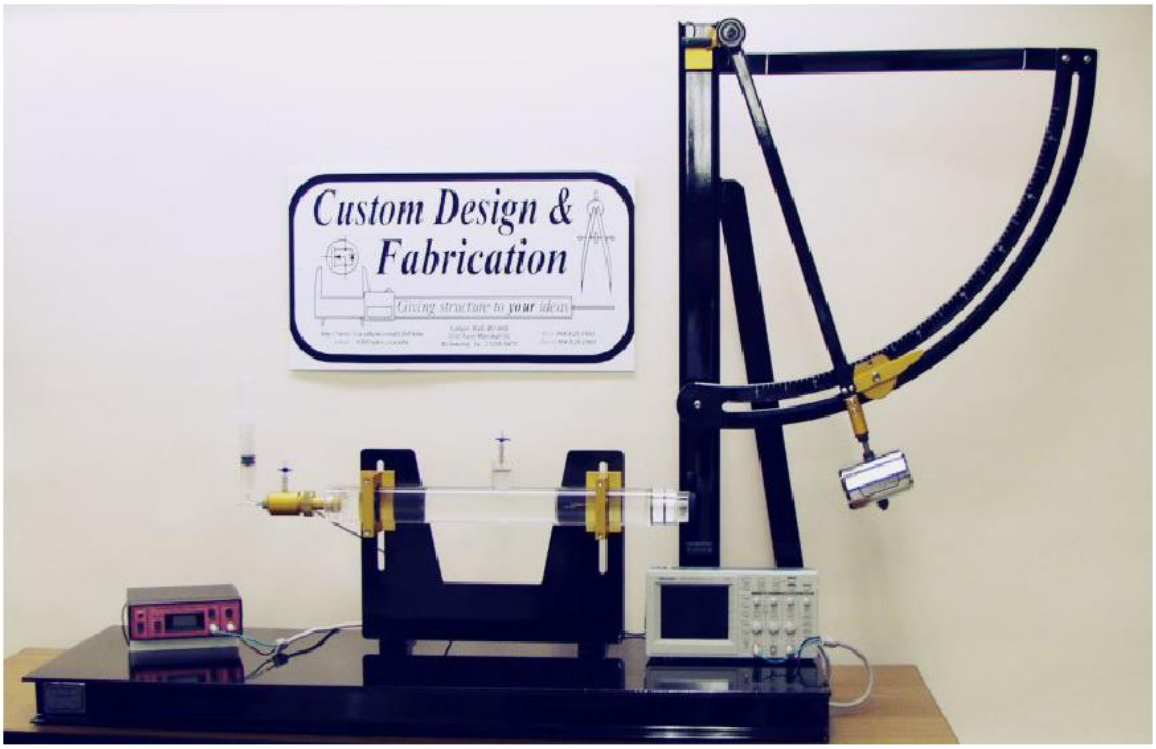
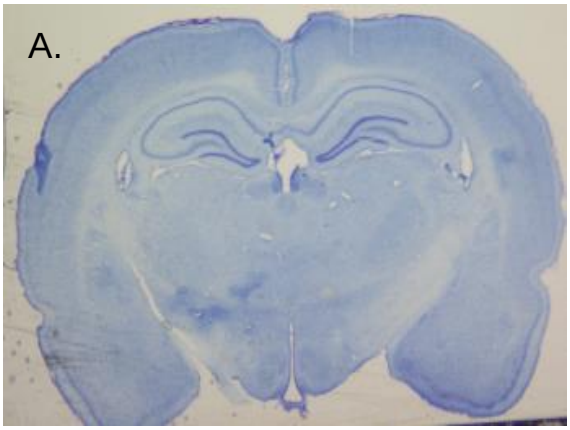
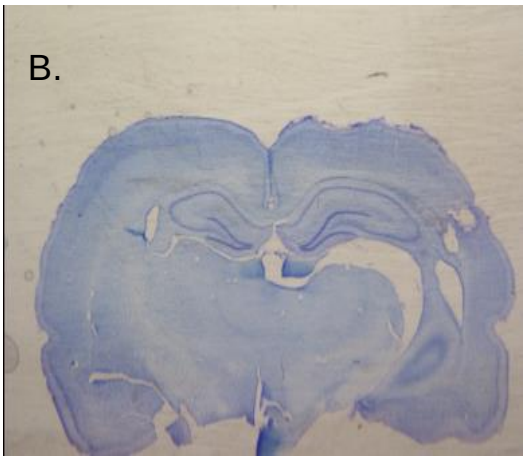
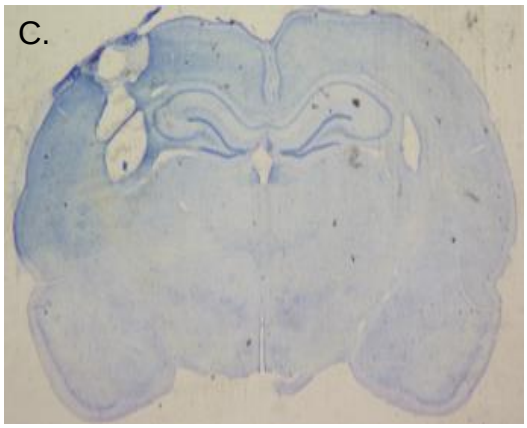
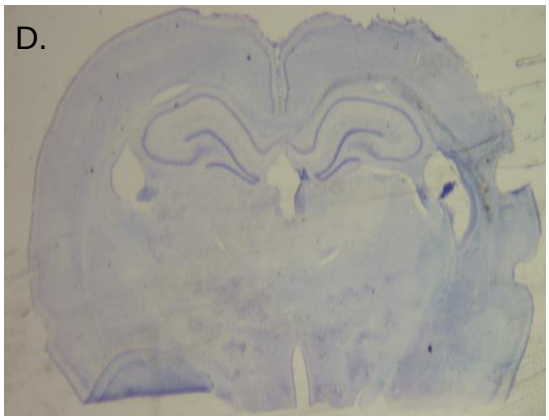

Custom Design &
Fabrication
Giving structure to your ideas

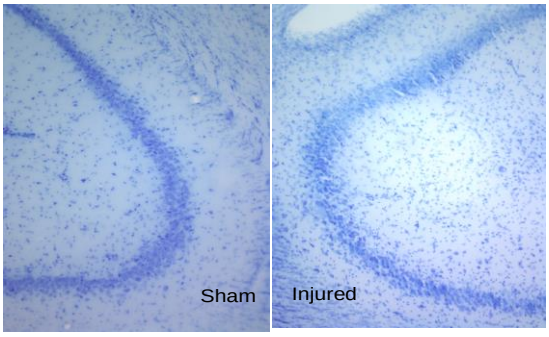
Figure 3 Histology Following Lateral-Fluid Percussion Injury. The top panel(A-D): Representative cresyl-violot stained photomicrographs from A. Sham, B. Injured-atomoxetine treated, and C-D. Injured-vehicle treated groups characterizing the L-FPI model. The bottom Panel Left: Representative Photomicrographs of the CA3 region of the hippocampus from Sham-injured and Injured-Vehicle treated animals. The bottom Panel Right: Magnification of the cortical contusion seen in both injured groups following L-FPI.

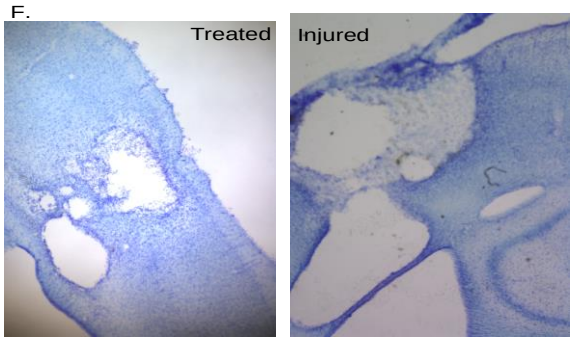

A.


B.


C.


D.


Sham
Injured


Treated
Injured

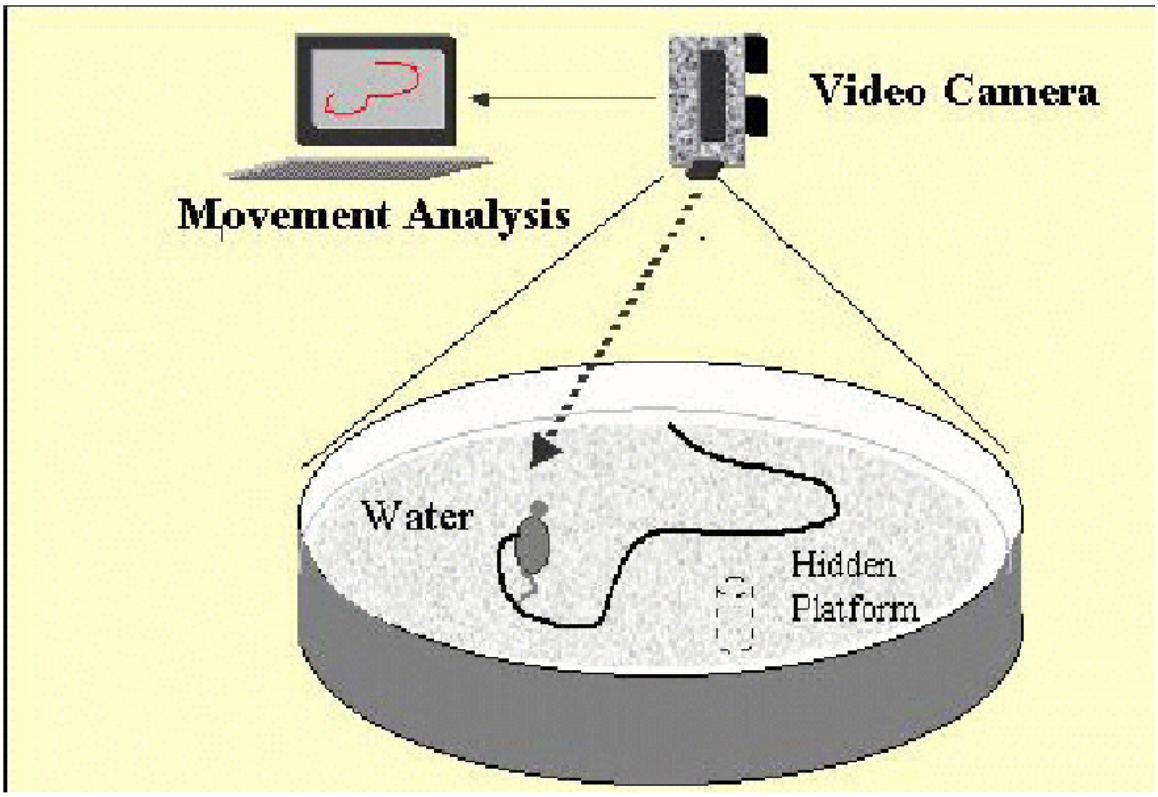
Behavioral Outcome Assessment

The impairments of cognitive function are the most significant and enduring consequences of human TBI. The Morris Water Maze (MWM) is a standard well established method for the assessment of cognitive function of rats (Brandeis et al., 1989; Morris et al., 1982), and has become a standard test procedure in assessment following experimental TBI. In the spatial reference memory task, animals learn to find a submerged escape platform occupying a fixed location in a large pool of water based on its location relative to extra-maze cues located in the testing room. There are several advantages for using the MWM. The MWM is known to be sensitive to hippocampal damage and is a relatively simple procedure that rats learn rapidly and has been shown to be sensitive to FPI-induced cognitive dysfunction for at least 65 days (Lyeth et al., 1991; Miyazaki et al., 1992). Although other paradigms exist, many require food deprivation which may influence final outcome and confound results. The MWM does not require food deprivation, and therefore is a superior model following injury. It has also been shown that the deficits observed on this task are not confounded by visual, motor, or other non-cognitive factors following injury (Hamm et al., 1993). In all behavioral experiments (except where otherwise noted), rats will be examined on post-injury Days 11-15. This time point coincides with large TBI-induced deficits, thus making drug effects more apparent. In addition, motor deficits produced following injury have abated. This time point is a common time point for assessing spatial discrimination following injury, and thus the data is comparable to other pharmacological studies.

Apparatus: The maze is a large circular tank (180 cm diameter by 45 cm high) filled with water to a depth of 30 cm. (Figure 4). The water temperature is maintained between 23°C and 26°C. The hidden goal platform is placed 45cm from the outside wall in the southeast quadrant and remains constant throughout testing. The pool is located in a room with numerous extra maze cues that remain constant throughout testing.

Procedure: Rats were given 4 trials per day for 5 consecutive days. On each day, rats were placed in a pool by hand in one of four locations (north, south, east, west) facing the wall. Each animal started a trial at each of the four locations on each day. The order of starting locations was randomized each day to minimize practice effects. The goal platform was placed 45cm from the outside wall in the southeast quadrant and remained constant throughout testing. Rats were given a maximum of 120 sec to find the goal. After this time, the rats were placed on the goal and held there for 30 sec. The rats were then removed from the maze and placed in an incubator until the next trial. Latency to reach the goal was recorded using a video tracking system (Videomex Columbus Instruments, Ohio). This device also recorded path length to the platform, which allowed for the calculation of swim speed. Latency to find the platform was the primary variable analyzed. Swim speed was also calculated to ensure there were no motor deficits or drug effects interfering with results.

Figure 4 A cartoon of the Morris Water Maze


Video Camera
Movement Analysis
Water
Hidden
Platform

CHAPTER 3

EXPERIMENTAL RATIONALE, DESIGN, & RESULTS

Experiment 1: Optimum Dose Determination

Rationale

Pharmocological strategies aimed at increasing neural activity in the chonic stages of TBI have demonstrated some success in increasing cognitive performance in the MWM following injury (Whiting and Hamm, 2005). Particularly, drugs that modulate NE and DA have been successful at enhancing specific aspects of cognitive dysfunction in human TBI. Atomoxetine is a nonstimulant selective NET inhibitor that increases extracellular levels of NE and DA. No previous studies have investigated the effects of atomoxetine on cognitive recovery following experimental TBI. Therefore, this experiment examined which dose of atomoxetine would be the most effective at reducing cognitive deficits associated with the consequences of brain injury. The lower doses (.03-1 mg/kg) were chosen based on the therapeutic concentration range of atomoxetine in humans and previous literature in rodents (Bymaster et al., 2000). The higher doses (3-9 mg/kg) were used to fully investigate the effects of different doses in TBI. Although, these doses may not be clinically relevant in ADHD patients, no investigations have examined atomoxetine

treatment following TBI. Therefore, this study used a full range of doses to demonstrate the effects of atomoxetine on cognitive recovery following TBI.

Design

The experiment was designed to find the most effective dose of atomoxetine for reducing post-injury cognitive deficits. All animals were randomly assigned to one of seven treatment groups: injured + .3 mg/kg atomoxetine (n=10), injured + 1 mg/kg atomoxetine (n=10), injured + 3 mg/kg atomoxetine (n=10), injured + 9 mg/kg atomoxetine (n=10), injured + vehicle (n=10), sham-injured + 1 mg/kg atomoxetine (n=10) and sham injured + vehicle (n=10). Each individual injection was administered at 3 mg/kg, 1mg/kg, 3mg/kg, or 9mg/kg dose, i.p., and animals in the injury + vehicle groups received an equal volume of saline (1 ml/kg). Vehicle or atomoxetine treatment was administered to animals beginning 24h after injury and continued once daily for 15 days. All animals were injected with either vehicle or atomoxetine at the same time each day. Morris water maze performance was determined on PIDs 11-15. On MWM testing days, administration of drug or vehicle occurred 1 hour before initiation of testing.

Results

No significant difference in recovery of righting reflex time was found between any injured group, demonstrating injury severity was consistent between all injured groups ($F(4,39) = 1.001$, $p=.419$, data not shown). Analysis of spatial learning acquisition by a split-plot [6 (Group) X 5 (Day)] analysis of variance (ANOVA) revealed a significant main effect on Group ($F(5,54) = 14.465$, $p < 0.001$), demonstrating a decreased latency to find the goal (Figure 5). Post-hoc comparisons with Tukey's Honestly Significant Differences

(HSD) test indicate that injured animals treated with .3 mg/kg, 1mg/kg, and 3 mg/kg atomoxetine differed significantly from injured animals treated with vehicle, demonstrated by the significant improvement in MWM performance ($P< 0.001$). In fact, injured animals receiving the .3 and 1 mg/kg atomoxetine treatment did not differ significantly from sham animals ($P>.05$). There were no significant differences between sham injured animals receiving either vehicle or 1 mg/kg atomoxetine, indicating that drug administration did not affect performance on the MWM in sham animals (Figure 6, $F (1,18) = 1.615$, $P > .05$). Analysis of swim speed found no significant group differences, suggesting there were no motor, or motivational factors caused by injury or drug effects (Figure 7, $F (6, 62) = 1.466$, $P = .205$).

Discussion

This is the first study to investigate the efficacy of atomoxetine in treating post-TBI cognitive impairments. The results demonstrate that daily chronic, post-injury treatment with low doses (.3, 1, 3 mg/kg) of atomoxetine reduces cognitive impairments following L-FPI in rats when initiated 24 h after injury. Treatment at the higher dose (9mg/kg) did not offer any benefit. The dose-dependant response suggests only low doses of atomoxetine are effective in attenuating cognitive deficits. Animals treated with 9 mg/kg atomoxetine appeared to be unusually aggressive and easily agitated (qualitative observation), which may have affected their performance in the MWM. However, no significant differences in swim speeds were observed between any of the groups, suggesting the drug did not affect their performance through an effect unrelated to injury. Atomoxetine has gathered much interest from the clinical TBI community for treating

post-injury cognitive deficits (Ripley 2006), but this is the first study to demonstrate that atomoxetine aids in cognitive recovery following experimental injury in animals. The results of the current study are similar to previous results using stimulant treatments such as methylphenidate (Kline et al., 2000; Napolitano et al., 2005); however, atomoxetine offers advantages over traditional stimulants, in that atomoxetine is not a controlled substance, has little abuse potential, and offers once-daily dosing (Christman et al., 2004). Therefore atomoxetine may be a more practical treatment for cognitive-related deficits in TBI patients.

Figure 5 MWM results for Experiment 1. The goal latencies for injured animals treated with .3, 1, and 3 mg/kg atomoxetine were significantly different than injured animals treated with vehicle alone ($p<.05$). The results also indicated latencies in the sham-vehicle group were not significantly different from injured animals treated with .3, and 1 mg/kg atomoxetine.

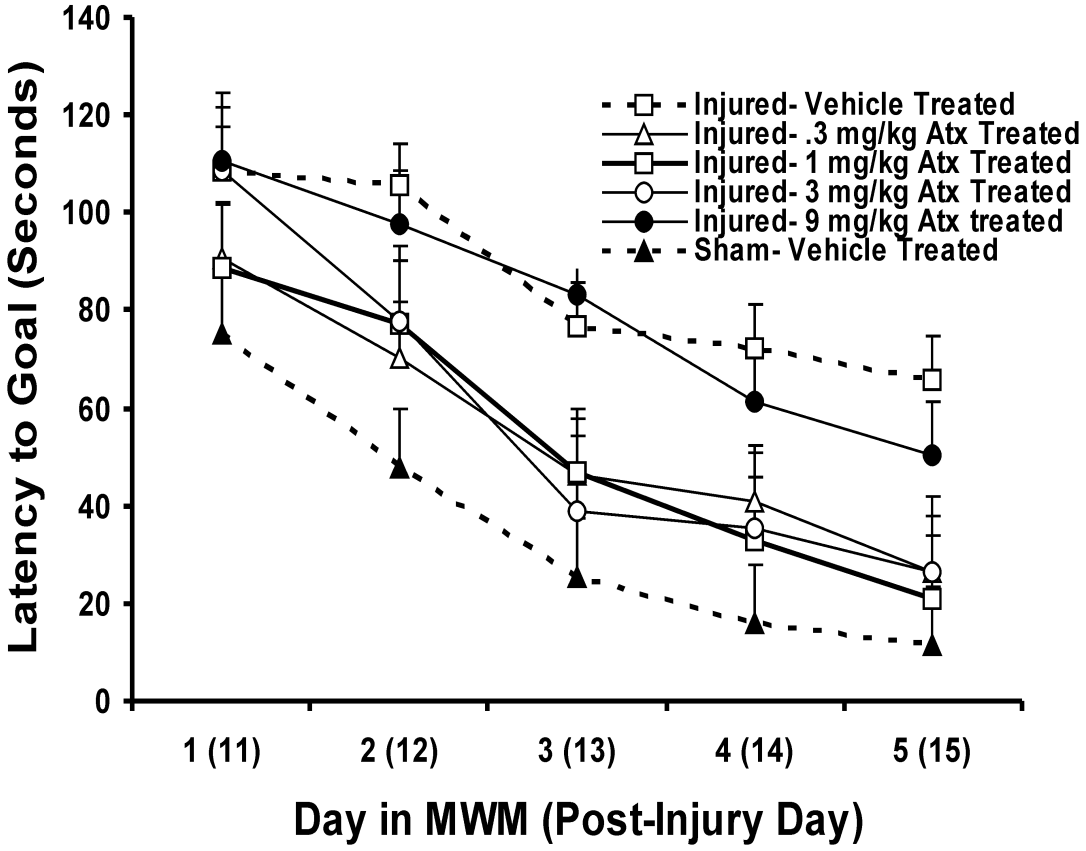

Latency to Goal (Seconds)
140
120
100
80
60
40
20
0
1 (11)
2 (12)
3 (13)
4 (14)
5 (15)
Day in MWM (Post-Injury Day)
Injured- Vehicle Treated
Injured- .3 mg/kg Atx Treated
Injured- 1 mg/kg Atx Treated
Injured- 3 mg/kg Atx Treated
Injured- 9 mg/kg Atx treated
Sham- Vehicle Treated

Figure 6 MWM results for Sham Groups. The mean (±SEM) latency (seconds) to locate a hidden platform in the Morris water maze on days 11-15 post-injury. Comparison of sham-vehicle treated and sham-1 mg/kg atomoxetine treated revealed no significant differences suggesting administration of atomoxetine did not affect MWM performance through mechanisms unrelated to injury.

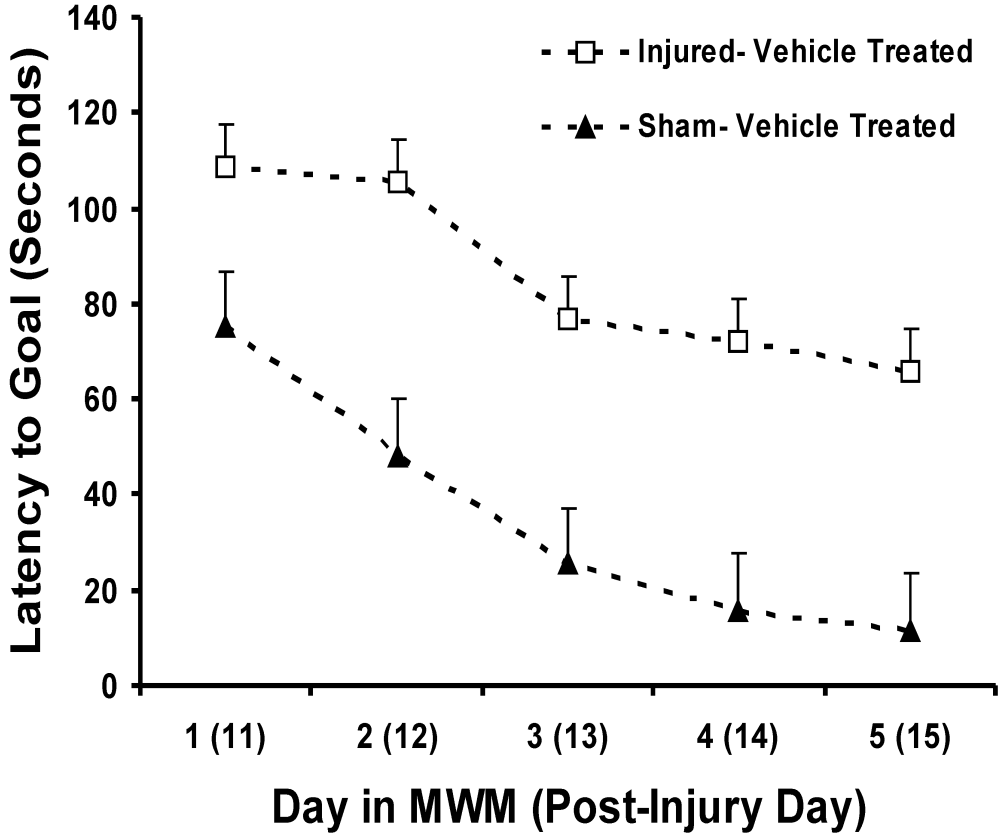
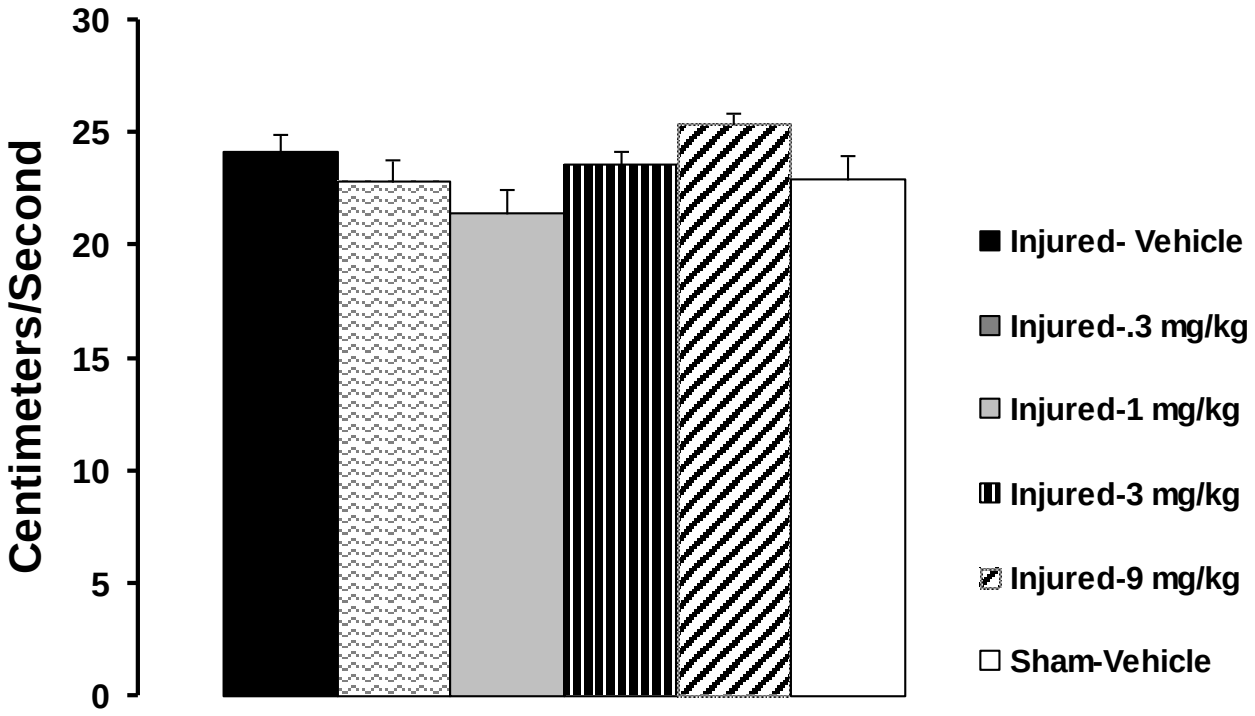

Latency to Goal (Seconds)
140
120
100
80
60
40
20
0
Injured- Vehicle Treated
Sham- Vehicle Treated
1 (11)
2 (12)
3 (13)
4 (14)
5 (15)
Day in MWM (Post-Injury Day)

Figure 7 Swim Speeds for Experiment 1. The mean swim speeds (cm/sec) for each group are shown. A one-way ANOVA found no significant differences between the groups, indicating the results of Experiment 1 are not due to injury or drug effects on swim speed.


Centimeters/Second
30
25
20
15
10
5
0
Injured- Vehicle
Injured-.3 mg/kg
Injured-1 mg/kg
Injured-3 mg/kg
Injured-9 mg/kg
Sham-Vehicle

Experiment 2: Examination of Therapeutic Window

Rationale

The aim of this experiment was to investigate the temporal therapeutic window for post-injury pharmacological treatment of cognitive impairments with atomoxetine. Delaying the initiation of chronic administration is clinically important because many of the cognitive deficits observed following TBI may not be apparent or may be masked by other disorders immediately after the injury. In addition, there are a number of people living with TBI-related disabilities that may benefit from treatments at delayed time points. Post-traumatic neural depression appears to be temporally bound to behavioral change, with both being impaired after injury and changing in parallel over time. Following injury, there may be a critical period for treatment to provide benefits in cognitive outcome. It is well known that a number of secondary injury cascades occur for hours to weeks following injury. Although, the exact time course for these injury induced changes varies according to model and outcome measure, it is hypothesized that drugs with neuroprotective mechanisms may have limited therapeutic windows, and therefore treatments initiated at later time points after injury may be ineffective due to irreversible damage. In addition to secondary injury mechanisms, there are also mechanisms of neural recovery, and the endogenous neural plasticity response begins to mount within the first week following injury. Therefore, drugs that enhance this endogenous plasticity response may be required to be initiated early after injury. In contrast, if drugs initiated at delayed time points (over 1 week) post-injury still provide cognitive benefit, it would suggest a different mechanism

of action. Therefore, this study also provides information to help clarify the potential mechanism.

Design

Animals were randomly separated into one of three groups: injured + saline treatment (n=10), injured + 1 mg/kg atomoxetine treatment (n=9), and sham-injured + vehicle (n=8). Following injury, animals did not receive any treatment on days 1 through 10 after the trauma. Beginning on post-injury day (PID) 11, animals were treated with atomoxetine or saline once daily at the same time for the remainder of the experiment. Cognitive performance was assessed in the MWM after 15 days of drug treatment on PID 26-30. On MWM testing days, administration of drug or vehicle occurred 1h before initiation of behavioral testing.

Results

A split-plot ANOVA [3 (Group) X 5 Day] indicated there was a significant effect of Group on Goal Latency ($F(2,24) = 7.542$, $P < .05$); however, post-hoc comparisons using Tukey's HSD test indicate that injured animals treated with 1 mg/kg atomoxetine were not significantly different from injured animals treated with vehicle (Figure 8, $P > .05$). There were no significant differences in mean righting times ($F(1,17) = 2.084$, $P = .174$) or swim speeds ($F(2,24) = 1.866$, $P = .177$) between groups (data not shown).

Discussion

The time course of TBI-induced changes and the timing of treatment are extremely important considerations when evaluating pharmacological interventions. This study was especially important as it looked at the therapeutic window over a very long period of time

post-injury. Clinically, this paradigm is important as it offers crucial information that could be used in patients where cognitive problems may not be apparent immediately following trauma. From an experimental point of view, this experiment offers important information about a potential mechanism for atomoxetine's observed effects. The results of this experiment suggest treatment early after injury is the critical time period for enhancing behavioral outcome with atomoxetine. Although few experimental studies have addressed therapeutic window along with dose response when investigating preclinical TBI pharmacotherapies, two previous studies in our lab found that beneficial treatments that were initiated at 24 h after injury were no longer effective if treatment initiation was delayed for 11 days post-injury (O'Dell et al., 1995; Pike et al., 1995). In fact, only one study, using aniracetam, found that chronic drug treatment initiated at 24 h and at 11 days post-injury were equally effective at reducing trauma-induced MWM deficits (Baranova et al., 2006). Clinically, this study suggests atomoxetine may have a limited therapeutic window for influencing post-injury cognition; however, controlled clinical trials are needed to fully examine when atomoxetine may be most effective in humans.

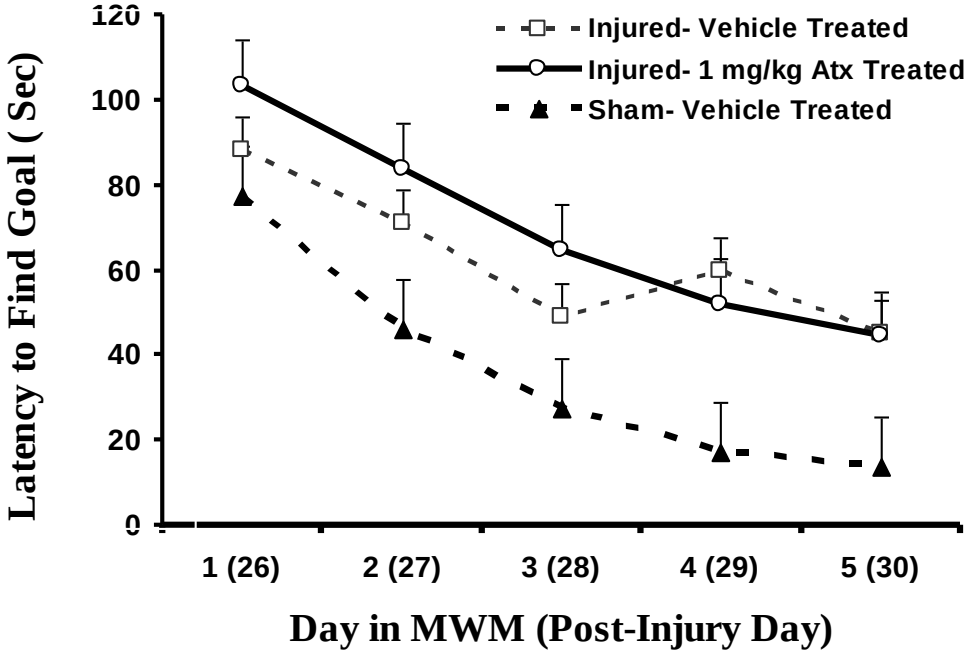
From a mechanistic perspective, the results offer valuable information regarding atomoxetine's mechanism for influencing cognition following experimental TBI. Previous studies, in several models have suggested that NE neural transmission begins to decrease as early as 6h post-injury and may remain depressed for as long as 8 weeks post-injury (Dunn-Meynell et al., 1998). A dramatic loss of cells in the LC has also been observed in the chronic stages following injury (Fujinaka et al., 2003). Likewise, several studies have found DA metabolism is decreased for several weeks following injury (McIntosh et al.,

1994; Massucci et al., 2004). Therefore, one hypothesis may be that atomoxetine, by increasing extracellualar levels of NE and DA, corrects chronic primary deficiencies in these neurotransmitters. In addition, because the neuromodulatory neurotransmitters are functionally interrelated, a derangement in one system can sometimes be felt "downstream" in another, e.g. influencing neuronal firing rates or the sensitivity of receptors. Therefore, increasing NE and DA may also correct chronic secondary deficiencies in other neurotransmitter systems. However, the results of this experiment suggest atomoxetine is not simply correcting NT deficiencies that exist in the chronic stages of injury. Initiating atomoxetine 10 days post- injury completely eliminated the beneficial effects observed in experiment 1. This suggests atomoxeteine's effects on cognition are related to TBI mechanisms occurring early after injury. Although animals do improve cognitively over time, there is still a significant difference between the injured-vehicle treated and sham-injured groups when MWM training is performed on PID 26-30. If atomoxetine treatment was simply correcting NT deficiencies that existed in the chronic stage, one prediction may be that treatment initiation at a delayed time point (10 days) would still be able to improve cognitive performance in the MWM. Therefore, it is unlikely that atomoxetine simply mediates the effects of a neurotransmitter-based diaschisis (RFD) in the chronic phase of injury, atleast in this model of TBI.

A more likely conclusion may be that increased extracellular catecholamines influence the events occurring in the first week following injury. This is a time period when a number of secondary injury mechanisms, as well as neural recovery mechanisms may influence functional outcome. The literature on NE suggests this neurotransmitter

may be neuroprotective and influence neural plasticity under certain circumstances via various mechanisms. In addition, neuronal depression occurring during this time period may affect many aspects of brain plasticity and neural recovery. Resolution of neural depression may depend on creating an optimal catecholaminergic environment. Future studies will be designed to explore this hypothesis.

Figure 8 MWM Results Following Delayed Atomoxetine Treatment. The mean (±SEM) latency (seconds) to locate a hidden platform in the MWM on post injury days 26-30 is shown. Injured animals treated with 1 mg/kg atomoxetine were not significantly different from injured animals treated with vehicle.


Injured- Vehicle Treated
Injured- 1 mg/kg Atx Treated
Sham- Vehicle Treated
Latency to Find Goal (Sec)
120
100
80
60
40
20
0
1 (26)
2 (27)
3 (28)
4 (29)
5 (30)
Day in MWM (Post-Injury Day)

Experiment 3: Termination of Chronic Administration before MWM

Rational

The aim of this study was to examine whether the continued administration of atomoxetine is necessary for the enhancement of performance on the spatial memory test in the MWM following TBI. This study determined if chronic atomoxetine treatment early after injury would lead to long-term enhancement of cognition, or if continued atomoxetine treatment was necessary to observe improvement. The experiment was designed to determine whether atomoxetine must be active in the organism during the time of MWM testing in order to provide cognitive enhancement, or if treatment during periods of postinjury-induced plasticity were sufficient to produce beneficial results.

Design

Animals were randomly separated into one of three groups: injured + saline treatment (n= 9), injured + 1 mg/kg atomoxetine treatment (n=10), and sham-injured + vehicle (n=11). Administration of the drug or vehicle began 24 h after injury, and continued daily for 7 days. Atomoxetine treatment was then terminated, and a drug wash out period was allowed before behavioral testing. Spatial memory performance in the MWM was assessed on post-injury days 11-15, and no drug treatment was given on testing days.

Results

A split-plot ANOVA [3 (Group) X 5 Day] indicated there was a significant effect of Group on Goal Latency (Figure 9, $F(2, 27) = 47.421$, $P < .001$). Post-hoc comparisons using Tukey's HSD test indicated that both sham-vehicle treated animals and injured

animals treated with 1 mg/kg atomoxetine were significantly different than injured animals treated with vehicle alone ($P < .001$). In fact, the injured group treated with atomoxetine had latencies that were not significantly different from sham-vehicle treated animals. There were no significant differences in mean righting times ($P=.186$) or swim speeds ($P=.095$) between groups.

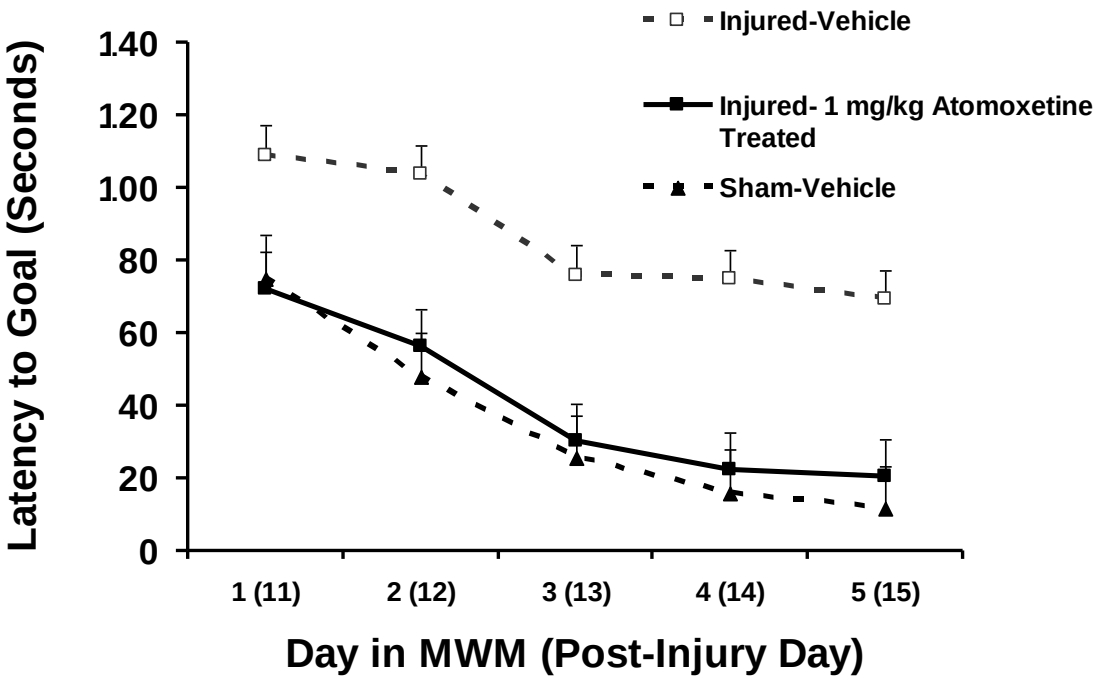
Discussion

The present study has demonstrated that cognitive dysfunction associated with L-FPI can be significantly attenuated with daily (7 day) administration of atomoxetine initiated at 24h after injury. Atomoxetine treatment was terminated prior to cognitive assessment and a wash-out period was allowed before MWM testing was performed. The injured-atomoxetine treated group exhibited significant cognitive improvement compared to the injured- vehicle treated group, suggesting chronic atomoxetine treatment in the early phase after injury may lead to long-term adaptive changes. The results also suggest that continued atomoxetine treatment during MWM testing is unnecessary to observe beneficial effects in cognitive performance.

Taken together with the previous experiments, the results strengthen the conclusion that atomoxetine's mechanism of action is related to the early phase following injury. Previous experiments, employing a 7 day drug-treatment paradigm following TBI, found drugs that enhance NE and DA, such as the MAO-inhibitor L-Deprenyl and the D1 agonist SK-38393, also enhance cognition in the MWM (Zhu et al., 2000). The behavioral effects were correlated with enhanced synaptic plasticity, and it has been suggested that these drugs may be able to affect neural remodeling. This is inline with previous studies of NE

and DA that suggest these NTs can affect plastic events, including intracellular signaling, changes in synaptic transmission, modification of neuronal architecture, and/or neurogenesis (Manji et al., 2003; and Millan, 2004). Therefore, one hypothesis that develops from this study is that chronic atomoxetine treatment during periods of neural plasticity following TBI may contribute to long-term adaptive structural reorganization. .

Figure 9 Results of Terminating Chronic Atomoxetine Treatment before MWM Testing. The mean (±SEM) latency (seconds) to locate a hidden platform in the MWM on post injury days 11-15 is shown. Injured animals treated with 1 mg/kg atomoxetine were significantly different from injured animals treated with vehicle.


Injured-Vehicle
Injured- 1 mg/kg Atomoxetine Treated
Sham-Vehicle
Latency to Goal (Seconds)
140
120
100
80
60
40
20
0
1 (11)
2 (12)
3 (13)
4 (14)
5 (15)
Day in MWM (Post-Injury Day)

Experiment 4: Administration of a Single Dose of Atomoxetine

Rationale

This experiment was designed to determine if a single dose of atomoxetine was sufficient to observe enhanced cognition or if once daily chronic (7 day) treatment was necessary to observe improved spatial learning. The experiment was also designed to help clarify a potential mechanism for the observed effects of atomoxetine following experimental TBI. Drugs that act through inhibition of catecholamine transporters have been shown to immediately increase levels of these NTs (Mattiuz et al., 2003); however, there tends to be a delay or a required period of chronic treatment before any observed behavioral changes are exhibited in various disorders such as depression and ADHD. The temporal mismatch between the rapid elevations in extracellular monoamine levels induced by selective norepinephrine reuptake inhibitors and their slow onset of action (weeks) in clinical use may reflect the initiation of plastic events including: alterations in receptor density, intracellular signaling changes in synaptic transmission, modification of neuronal architecture and/or neurogenesis (Manji et al., 2003; Millan, 2004). Likewise, following TBI, these drugs may act in a similar fashion and therefore require chronic administration to be effective.

In contrast, many studies have found that pretreatment or post-treatment with a single dose of catecholaminergic drugs following injury affords protection and enhances neurological outcome. For example, studies with amphetamine have found that a single oral dose following injury, combined with physical training, can have beneficial and lasting effects on motor outcome (Bash-y-Rita and Bjelke, 1991; Queen et al., 1997).

Likewise, a single dose of atomoxetine has been shown to improve outcome in stroke patients (Foster et al., 2006). Clinically, a single-dose of methylphenidate administered early after injury has been shown to increase cognitive performance in various tasks of attention, memory, and speed of processing in TBI patients (Kim et al., 2006). Improved cognition following a single dose of treatment at 24h may suggest the mechanism is more related to one or several of the secondary injury cascades observed following TBI. Still, it can not be ruled out that a single dose of these drugs may act though other mechanisms. Therefore, this study was not intended to provide conclusive evidence about the mechanism of atomoxetine, but rather to help clarify how this drug works following TBI and to determine a course of future outcome measures that may be related to the mechanism.

Design

Animals were randomly separated into one of three groups: injured + saline treatment (n=10), injured + 1 mg/kg atomoxetine treatment (n=9), and sham-injured (n=10). Animals in the injured groups received a L-FPI of moderate severity, and animals in the sham group underwent surgical preparation for received no injury. Twenty-four h after injury, animals received a single injection of either atomoxetine (1 mg/kg), or an equal volume of saline. Animals were returned to their cages and handled and weighed daily for the remainder of the experiment. No further treatments were given after PID 1. Spatial memory performance in the MWM was assessed on post-injury days 11-15.

Results

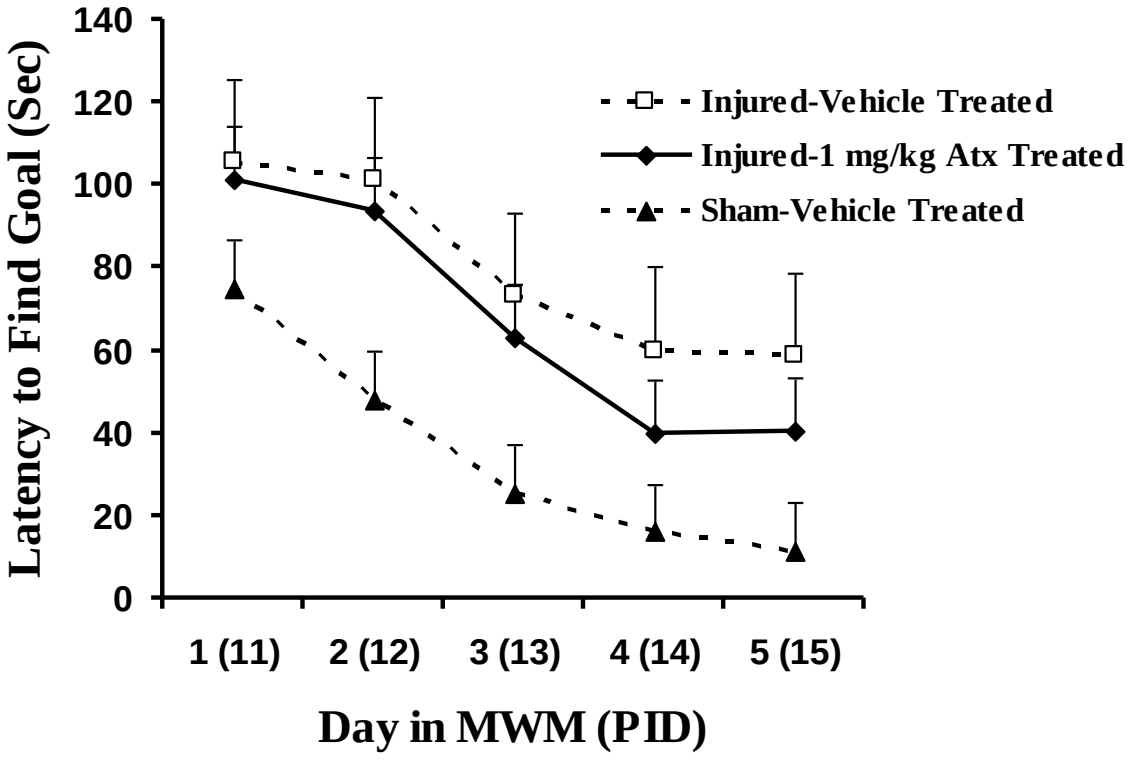
A split-plot ANOVA [3 (Group) X 5 Day] indicated there was a significant effect of Group on Goal Latency (Figure 10, $F(2, 26) = 37.799$, $P < .001$). However, post-hoc comparisons using Tukey's HSD test indicated there were no significant differences between the injured-vehicle and injured-atomoxetine treated groups ($P = .110$). A one-way ANOVA was performed to observe group differences for each day. Although, no differences were observed between the two injured groups on days 1-3 of MWM testing, there was a mild effect on latency in the atomoxetine treated group on day 4 and 5. There were no significant differences in mean righting times ($P = .715$) or swim speeds ($P = .118$) between groups (data not shown).

Discussion

The results found no significant differences between the two injured groups when a single-dose of atomoxetine was given 24 h after injury. There does appear to be a mild effect in the injured-atomoxetine treated group on the final two days of MWM training, although it was not significant over all five days of testing. Previous studies have shown blockade of α-adrenoreceotors prior to brain injury impairs recovery (Dunn-Meynell et al., 1997), whereas intraventricular NE infusion prior to injury improves recovery (Boyeson et al., 1990). In addition, several studies in humans and animals have demonstrated that a single dose of d-amphetamine or atomoxetine following stroke enhances functional motor outcome (Sawaki et al., 2002; Foster et al., 2006). In this study, a single dose of atomoxetine at 24h after injury was unable to augment cognitive outcome significantly. It is unknown what effects atomoxetine may have if initiated at earlier, more acute time

points, in which NE may be neuroprotective against one or many of the secondary injury cascades occurring following injury. Although single treatments with NET inhibitors are known to increase the extracellular levels of NE through transporter blockade, it appears effective therapy in certain disorders requires chronic administration of these drugs. This suggests long-term adaptive changes in neuronal function are necessary for their therapeutic effects. Similarly, in this experiment, a single treatment with atomoxetine following TBI did not produce the same benefit in cognition as chronic treatment (7days). Although, the mechanism underlying this therapeutic effect is not understood, several studies have demonstrated chronic, but not acute treatments, with catecholamine-enhancing drugs are able to up-regulate the cAMP signal transduction system, increase neurotrophins and other factors involved in cell survival and growth and promote neurogenesis (Thome et al., 2000; Chen et al., 2003; Calabrese et al., 2007). Therefore, a single treatment may not be sufficient to elicit significant adaptive structural changes that could influence cognitive outcome. Future studies will explore this possibility.

Figure 10 MWM Results Following Administration of a Single Dose of Atomoxetine 24h after L- FPI. The mean (±SEM) latency (seconds) to locate a hidden platform in the MWM on post injury days 11-15 is shown. Post-hoc comparisons found no overall significant differences between the two injured groups; however, there is a trend on day 4 and day 5 of MWM training towards improved latency in the atomoxetine-treated group.


Latency to Find Goal (Sec)
140
120
100
80
60
40
20
0
1 (11)
2 (12)
3 (13)
4 (14)
5 (15)
Day in MWM (PID)
Injured-Vehicle Treated
Injured-1 mg/kg Atx Treated
Sham-Vehicle Treated

Experiment 5: Effects of Atomoxetine on Protein Expression of Plasticity Markers

Rationale

The behavioral studies found low doses of atomoxetine are effective at attenuating cognitive deficits when initiated early and given daily (7 days) after injury. In fact, improved cognitive performance was even observed after chronic atomoxetine treatment was terminated, suggesting the potential for this drug to have long-term effects. This study was designed to investigate changes in plasticity markers by correlating the observed behavioral responses following atomoxetine treatment with changes in structural plasticity markers. Several studies suggest the catecholamines and catecholamine-enhancing drugs may influence neuroplasticity. Therefore, we decided to investigate changes in the expression of GAP-43, synaptophysin, and BDNF following post-injury atomoxetine treatment. We chose these markers because they have been previously characterized in TBI models and are sensitive to injury induced changes during the period of drug treatment. GAP-43 has been implicated in axon guidance, synaptic plasticity, and neuroregeneration, and its expression serves as a marker of these processes following injury. Synaptophysin is a marker for the total number of synapses, which may reflect both the loss of synapses as well as synaptic reorganization. Finally, BDNF is a neurotrophin that is sensitive to both TBI and NE-enhancing drugs. Therefore, we posit that atomoxetine treatment will enhance the expression of these markers, which may represent adaptive changes that are correlated with the observed behavioral responses.

Design

Animals were randomly separated into one of three groups: injured + saline treatment (n=5), injured + 1 mg/kg atomoxetine treatment (n=5), and sham injured (n=5). Animals were injured and sham injured as described previously. Beginning 24h after injury, animals received either saline or 1 mg/kg atomoxetine depending on group assignment. Treatment continued for 7 days, in which animals were sacrificed and brain tissue collected for Western blot analysis. Contralateral hippocampi were removed from 4 brains for each group (n=4).

Tissue Preparation & Western Blot Analysis*:* At 7 days post-injury, animals were deeply anesthetized with 4% isofluorane and decapitated. After removing the full brain, ipsilateral and contralateral hippocampus sections were dissected and separated on ice-cold glass plates and immediately transferred to a centerfuge tube containing 150µl of Ripa lysis buffer (1X Ripa, Upstate; 1 tablet Complete Protease Inhibitor, Roche). Samples were homogenized and then centrifuged at 14,000g for 20 minutes at 4°C. Total protein concentrations were determined using a Bio-Rad microassay, and a standard regression equation was used to calculate protein quantity from spectrophotometer readings. Samples were diluted 1:20 before water was added in appropriate concentrations to ensure equal quantities of protein loaded in each lane. 7.5µl of sample buffer and 1.5µl of reducing agent were added to each tube, before heating the samples for 5 minutes at 95°C. Samples were then loaded on to a SDS–PAGE precast gels (4-12% Bis-Tris Criterion XT, Bio-Rad), and run, using 1X MES Running Buffer or 1X Mops Running Buffer (Bio-Rad) at 150V for 1h. The separated gels were then transferred to PVDF membranes (Bio-Rad,

100V, 1H) using Transfer Buffer (1x Tris/Glycine Buffer (Bio-Rad) + MeOH). Following transfer, the gels were removed and stained with Coumassie Blue to ensure complete transfer to the membranes. The membranes were blocked in .5% non-fat dry milk +.05% Tween 20 in phosphate-buffered saline for 1 hour at room temperature on a shaker. The membranes were then incubated overnight at 4°C in TBS-T(.05%Tween 20) and the appropriate dilution of primary antibody. The following monoclonal antibodies and dilutions were used: GAP-43 (1:1000, 7B10, Invitrogen), synaptophysin (1:300, SVP-38, Sigma), BDNF (1:300, sc-546, Santa Cruz Biotechnology, Inc.). The membranes were washed six times in TBS-T (.05%) and incubated for 1 hour with a goat anti-mouse secondary antibody conjugated to horseradish peroxidase (1:20,000, Rockland, Lot # 18329). Membranes were thoroughly washed before being developed for 5 minutes with SuperSignal (Pierce). The blots were then imaged and quantified using GeneSnap and GeneTools software (Syngene). Negative controls included lanes that received the same treatments except the primary antibody was omitted.

The membranes were re-probed using monoclonal β-Actin (1:3000, Sigma) as a loading control. Each membrane was washed in methanol for 5-10 minutes, followed by 2 washes (5minutes) in DI water. The membranes were then placed in 1X Western Re-Probe Solution (G-Biosciences) for 1 hour on a shaker, and then washed 3 times (5 min each) in PBS-with .1% Tween-20. The membranes were then blocked in .5% non-fat dry milk + PBS-with .05% Tween-20 for 1 hour, and then incubated overnight on a shaker with primary antibody + PBS-T with milk. The membranes were washed 3 times in PBS-T + milk and 3 times with PBS before being incubated for 1 hour in goat anti-mouse secondary

antibody (1:20,000, Rockland). Membranes were washed, developed, and analyzed as previously described.

Results

GAP-43 is expressed at elevated levels in growth cones during axonal extension and is believed to be one component of injury-induced plasticity. Immunoblot analysis was performed to confirm changes in GAP-43 expression following injury and to quantify any changes in the amount of GAP-43 following 7 day post-injury treatment with atomoxetine. The GAP-43 antibody (7B10) appeared at approximately 46 kDA and produced 1 consistent band across all lanes (Fig. 11A). The omission of primary antibody was used as a negative control to ensure specific binding of the antibody. The negative control consistently produced no staining in multiple blots (Fig. 11B). Beta-Actin (AC-15, 42 kDA) was used as a control to ensure equal loading of protein between lanes (Fig. 11C). A one-way ANOVA found no significant differences in the expression of Beta-Actin between the groups ($F(2,12)=2.224$ $P=.151$). Statistical analysis using a one-way ANOVA indicated there was a significant group effect on GAP-43 expression in the ipsilateral hippocampus (Fig. 11D, $F(2,12)=7.189$, $P< .01$). Post-hoc analysis using the LSD test indicated there was a small but significant decrease in GAP-43 expression in the ipsilateral hippocampus of injured animals compared to sham levels ($P=.034$). The injured group treated with atomoxetine showed significantly increased GAP-43 levels when compared to injured-vehicle alone ($P=.003$). In fact, there were no differences in GAP-43 expression between the Injured- Atomoxetine-Treated and Sham-groups ($P=.204$), indicating the injury-induced decreases in GAP-43 seen at Day 7 post-injury can be

counteracted by treatment with atomoxetine post-injury. The contralateral hippocampus was also dissected and used for Western Blot analysis (Figure 12). A one-way ANOVA found no significant differences in GAP-43 expression in the contralateral hippocampus between any of the groups ($F(2, 9) = 1.482$, $P = .278$).

Synaptophysin (38kDA) has been used previously to quantify changes in synapses following injury and during synaptic reorganization. Immunoblot analysis was performed on synaptophysin to observe any injury-induced changes at PID 7 following L-FPI. The experiment was designed to compare differences in injury-vehicle treated and injury-atomoxetine treated animals. A one way ANOVA found no statistical differences between the groups ($P = .501$), indicating that the amount of protein expression was not significantly different between injured-vehicle and sham groups and between the two injured groups (Figure 13).

The expression of BDNF (14kDa) protein in the ipsilateral and contralateral hippocampus was analyzed using a one-way ANOVA (Figure 14). Although, no significant differences were detected between the ipsilateral groups ($P = .065$), post-hoc analysis using the Least Significant Differences (LSD) test found a significant increase in the expression of BDNF in the ipsilateral hippocampus of injured- atomoxetine treated animals over injured-vehicle treated animals ($P = .027$), suggesting atomoxetine treatment may influence neurotrophins when given chronically after injury(Fig. 14a). Analysis of the contralateral hippocampus (Fig. 14b) found a significant effect on group ($F(2,10) = 5.455$, $P = .025$). Post-hoc comparisons using LSD found there was a significant

increase in BDNF expression in atomoxetine treated animals over sham-injured animals (P=.020).

Discussion

In this experiment we measured the levels of GAP-43 and synaptophysin, which are normally found in growth cones and synaptic vesicles, to obtain an estimate of the effects of L-FPI on these markers and to show how atomoxetine treatment may influence plasticity. We also looked at changes in BDNF, a neurotrophin that may play an important role in post-injury plasticity. Time-dependant changes in GAP-43 and synaptophysin expression appear to be a common event following brain injury (Christman et al., 1997; Emery et al., 2000). Because GAP-43 has been implicated in many processes including neuronal growth, the formation of novel neuronal connections, synaptic remodeling, and neuronal sprouting following insult, it has been widely used as a marker of axonal and synaptic plasticity. Although TBI is generally followed by a period of increased axonal sprouting, characterized by increased GAP-43 and synaptophysin staining, the regenerative response has been shown to be short-lived and varies depending on the injury model and severity (Thompon et al., 2006). A previous study using L-FPI found GAP-43 immunoreactivity was elevated bilaterally at 24 h and 48h after injury; however, on PID 7 the levels of GAP-43 had returned to sham levels, or had even decreased below sham levels (Emery et al., 2000; Marklund et al., 2007). This study suggested that the posttraumatic brain may have a transient period for increased regenerative potential, in which therapeutic interventions may be able to enhance the endogenous plasticity response. In the current study, which also employed the L-FPI model, we found that GAP-

43 levels were significantly decreased at PID7 in the ipsilateral hippocampus when compared to sham levels, with no significant differences observed in the contralateral hippocampus. When atomoxetine treatment was administered for 7 days post-injury, GAP-43 expression returned to sham levels, suggesting atomoxetine treatment may have an effect on post-injury regeneration. The atomoxetine treated animals, like the injured-vehicle treated group, did not show any significant differences from sham levels in the contralateral hippocampus, suggesting the change in GAP-43 protein expression observed in the ipsilateral cortex was related to atomoxetine's effects on the injured brain.

The decrease in GAP-43 observed in injured- vehicle treated animals may indicate an inhibition of axonal plasticity that occurs following injury or may be a result of cell loss in the hippocampus. Previous studies have found that GAP-43 levels are increased at 24 h and 48 h following mild or moderate TBI, but that this response may be limited (Thompson et al., 2006). Successful neurite outgrowth resulting in functional regeneration likely requires a delicate balance between growth promoting and growth-inhibiting cues. Although increased GAP-43 expression can not be termed successful regeneration alone, it has been postulated to correlate with behavioral recovery following TBI (Hulsebosch et al., 1998). Overexpression of GAP-43 has also been shown to increase spinal cord regeneration *in vivo* (Bomze et al., 2001) and play an important role in hippocampal synaptic remodeling following denervation (Masliah et al., 1991). Furthermore, previous studies using L-FPI have found that the decrease in GAP-43 expression following injury at 1 week post-injury can return to sham values with drug treatments initiated during the early phase of injury (Marklund et al., 2007). Although it is unknown whether the increase

in GAP-43 following atomoxetine treatment represents successful regeneration based solely on this study, taken together with our previous behavioral studies that found atomoxetine treatment for seven days following injury leads to long-term cognitive enhancement, it appears the increased levels of GAP-43 may contribute to the cognitive enhancement observed in previous experiments, although this is merely correlative. The exact mechanism by which atomoxetine influenced GAP-43 expression remains unknown, but may involve changes in neurotrophins elicited by extracellular catecholamines and their downstream effects on signal transduction.

Atomoxetine treatment also led to bilateral increases in the neurotrophin BDNF. BDNF is an activity-dependant neurotrophic factor, with receptors densely distributed throughout the CNS (Altar et al., 1994). BDNF has been implicated in hippocampal learning and memory, and enhanced expression of BDNF has been correlated with improved cognition (Bramham and Messaoudi, 2005, Ando et al., 2002). Following TBI, increases in the levels of BDNF have been reported at different time points (Hicks et al., 1997; Chen et al., 2005). In general, changes in BDNF expression have been explained by alterations in BDNF production, degradation, or neuronal transport. In this study performed at day 7 post-injury, BDNF expression in injured-vehicle treated animals was not significantly different from sham animals in the ipsilateral or contralateral hippocampus. The injured-atomoxetine treated group displayed small, yet significant increases in the levels of BDNF in both the ipsilateral and contralateral hippocampus compared to both sham and injured-vehicle treated groups. This is suggestive of an enhanced structural recovery correlated with improved behavioral outcome following

atomoxetine treatment; however, the exact mechanism remains unknown. One conclusion may be that increased BDNF in the hippocampus, created a more favorable environment for regeneration, increased survival and maturation of newborn granule cells, and/or enhanced synaptic plasticity, which as a consequence enhanced cognition. Functionally, BDNF plays a role in facilitating synaptic transmission and is associated with LTP (Patterson et al., 1996). This increased plasticity may allow for increased cognitive flexibility, which enables animals to perform better in cognitive tasks (for a review, see Cui, 2006). Moreover, *in vitro* studies have shown that neurons stimulated with BDNF have an enhanced ability to counteract the effects of myelin-associated inhibitors through a mechanism involving the action of protein kinase A (Cai *et al*., 1999; Gao *et al*., 2003). Therefore, increased BDNF levels have been associated with increased levels of GAP-43 and may have contributed to the increase in GAP-43 observed in the current study. In addition, several studies have found that increased BDNF following injury is correlated with improved behavioral outcome. For example, environmental enrichment and delayed exercise have both been shown to increase hippocampal BDNF levels and improve cognition following injury (Chen et al., 2005; Chytrova et al., 2008). The increase in BDNF expression in the contralateral hippocampus following atomoxetine treatment, suggests atomoxetine may have an effect globally on BDNF levels, which may represent a compensatory recovery mechanism stimulated by atomoxetine in areas remote from the injury. Previous studies have found elevated BDNF levels in the contralateral cortex at 72h after injury in rats (Hicks et al., 1997), however, we found no differences in BDNF expression from sham in the injured- vehicle treated animals. Therefore, it may be that

atomoxetine treatment is necessary for such compensatory mechanisms. Alternatively, the bilateral expression of BDNF may suggest atomoxetine affects BDNF expression through mechanisms that are unrelated to TBI. No previous studies have investigated atomoxetine's effects on BDNF in naïve animals.

BDNF is partially localized to NE nerve fibers and terminals and is synthesized and transported by LC neurons (Fawcett et al., 1998). Overexpresion of BDNF in NE neurons results in increased activation of trkB and long-lasting changes in neuronal survival and organization. The increase in BDNF expression following atomoxetine treatment in the current study is also in line with several studies that suggest NE-enhancing drugs can increase the levels of neurotrophins in the adult brain (Aloyz et al., 1999; Calabrese et al., 2007). Several studies have found that antidepressant treatment induces up-regulation of BDNF (Nibuya et al., 1995), and increases neurogenesis in the adult rat hippocampus (Malberg et al., 2000), although it is unknown which neurotransmitter system is most responsible for these effects. These studies also suggest that chronic treatment, rather than a single dose, is necessary for significantly increased BDNF expression (Calabrese et al., 2007), which is inline with our behavioral results that found only a modest non-significant change in cognition following a single treatment. Therefore, increased neurotrophin expression may represent adaptive structural changes after repeated atomoxetine treatments. Moreover, alterations in neuronal activity can up-regulate BDNF mRNA and induce prolongation of its stability (Ghosh et al., 1994; Fukuchi et al., 2005), thus the downstream effects of atomoxetine on other neurotransmitters and an overall stimulation of neuronal activity may also be responsible for the increase in BDNF. Further evidence

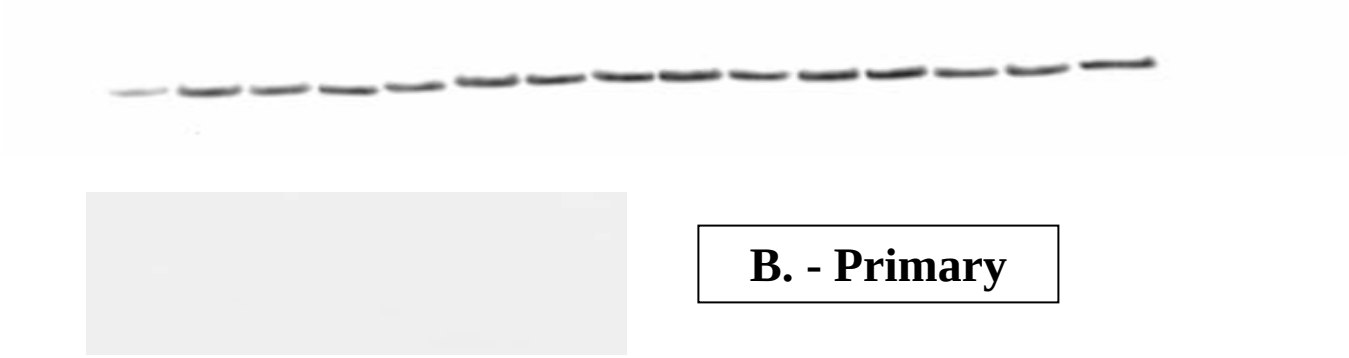
comes from the studies that show neural activity regulates the induction and release of BDNF (Poo, 2001; Lessmann et al., 2003), and NE modulates BDNF expression in neurons and astrocytes (Juric et al., 2006). Therefore, another potential mechanism for atomoxetine's effects on BDNF expression may involve activation of astrocytes.

The present study at 7 days following injury found no differences in the levels of synaptophysin between any groups. Changes in synaptophysin immunoreactivity have been used previously to characterize both synaptic loss and formation of new synapses. Synaptophysin expression has been shown to increase in parallel with the formation of synapses during development and reorganization (Calhoun et al., 1996). Previous studies found synaptophysin is increased beginning at 10 days post-injury and lasts as long as 60 days following experimental brain injury (Scheff et al., 2005; Thompson et al., 2006). In the current study, it may be possible that atomoxetine affected synaptophysin but that this effect was masked by the loss of synapses. However, this is unlikely because we found no significant differences between injured-vehicle treated and sham groups. One caveat is that only one time point was analyzed. Several studies have previously characterized the time course for the expression of synaptophysin following injury and suggested that increases in synaptophysin follow increases in the expression of GAP-43. Therefore, a later time point may be more appropriate to investigate the effects of atomoxetine on the expression of synaptophysin.

Figure 11 Ipsilateral GAP-43 Western Blot (A-D). A. Western blot for GAP-43 protein from the hippocampus of injured-vehicle treated, sham, and injured-atomoxetine treated groups B. The negative control, in which primary antibody was omitted, showed no staining, demonstrating the primary antibody is specific for GAP-43. C. A one-way ANOVA found no significant differences in the expression of beta-Actin between the groups ($F(2, 12) = 2.224$ $P = .151$), indicating equal loading of protein in each lane. D. The effect of atomoxetine on GAP-43 expression following injury. The graph shows the quantified optical density measurements from the Western Blot. There was a significant decrease from sham levels in the injured-vehicle treated groups ($P = .034$). Injured animals treated with atomoxetine returned GAP-43 expression to sham levels ($P = .204$). (* is $P < .05$, ** is $P < .01$, compared to Injured-Vehicle Treated Group)

A. Ipsilateral GAP-43

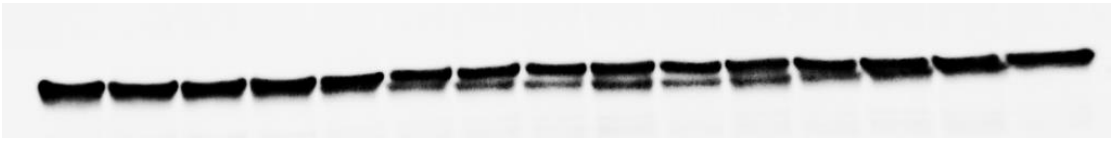
----------Injured-------- ----------Sham---------- ---------Treated---------



B. - Primary

C. Ipsilateral Actin- Loading Control

---------Injured------- ----------Sham--------- ---------Treated---------



D. OD measurements for GAP-43

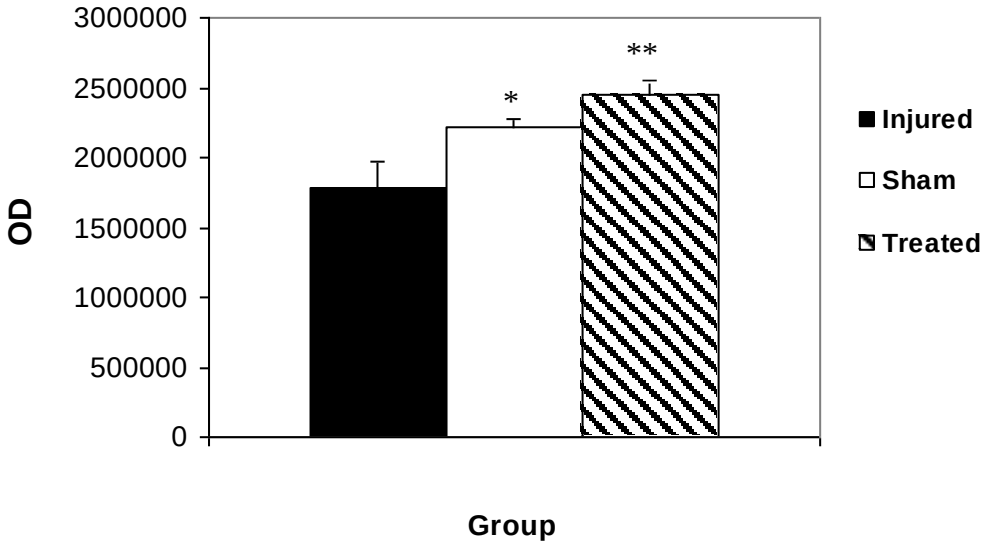


Figure 12 Contralateral GAP-43 Western Blot. The western blot for GAP-43 (46 kDA) expression in the contralateral hippocampus is shown. All three groups (n=4) were run on the same gel. No significant differences were found between the groups (P=.278).

Contralateral GAP-43

---------Injured---------- *----------Sham----------* *----------Treated----------*

Contralateral Actin-Loading Control

OD Measurements for CL GAP-43

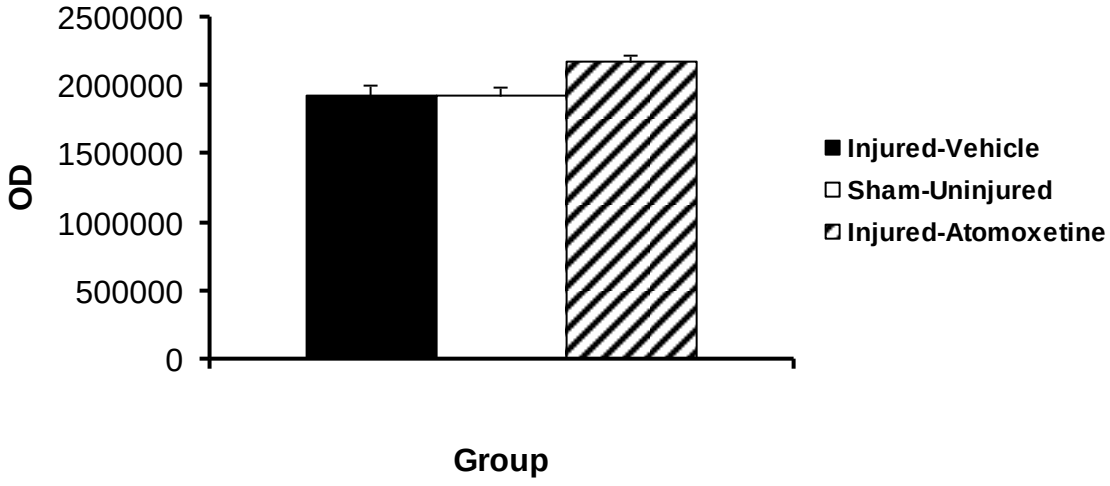


Figure 13 Ipsilateral Synaptophysin Western Blot. The western blot for Synaptophysin (38 kDA) expression in the ipsilateral hippocampus is shown. All three groups (n=5) were run on the same gel. No significant differences were found between the groups (P= .501). The actin blot also revealed no significant differences among groups.

Ipsilateral Synaptophysin

---------Injured---------- *----------Sham----------* *----------Treated----------*

Actin Reprobe for Synaptophysin

OD Measurements for Synaptophysin

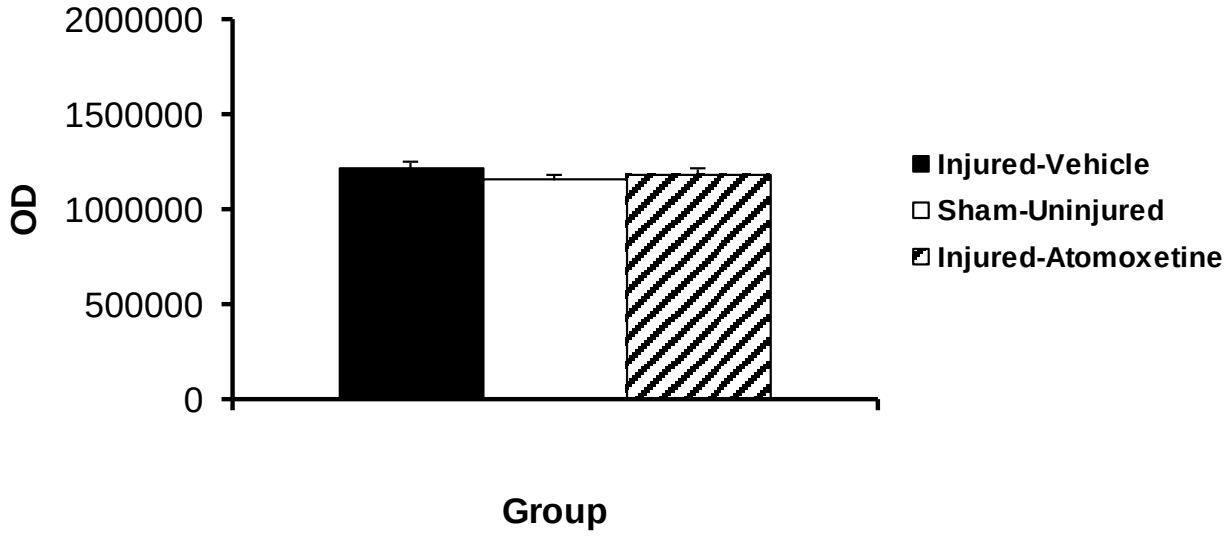
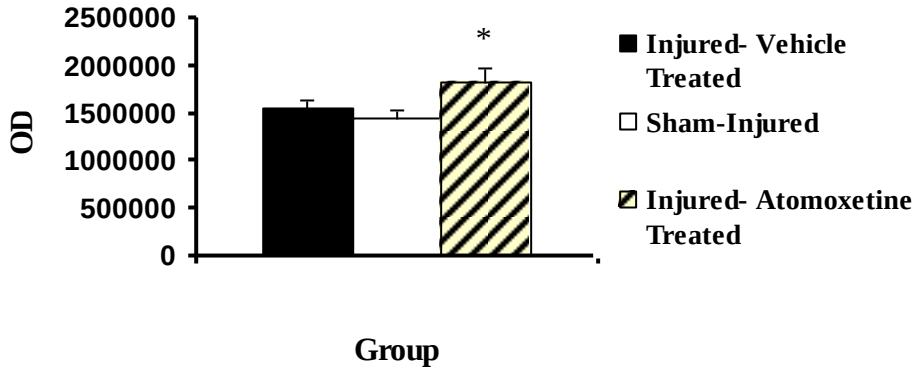


Figure 14 The expression of BDNF in the Ipsilateral and Contralaterl Hippocampus.
a. The western blot for BDNF (14 kDA) expression in the ipsilateral hippocampus of injured-vehicle, sham-injured, and injured-atomoxetine treated animals. A one-way ANOVA found no significant differences between the groups ($F(2,12)=3.441$, $p=.066$); however, pare-wise comparisons using the Least Significant Differences (LSD) test found a significant increase in the expression of BDNF in the hippocampus of injured-atomoxetine treated animals over sham-injured animals ($P= .027$), suggesting atomoxetine treatment may influence neurotrophins when given chronically after injury.
b. The western blot for BDNF expression in the contralateral hippocampus of injured-vehicle, sham-injured, and injured-atomoxetine treated animals. Analysis of the contralateral hippocampus (Fig. 13b) found a significant effect on group ($F(2, 10) =5.455$, $P=.025$). Post-hoc comparisons using the LSD test found there was a significant increase in BDNF expression in atomoxetine treated animals over sham-injured animals ($P=.020$).

A. BDNF (Mature)- Ipsilateral

---------Injured---------- ----------Sham---------- ----------Treated----------

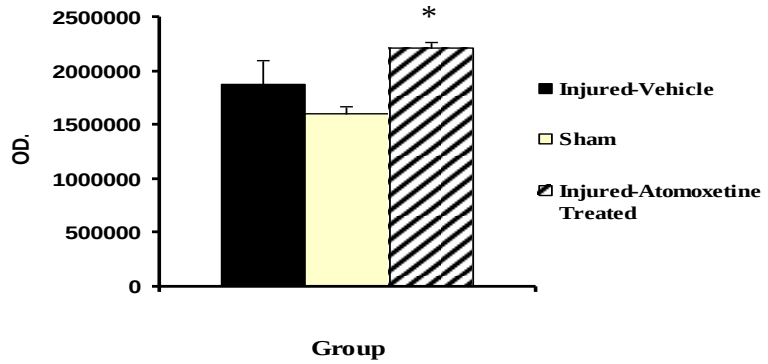
Ipsilateral Hippocampus BDNF



B. BDNF (Mature)- Contralateral

---------Injured---------- ----------Sham---------- ----------Treated---------

Contralateral BDNF



Experiment 6: Immunohistochemistry for GAP-43 in the Hippocampus

***Rationale**:* Western Blot analysis found a significant change in the expression of GAP-43 protein levels at PID 7. Therefore, we decided to employ immunohistochemistry techniques to observe specific changes in GAP-43 expression in different regions of the hippocampus.

Design: Animals were randomly separated into one of three groups: injured + saline treatment (n=5), injured + 1 mg/kg atomoxetine treatment (n=5), and sham-injured (n=5). Animals were injured and sham-injured as described previously. Beginning 24h after injury, animals received either saline or 1 mg/kg atomoxetine depending on group assignment. Treatment continued for 7 days. On PID 7, animals were anesthetized with 100 mg/kg pentobarbital and fixed via cardiac perfusion with saline followed by 4% paraformaldyhide, in .1M phosphate buffer, pH 7.2. Sham animals were likewise sacrificed at the same time points. Following perfusion, brains were removed and placed in paraformaldyhide for 1-3 days, and then placed in Millonig's Phosphate Buffer Solution and stored at 4°C. Several naïve animals were used as controls.

Immunohistochemistry Procedure

The brains were sliced on a vibratome into 40µm thick sections, and sections containing rostral (-3.6 mm from bregma) to caudal (-4.2mm from bregma) hippoampal regions were used for immunohistochemistry. The immunohistochemistry procedure was performed on 5 sections from each brain for all three groups. The slices were washed in phosphate buffer saline (PBS) three times for five minutes, and then incubated in 5% $H_2O_2$for 30 minutes to quench endogenous peroxidases. After three additional 5 minute

washes in PBS, the slices were incubated in .1M PBS with Triton X/ 10%normal horse serum. Sections were then incubated overnight at 4°C in a 1:2000 concentration of a mouse anti-GAP-43 (7B10, Invitrogen). On day two, the sections were washed 3 times for 10 minutes, and then incubated at room temperature for 1h in biotinylated secondary antibody (1:200, ImmunoPure Ultra-Sensitive ABC Mouse IgG Staining Kit, Pierce). After additional washes, sections were incubated for 1h at room temperature with ABC reagent (Pierce) and developed with diaminobenzadine (DAB substrate kit, Zymed). Sections were mounted onto gelatin-coated slides, dehydrated and cover-slipped. Application of control serum instead of primary antibody on selected sections of rat tissue provided a negative control.

Densitometric Analysis

Slides were coded and the data was collected by an observer unaware of treatment group. Each group contained 5 animals, and 5 slices were analyzed from each animal. Images were captured using a Nikon Optiphot-2 microscope equipped with a MRI-103 driver for a MAC 2000 motorized stage and a MTI 3CCD video camera interfaced with the MicroBrightfield Neurolucida software. Images were analyzed using IPLab 3.7 (BD Biosciences). The igages were first converted to grey scale before analysis. The borders of each region were traced manually and the pixels per area were then automatically calculated using image analysis software after defining a threshold for the background.

Results

The IR for GAP-43 was observed in the stratum oriens, stratum radiatum, stratum lacunosum moleculare, and inner molecular layer of the denate gyrus. GAP-43

immunoreactivity was characterized by a laminar immunostaining pattern. There was no visible staining in the negative controls (data not shown). Compared to uninjured-sham animals (Figure 15A) increases in IR for GAP-43 were observed at 7 days post-injury within the IML of the dentate gyrus and SLM of CA1 of the hippocampus. Densitometric analyses using a one-way ANOVA found a significant effect of group in the IML of the dentate gyrus (Fig 15B, $F(2, 12) = 15.280$ $P = .001$). Pair-wise comparisons using Tukey's HSD test revealed there was a significant increase in GAP-43 expression from sham levels in both injured-vehicle treated ($P < .05$) and injured-atomoxetine treated animals in the IML ($P < .001$). Although GAP-43 IR was not significantly higher in the injured-atomoxetine-treated group compared to injured-vehicle treated in this region, there was a trend towards increased GAP-43 in the injured-atomoxetine-treated group that reached significance over the injured-vehicle treated group when a less stringent post-hoc test, the LSD test, was used ($P = .030$). A one-way ANOVA also found a significant effect on GAP-43 IR in the SLM in CA1 of the hippocampus ($F(2, 12) = 5.628$ $P = .019$). Tukey's HSD found a significant increase in the expression of GAP-43 in the SLM region relative to sham levels in the atomoxetine treated group ($P < .05$). There were no significant differences found between sham and injured-vehicle groups or between the two injured groups in this region of the hippocampus. All other regions of the hippocampus analyzed revealed no significant differences between groups (data not shown).

The contralateral hippocampus was also analyzed for differences in GAP-43 IR (Figure 16). There were no significant differences found between the uninjured-sham group and the injured-vehicle treated group in any of the regions analyzed in the CL

hippocampus. However, there was a significant increase in GAP-43 IR found between the injured-atomoxetine treated group in the IML of the hippocampus compared to both Sham ($P<.001$) and injured-vehicle treated groups ($P<.001$), suggesting atomoxetine treatment affected GAP-43 expression in both hemispheres of the IML. In the SLM of CA1 in the hippocampus, GAP-43 IR was increased modestly, yet significantly, in the injured-atomoxetine treated group over the sham ($P=.03$), although no differences were observed in the SLM between atomoxetine-treated animals and sham animals, or between sham animals and injury-vehicle treated animals.

Discussion

In the present study, we investigated the effects of chronic atomoxetine treatment on injury-induced changes in GAP-43 using immunohistochemistry and image analysis. The results indicated that (1) GAP-43 expression was increased in the IML of the dentate gyrus of the hippocampus in injured-vehicle treated animals compared to sham-injured animals, (2) atomoxetine treatment for 7 days following L-FPI increased GAP-43 expression in the ipsilateral and contralateral IML and SLM region of CA1 when compared to shams and (3) atomoxetine treatment following injury increased GAP-43 expression relative to injured-vehicle treated in the IML of the contralateral hippocampus.

Numerous studies have demonstrated that GAP-43 plays an important role in axonal outgrowth, regeneration, and neuroplasticity (for review, see Emery et al., 2003). Overexpression of GAP-43 following injury has been correlated with improved behavioral recovery (Holesbosch et al., 1998; Marklund et al., 2007). Previous studies using immunohistochemistry techniques found that GAP-43 expression is increased early after

injury but returns to sham levels by PID 7, indicating a limited regenerative response (Emery et al., 2000; Marklund et al., 2007). In the current study using immunohistochemistry performed on PID 7, we found that GAP-43 levels were significantly increased in the IML of the dentate gyrus of the ipsilateral hippocampus of injured-vehicle treated animals relative to sham. No significant differences were found between injured-vehicle treated animals and sham animals in any of the other regions of the hippocampus (ipsilateral and contralateral) that were analyzed. These results using immunohistochemistry differ from those obtained in the previous experiment using western blot analysis, in which we found a decrease in GAP-43 in the ipsilateral hippocampus that was restored to sham levels by daily administration of atomoxetine treatment for 7 days following injury. The current study using immunohistochemistry also found that atomoxetine treatment following injury increased GAP-43 expression compared to Sham, specifically in the dentate gyrus and SLM region of CA1. In contrast to the previous study, we found no decreases in injured-vehicle treated animals from sham levels in GAP-43 expression in any of the regions analyzed and even found an increase over sham levels in the IML. Therefore, the immunohistrochemistry results do not directly confirm the results obtained from western blot analysis. The different results from these two studies may partially be explained by the experimental differences in the techniques used. For example, western blot analysis uses tissue from the entire hippocampus (dorsal and ventral), whereas the results from the immunohistochemistry reflect specific regions from only dorsal sections of hippocampus. Some of the subtle changes that are expressed in specific regions of the hippocampus following immunohistrochemistry may be missed

using western blot. The differences also may be explained on the basis of injury variability between animals.

The increased expression of GAP-43 observed in the IML following injury likely represents sprouting of associational/commissural fibers and synaptic reorganization occurring following injury. The mossy cells of the hilar region of the hippocampus constitute the majority of the fibers innervating the inner third of the dentate gyrus. There was a trend towards increased GAP-43 expression in the IML of atomoxetine treated animals following injury compared to injured-vehicle treated groups. Functionally, it is unknown what an increase in GAP-43 in this region may represent. Several studies have found aberrant sprouting of mossy fibers in this region is associated with maladaptive plasticity (Longo et al., 2005). Our behavioral results indicated that atomoxetine improved cognitive function following injury, suggesting the small increase in GAP-43 in this region may not be representative of maladaptive plasticity. Although it is unknown what the small increase in GAP-43 following atomoxetine treatment represents functionally, several studies have shown drugs that affect the NE system may influence GAP-43 in this region. For example, imipramine, a NE reuptake inhibitor that also influences other monoamines, increases GAP-43 specifically in the IML and SLM of the hippocampus of naïve adult male Wistar rats (Sairanen et al., 2007). Desiparmine, a NE reuptake inhibitor, specifically increased GAP-43 expression in the dentate gyrus. Furthermore, *in vitro* studies in hippocampal cultured cells have found administration of NE leads to increased GAP-43 expression, concurrent with increases in neurite outgrowth-promoting genes (Laifenfeld et al., 2002). Although, the mechanism is unknown, it may involve the role of NE in LTP in

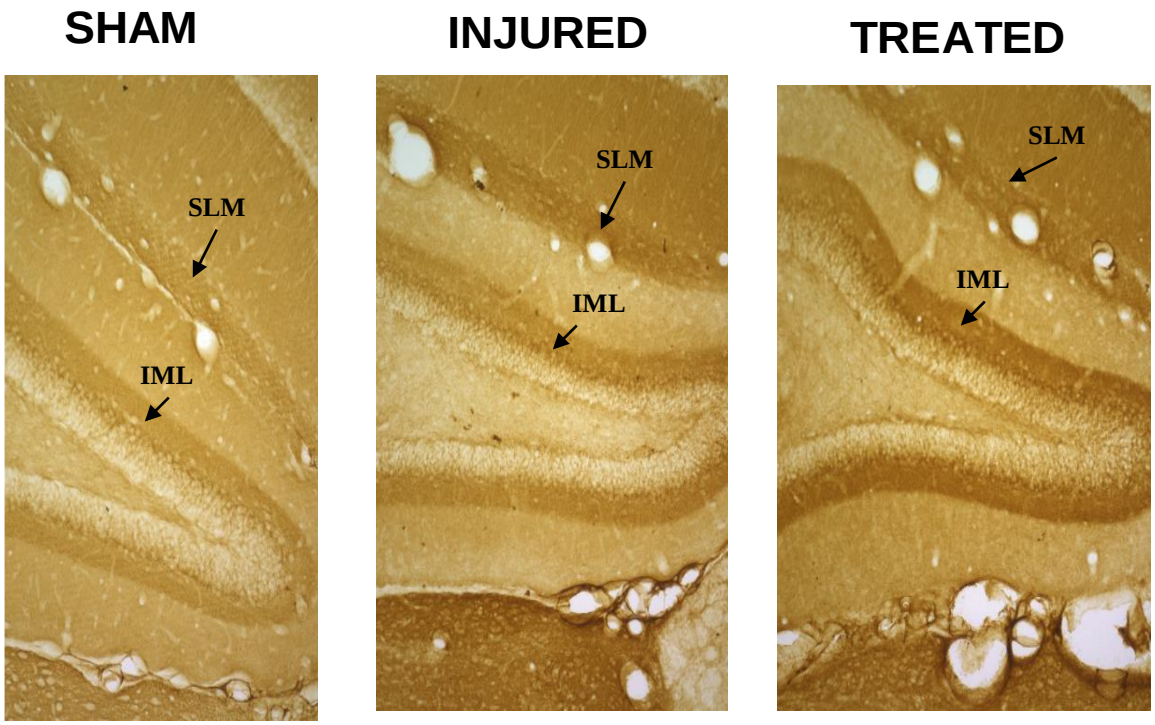
the hippocampus and may be related to activation of cAMP AND PKA. In the hippocampal formation, noradrenergic innervation is particularly dense in areas receiving mossy fiber inputs, including the hilus of the dentate gyrus and stratum lucidum of the CA3 (e.g., Moudy et al., 1993). Interestingly, GAP-43 expression was increased over sham animals in the iml of both the ipsilateral and contralateral hippocampus, suggesting the changes in GAP-43 following atomoxetine treatment may not be directly related to TBI pathology.

The other region of the hippocampus that displayed a change in GAP-43 was the SLM of CA1. The SLM region of CA1 receives lateral perforant pathway input, as well as Shaffer collaterals from CA3. GAP-43 expression was increased relative to sham animals in this region following atomoxetine treatment for 7 days post-injury in both the ipsilateral and contralateral hippocampus. Therefore, atomoxetine treatment may have an influence on synaptic plasticity and reorganization occurring in this region. The influence of dopamine may be particularly important regarding synaptic plasticity in this region. D1 receptor activation can mimic LTP-inducing electrical stimulation to produce GAP-43 up-regulation in CA1 (Williams et al., 2006). The occurrence of late phase-LTP in CA3-CA1 and subsequent up-regulation of GAP-43 in hippocampus is simultaneously blocked by SCH23390, a selective DA1R antagonist (Chirwa et al., 2004).

.

Figure 15 GAP-43 Immunohistochemistry in the Ipsilateral Hippocampus. A. Representative photomicrographs of GAP-43 staining in the ipsilateral hippocampus of Sham, Injured-Vehicle treated, and Injured- Atomoxetine Treated Animals (10X) at 7 days post-injury. B. Densitometric analysis of GAP-43 immunoreactivity within individual hippocampal regions revealed statistically significant increases. Pair-wise comparisons using Tukey's HSD test revealed there was a significant increase in GAP-43 expression from sham levels in both injured-vehicle treated ($P<.05$) and injured-atomoxetine treated animals ($P<.001$) in the IML of the dentate gyrus. Although GAP-43 IR was not significantly higher in the atomoxetine treated group compared to injured-vehicle treated, there was a trend towards increased GAP-43 in the atomoxetine-treated group that reached significance over the injured-vehicle treated group when a less stringent post-hoc test, the LSD test, was used ($P=.030$). A one-way ANOVA also found a significant effect on GAP-43 IR in the SLM of the hippocampus ($F(2, 12) = 5.628$ $P=.019$). Tukey's HSD found a significant increase in the expression of GAP-43 in the SLM of CA1 from sham levels in the atomoxetine treated group ($P<.05$).

A. Ipsilateral Hippocampus



B.

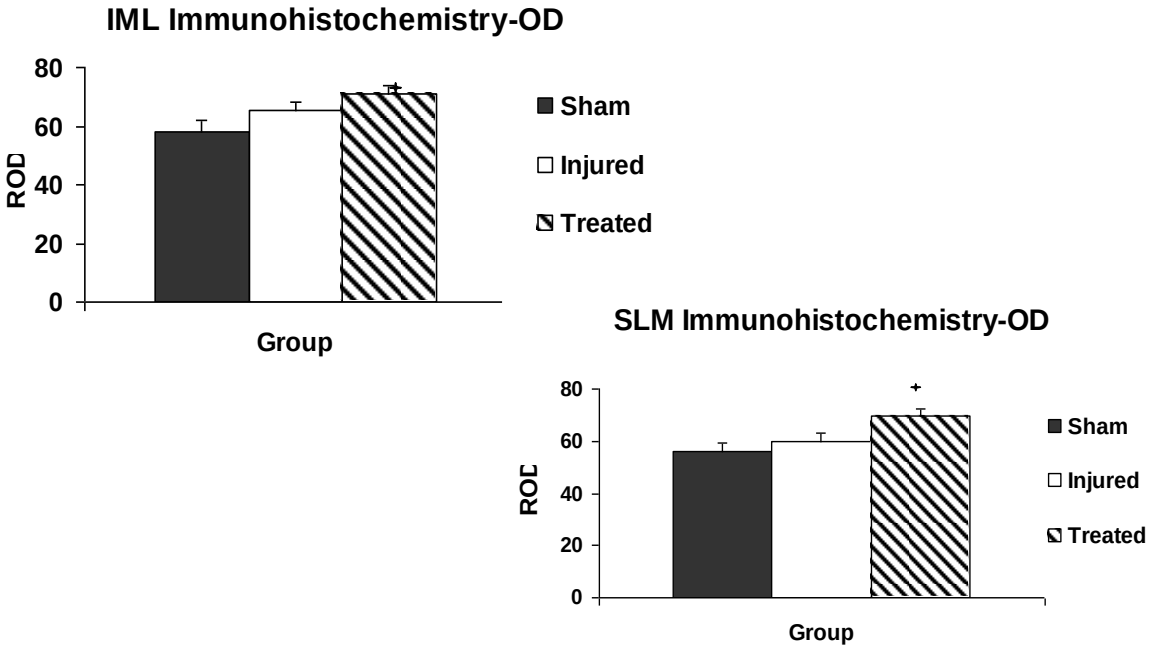
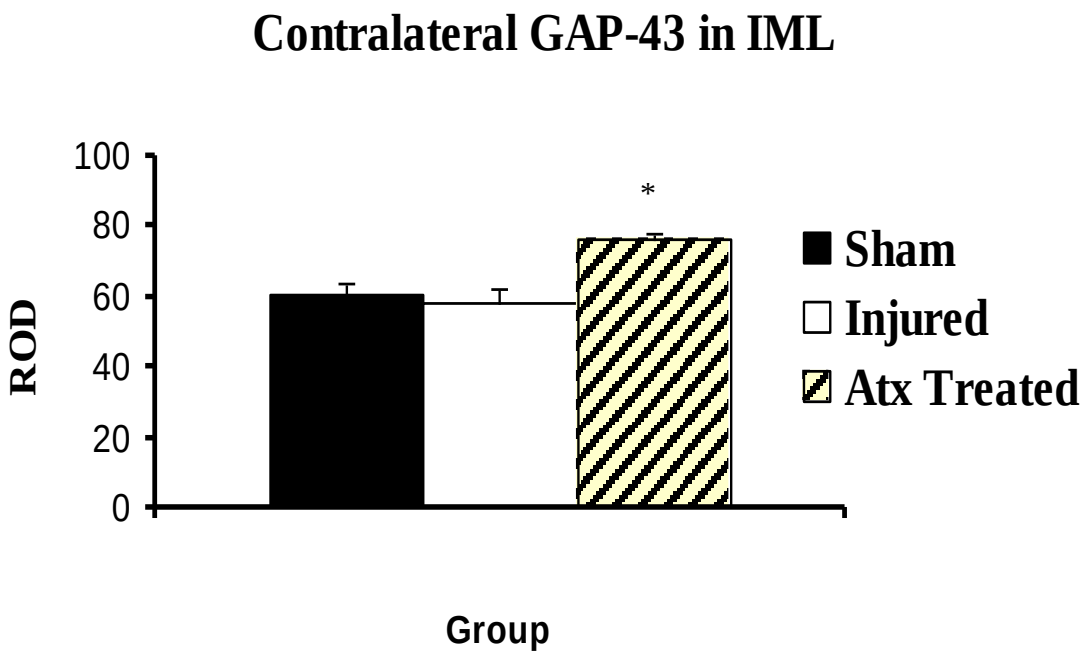
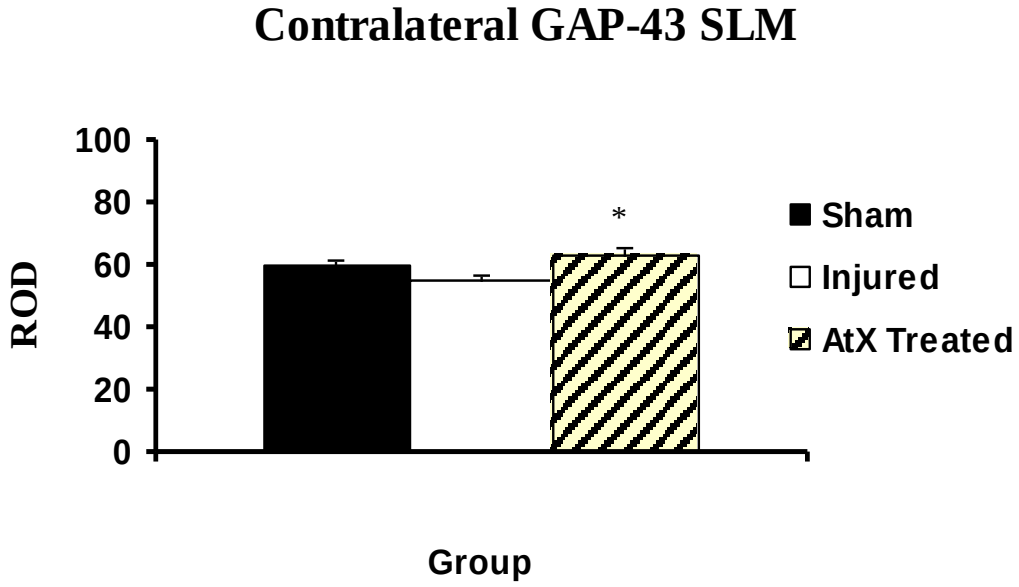


Figure 16 GAP-43 Densitometric analysis in the Contralateral Hippocampus. No significant differences found between the uninjured-sham group and the injured-vehicle treated group in any of the regions analyzed in the CL hippocampus. A. There was a significant increase in GAP-43 IR found between the injured-atomoxetine treated group in the IML of the hippocampus compared to both Sham ($P<.001$) and injured-vehicle treated groups ($P<.001$). B. In the SLM of the hippocampus, GAP-43 IR was increased modestly, yet significantly, in the atomoxetine-treated group over the injury-vehicle treated group ($P=.03$).

A.


Contralateral GAP-43 in IML
100
80
60
40
20
0
ROD
*
Sham
Injured
Atx Treated
Group

B.


Contralateral GAP-43 SLM
100
80
60
40
20
0
ROD
*
Sham
Injured
AtX Treated
Group

CHAPTER 4 GENERAL DISCUSSION

Summary of Results

The studies presented here investigated whether the pharmacological enhancement of catecholamines with atomoxetine treatment following TBI affects cognitive outcome and the potential mechanism for any beneficial effect. Several different behavioral paradigms were used to investigate important questions of treatment dose, therapeutic window, and duration of treatment. In addition, several markers were used to investigate how atomoxetine may affect post-injury plasticity and reorganization. 1.) Low doses of atomoxetine are effective an attenuating cognitive deficits caused by experimental TBI when daily treatment was initiated 24h after injury. 2.) When chronic treatment was initiated at a delayed time point of 10 days, atomoxetine did not provide the same beneficial effects, suggesting atomoxetine's mechanism for enhanced cognition may be related to post-injury mechanisms that are occurring within the first week post-injury. 3.) We further demonstrated that daily atomoxetine treatment during the first seven days post-injury can provide an enhancement of cognitive deficits even after its termination, but was not as effective when a single dose was given, suggesting chronic treatment is necessary for behavioral enhancement. 4.) Our final studies found that daily atomoxetine treatment for 7 days following injury increases GAP-43 protein expression and modestly increases

BDNF protein expression in the hippocampus. The western blot results found atomoxetine treatment can increase ipsilateral GAP-43 expression to sham levels, in comparison to a decrease relative to sham observed in injured-vehicle treated animals. Furthermore, we found a bilateral effect of atomoxetine treatment on BDNF levels in the hippocampus, suggesting atomoxetine may influence plasticity by increasing neurotrophins. Immunohistochemistry analysis found that atomoxetine treatment increased GAP-43 in both the IML of the dentate gyrus and SLM of CA1 in the ipsilateral and contralateral hippocampus. Collectively, these observations support the hypothesis that catecholamines are active participants in the recovery process following injury, and that pharmacological enhancement of these systems may be able to influence the structural and functional reorganization following injury.

Clinical Implications

In terms of post-injury therapeutic treatments, perhaps one of the more important observations from this research is that daily atomoxetine treatment during the early phases of injury enhances cognition. Several experimental studies have found similar results using pharmacological agents that affect the monoamines, including stimulants, antidepressants, dopamine agonists, and MAO-inhibitors (Kline et al., 2000; Kline et al., 2002; Zhu et al., 2000). A percent improvement score was calculated for several treatments to better understand overall effectiveness and allow for a comparison between atomoxetine and previous experimental pharmacological treatments. The score is a ratio of treatment improvement over total deficit (Figure 17, Whiting and Hamm, 2006). Although

this score is an approximation that does not take into account differences in injury models and experimental parameters, it does allow for a basic comparison between the effects of pharmacological treatments on cognitive enhancement after injury. Chronic atomoxetine (1 mg/kg) treatment produced a percent improvement score of 65%, similar to that observed in experimental studies with methylphenidate (53%), bromocriptine (56%), and L-Deprenyl (66%), confirming that catecholaminergic agonism is an effective target for therapeutic treatments following injury.

One of the biggest challenges in TBI research is the translation of experimental research to the clinical setting. Clearly there are differences between humans and animals that may contribute to failed clinical trials; however, there are several factors that can be addressed in experimental studies. Preclinical studies need to address the important issues of treatment dose, timing of treatment initiation and duration. The behavioral studies in this study were especially important because they incorporated a complete dose-response analysis and investigated the therapeutic potential of atomoxetine over a relatively long post-injury time window. In addition, this study addressed the effects of terminating pharmacological treatment before behavioral testing to examine atomoxetine's effects on long-term outcome. The behavioral data revealed that low doses of atomoxetine are effective when initiated early after injury, but not as effective when delayed for several days (10 days). Although few experimental studies have addressed this issue, these results are similar to previous studies with pharmacological agents such as BIBN-99 and MDL 26479 that found enhanced cognitive recovery when initiated at 24 h becomes ineffective when initiated at 10 days post-injury (O'Dell and Hamm, 1995; Pike and Hamm, 1995).

Atomoxetine was also able to enhance cognition after cessation of treatment, suggesting the early phase following injury is the critical time point for effective therapeutic interventions with this drug. The results also suggest daily atomoxetine treatment may have long-term effects on functional outcome if initiated during the time period of post-injury-induced plasticity and continued chronically. Similar results were found using L-Deprenyl following TBI, in which improved cognitive outcome was correlated with changes in dopamine beta-hydroxylase IR and acetylcholinesterase histochemistry in the injured hippocampus (Zhu et al., 2000). The authors of this study concluded that catecholaminergic enhancement facilitated cognitive recovery and was correlated with enhanced synaptic plasticity in the hippocampus. The results of the current studies suggest atomoxetine may have similar effects on post-injury plasticity, although this should be confirmed in other experimental injury models and different laboratories. Taken together, the results provide valuable information that may be used as a basis for clinical trials with atomoxetine.

Atomoxetine has several advantages over some of the current treatments being used following injury in that atomoxetine is a non-stimulant NET inhibitor and therefore has little addictive potential. Further, it is effective with once daily dosing and has been shown to effectively alleviate symptoms of ADHD, as well as co-morbid symptoms of depression and anxiety, which are all common complaints following injury (Pataki et al., 2004; Ripley et al., 2006). There are also studies that suggest atomoxetine may enhance memory in both animals and humans (Foster et al., 2006; Tzavara et al., 2006). Although attention, memory, information processing and emotional disorders all depend on specific

neural networks and specific cellular and molecular events, there is significant evidence that these circuits overlap and are less differentiated. For example, arousal and information processing speed underlie virtually every aspect of cognitive performance. Depression, likewise, is associated with cognitive dysfunction at many levels of analysis. Drugs with potent noradrenergic effects have been suggested to be overall cognitive enhancers due to the effects of NE on arousal and its faciliatory role in LTP. Therefore, atomoxetine may have similar effects in the TBI population.

In summary, atomoxetine is a FDA approved drug with relatively few side effects in the dose range of .3-3 mg/kg. At these doses, the drug did not induce any significant changes in animal body weight or produce observable side effects. Atomoxetine did produce significant improvements in the MWM by i.p. injection, which is an accepted method for delivery in the clinical setting (Magyar et al., 1995). The current studies demonstrated that low doses of atomoxetine improved cognitive performance when initiated early and given daily for 7 days following L-FPI.

Potential Mechanisms for Atomoxetine's Effects

The present studies further examined the possibility that the therapeutic effect of atomoxetine was related to catecholaminergic effects on neural plasticity and reorganization following TBI, similar to that observed by Zhu et al. (2000). Beneficial effects of pharmacological agents on brain plasticity and reorganization have previously been demonstrated in TBI. Drugs that affect the catecholamines, such as amphetamine, methylphenidate, and bromocriptine have all been shown to affect cortical motor plasticity

through mechanisms that likely involve LTP (Schwenkreis et al., 1999). Numerous studies following experimental TBI have also demonstrated that pharmacological enhancement with monoamines leads to improvements in cognitive function that are associated with structural reorganization (for a review, see Whiting and Hamm, 2006; Tenovuo, 2007). Furthermore, perturbations of NE activity play a central role in the development of diaschisis and hinder neural recovery. Although few experimental studies have been done with atomoxetine, several studies have found that drugs that affect extracellular levels of monoamines can affect synaptic plasticity and/or neurogenesis. For example, depression and chronic stress have been associated with impairments of structural plasticity (Fuchs et al., 2004), and there are multiple theories regarding antidepressant treatment effects on cell survival and function and the reversal of stress-induced effects (Duman et al., 2000; Morishita and Aoki, 2002). In addition, the actions of NE and DA alter intracellular signaling pathways and neurotrophic factors, thus changing the electrophysiological and morphological properties of neurons (Stewart and Reid, 2000). NE has been associated with changes in BDNF expression and NGF, which may positively influence the post-injury environment (Fawcett et al., 1998; Ivy et al., 2003). NE may also effect cell proliferation and/or differentiation of new neurons or non-neuronal cells (Kulkarni et al., 2002). The ability of NE to influence such processes is in line with the latter's documented role in neuroplasticity as expressed in the modulation and induction of LTP and in memory formation. Finally, the current study is important in that it confirms previous work that suggested pharmacological enhancement of GAP-43 during periods of

post-injury plasticity is correlated with improved behavioral (cognitive and motor) outcome (Ramic et al., 2006; Chytrova et al., 2007; Marklund et al., 2007).

The exact cellular mechanisms underlying atomoxetine-induced cognitive improvements and changes in GAP-43 and BDNF expression after TBI remain unclear. One mechanism by which atomoxetine resulted in the observed effects likely involves the role of NE is regulating neuronal activity. Increased NE facilitates positive signaling and trophic influences through the recruitment of adrenergic receptors, activation of ion channels and the second and third messenger cascades downstream of these receptors (e.g., cAMP, protein kinase A, CREB). The effects observed in these experiments following atomoxetine treatment may be a direct effect of NE agonism at the synapse. Experimental TBI has been associated with depressed levels of cAMP and PKA for several days following injury (Atkins et al., 2007). NE and DA activate G proteins that in turn alter membrane excitability and act through second messengers within the neurons. Protein kinases are important second messengers that phosphorylate proteins and alter the sensitivity of receptors, the responsiveness of ion channels and the regulation of gene expression. These cascades drive the release of other neurotransmitters and upregulate endogenous neurotrophin systems. For example, the basolateral cholinergic system receives noradrenergic innervation, and drugs that increase norepinephrine, including atomoxetine, also increase the release of acetylcholine, which is affected following TBI (Dalley et al., 2004; Tzavara et al., 2005). In light of the role of acetylcholine in memory functions, any drug that increases levels of acetylcholine would in theory enhance cognition. Indeed, CDP-choline significantly increases extracellular levels of

acetylcholine, and significantly improves performance in the MWM following experimental TBI (Dixon et al., 1997). NE can also regulate both glutamate and GABA transmission and, therefore, balance excitatory/inhibitory homeostasis in specific circuits and contribute to the tuning of memory processes (Tan et al., 2006; Zsiros et al., 2008). NE, acting through beta-adrenergic receptors, affects both PKA and cAMP. PKA and cAMP signaling have been shown previously to regulate the capacity of dorsal root ganglion and retinal ganglion cell neurons to grow axons in both permissive and inhibitory cultures (Cai et al., 2001; Shearer et al., 2003). Furthermore, PKA inhibitors decreased the ability for growth cone formation following *in vitro* axotomy, and pharmacological treatments that increase cAMP and PKA promote growth cone regeneration in adult retinal ganglion cell axons in culture (Chierzi et al., 2005). NE application to cultured hippocampal cells has been shown to increase GAP-43, as well as growth promoting genes, suggesting there may be a similar effect *in vivo,* following an increase in extracellular NE. Dopamine has similar effects on cAMP via activation of D1 like receptors. Administration of D1/D5 receptor agonists increases cAMP levels and activates PKA (Bach, 1999). PKA activation has been linked with memory functions that depend on the hippocampus, and has been shown to reverse long-term memory deficits (Kandel, 2001). However, other studies have found that long-term elevation of intracellular cAMP levels in rat primary cortical cultures results in a persistent downregulation of GAP-43 (Krueger and Narin, 2007). Therefore, it appears that increasing cAMP and PKA may lead to different effects depending on the circuits and brain regions they are located.

NE may also modulate BDNF expression in both neurons and astrocytes (Juric et al., 2006). BDNF is thought to mediate activity-dependent synaptic plasticity in the mature nervous system, and increases in neural activity lead to increases in BDNF (Poo, 2001; Lessmann et al., 2003). A positive cooperativity between increased extracellular levels of NE and enhanced BDNF may interact to influence plasticity and regeneration in the injured brain. BDNF shows a high-affinity for tropomyosin-related kinase (TrK) B. Several studies have implicated BDNF-TrKB signaling in gene regulatory events that contribute to neuronal survival, synaptic plasticity and learning and memory (Cui, 2006). I*n vitro* studies have found BDNF application to cultured hippocampal neurons elicits a rapid potentiation of excitatory synaptic transmission and facilitates LTP (Lessmann et al., 1994; Figurov et al., 1996). These rapid changes can lead to structural changes, such as axonal branching and dendritic growth after exposure to BDNF for longer periods of time (Cohen-Cory et al., 1995; McAllister and Katz, 1999). In addition, several *in vitro* studies have demonstrated that neurons primed with BDNF become resistant to the inhibitory actions of MAG (Myelin Associated Glycoproteins), and thus elevate the capacity of the injured brain to overcome the limitations in regeneration (Cai et al., 1999; Gao et al., 2003; Spencer and Filbin, 2004). NE receptors are also located on glial cells, and these receptors may indirectly modify the extracellular milieu by increasing neurotrophins (Aoki et al., 1987; Tsacopoulos et al., 1996).

BDNF has previously been associated with postinjury recovery periods, and changes in GAP-43 have been correlated to changes in BDNF (Chytrova et al., 2008). Therefore, the small, yet significant, increases in BDNF observed following atomoxetine

treatment may contribute to the changes in GAP-43 that are observed, although this theory requires further studies. The changes in hippocampal GAP-43 protein expression in the current studies were observed in areas of the hippocampus that contain dense NE projections, suggesting local increases in extracellular NE may influence regeneration, possibly through a mechanism involving BDNF. BDNF may positively influence the cellular environment and thus affect synaptic reorganization following injury. Although we did not observe any changes in BDNF in our injured-vehicle treated animals in this experiment, recovery following TBI may require increased BDNF for restoration of function. In addition, it can not be ruled out that atomoxetine's effects on cognition were not related to changes in brain regions other than the hippocampus. Therefore, future studies will investigate changes in these markers in other brain regions.

Alternatively, atomoxetine may act through other mechanisms that are involved in TBI pathology. NE is released at the synapse as well as extrasynaptically. Extrasynaptic NE has been hypothesized to play a role apart from its role as a neurotransmitter. Immune cells express various neurotransmitter receptors that are sensitive to monoamines, and the production of cytokines and other immune/inflammatory mediators (such as free radicals) are modulated by activation of these receptors (Elenkov et al., 2000). The interactions of the catecholamines and the immune system, including cytokines may be another mechanism by which atomoxetine acts following injury. Although not addressed in this dissertation, neurotransmitter receptors found on immune cells are sensitive to monoamines, and the production of cytokines (and other immune/inflammatory mediators

such as chemokines and free radicals) is modulated by activation of these receptors (Elenkov et al., 2000).

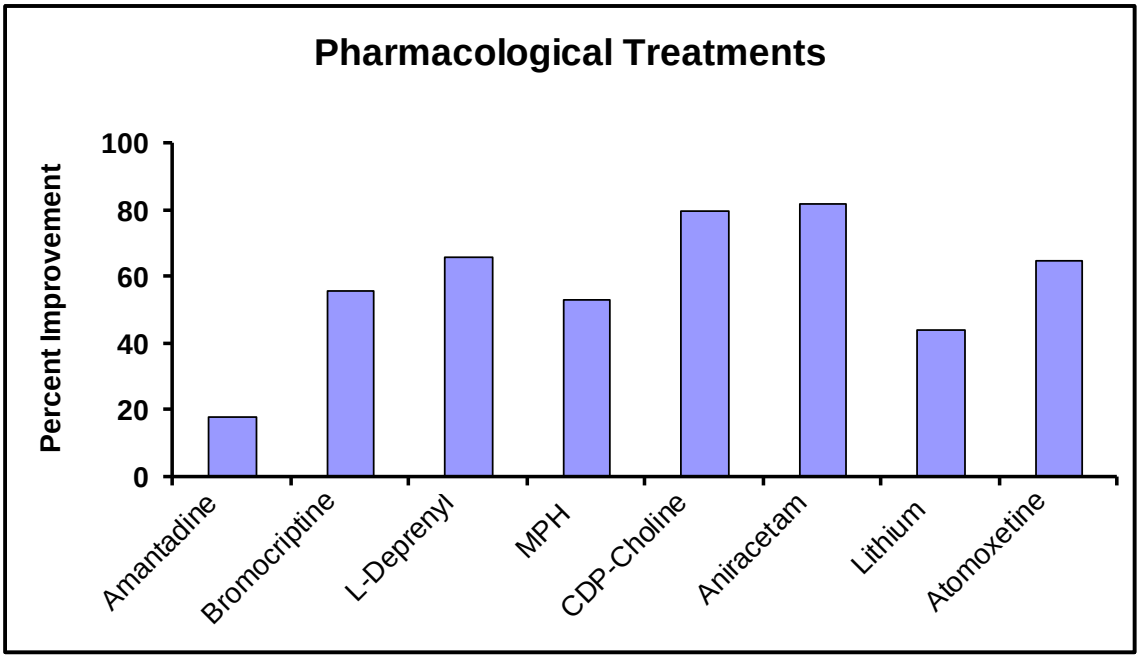
Neurotransmitters can initiate signal transduction pathways that modulate the cytokine production of the cell. NE alters functions such as cytokine production and cellular responses to cytokines, and inflammatory gene expression (Hu et al., 1991). For example, NE blocks the expression of inflammation-induced proteins, including major histocompatibility complex class II (Frohman et al., 1988) tumor necrosis factor-α (Hu et al., 1991), interleukin-1B (IL-1B) (Willis and Nisen, 1995), and inducible nitric oxide synthase (iNOS) in astrocytes and microglia (Feinstein et al., 2002). In contrast, NE depletion reinforces the increase of iNOS, IL-1B, and cyclooxygenase-2 (Heneka et al., 2002). The process of neurodegeneration has been closely linked with a shift to the production of pro-inflammatory cytokines (Feuerstein et al., 1998), and the modulation of cytokine balance is governed by the amount of catecholamines in the extracellular space (Selmeczy et al., 2003). It appears that NTs that activate cAMP and adenylate cyclase (such as NE) also stimulate anti- inflammatory cytokines (Szelenyi and Vizi, 2006). For these reasons, NE has been suggested to act as an endogenous anti-inflammatory agent-reducing pro-inflammatory cytokines and encouraging anti-inflammatory properties (reviewd by Feinstein et al., 2002). Therefore, drugs that affect the NE system may influence the immune response directly through adrenergic receptors expressed on macrophages and other immunological cells. It has been suggested that impairments to the LC-NE system, because of its immunomodulatory role, may enhance inflammatory responses to beta-amyloid and contribute to the pathogenesis of Alzheimer's. Drugs that

increase NE availability may be able to prevent these inflammatory processes (Heneka et al., 2002, 2003). In conclusion, there are a number of burgeoning theories supported by the literature for NE's role in various processes; however, significant research in these fields is required to fully determine the specific mechanism for the results observed. It is possible that atomoxetine may affect functional outcome through multiple mechanisms, which will require further research in the context of TBI.

Conclusions

Atomoxetine offers potential for a therapeutic treatment to enhance cognition following TBI. These studies suggest low doses of daily atomoxetine initiated 24h after injury may also be able to influence post-injury structural and functional outcome. To date, there have been no clinical trials investigating the potential of this drug. Although the results presented in this study are convincing, several questions surrounding atomoxetine's mechanism remain to be answered. Before clinical trials begin, details about atomoxetine's mechanism of action should be fully investigated and the results confirmed in other experimental models. These studies are the first to show that atomoxetine improves cognition that is correlated with structural hippocampal changes. Changes in GAP-43, BDNF, synaptophysin, and other markers of synaptic plasticity should be investigated at several time points and in additional brain regions. Atomoxetine is a well tolerated drug with few side effects and low addictive potential. Therefore, atomoxetine may be effective alone or in combination with other pharmaceutical and rehabilitative therapies, which may provide more successful functional outcomes for brain injured patients.

Figure 17. Pharmacological Treatments Following TBI. The percent improvement was calculated as a ratio of treatment improvement over total deficit. Treatment improvement was calculated as the difference in water maze performance between injured-vehicle and injured- Treated groups. The total deficit was calculated as the difference in water-maze performance between sham and injured-vehicle treated groups. The percent improvement was based on estimates from published graphs. Higher percentage scores indicate that the treatment was more effective at eliminating injury-induced deficits.


Pharmacological Treatments
Percent Improvement
100
80
60
40
20
0
Amantadine
Bromocriptine
L-Deprenyl
MPH
CDP-Choline
Aniracetam
Lithium
Atomoxetine

Literature Cited

Literature Cited

Aigner, L., Arber, S., Kapfhammer, J. P., Laux, T., Schneider, C., Botteri, F., Brenner, H. R. & Caroni, P. (1995) Overexpression of the neural growth-associated protein GAP-43 induces nerve sprouting in the adult nervous system of transgenic mice. *Cell*, **83,** 269-278.

Alessandri, B., Rice, A. C., Levasseur, J., DeFord, M., Hamm, R. J. & Bullock, M. R. (2002) Cyclosporin A improves brain tissue oxygen consumption and learning/memory performance after lateral fluid percussion injury in rats. *J.Neurotrauma*, **19,** 829-841.

Anagnostaras, S. G., Gale, G. D. & Fanselow, M. S. (2001) Hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus*, **11,** 8-17.

Aoki, C., Venkatesan, C., Go, C. G., Forman, R. & Kurose, H. (1998) Cellular and subcellular sites for noradrenergic action in the monkey dorsolateral prefrontal cortex as revealed by the immunocytochemical localization of noradrenergic receptors and axons. *Cereb.Cortex*, **8,** 269-277.

Aquilani, R., Iadarola, P., Boschi, F., Pistarini, C., Arcidiaco, P. & Contardi, A. (2003) Reduced plasma levels of tyrosine, precursor of brain catecholamines, and of essential amino acids in patients with severe traumatic brain injury after rehabilitation. *Arch.Phys.Med.Rehabil.*, **84,** 1258-1265.

Arciniegas, D. B. (2003) The cholinergic hypothesis of cognitive impairment caused by traumatic brain injury. *Curr.Psychiatry Rep.*, **5,** 391-399.

Arnsten, A. F. & Dudley, A. G. (2005) Methylphenidate improves prefrontal cortical cognitive function through alpha2 adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in Attention Deficit Hyperactivity Disorder. *Behav.Brain Funct.*, **1,** 2.

Arnsten, A. F., Ramos, B. P., Birnbaum, S. G. & Taylor, J. R. (2005) Protein kinase A as a therapeutic target for memory disorders: rationale and challenges. *Trends Mol.Med.*, **11,** 121-128.

Aston-Jones, G., Chiang, C. & Alexinsky, T. (1991) Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. *Prog.Brain Res.*, **88,** 501-520.

Atkins, C. M., Oliva, A. A., Jr., Alonso, O. F., Pearse, D. D., Bramlett, H. M. & Dietrich, W. D. (2007) Modulation of the cAMP signaling pathway after traumatic brain injury. *Exp.Neurol.*, **208,** 145-158.

Axelrod, J. (1971) Brain monoamines. Biosynthesis and fate. *Neurosci.Res.Program.Bull.*, **9,** 188-196.

Bach, M. E., Barad, M., Son, H., Zhuo, M., Lu, Y. F., Shih, R., Mansuy, I., Hawkins, R. D. & Kandel, E. R. (1999) Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc.Natl.Acad.Sci.U.S.A*, **96,** 5280-5285.

Baker, A. J., Moulton, R. J., MacMillan, V. H. & Shedden, P. M. (1993) Excitatory amino acids in cerebrospinal fluid following traumatic brain injury in humans. *J.Neurosurg.*, **79,** 369-372.

Baldwin, S. A., Gibson, T., Callihan, C. T., Sullivan, P. G., Palmer, E. & Scheff, S. W. (1997) Neuronal cell loss in the CA3 subfield of the hippocampus following cortical contusion utilizing the optical disector method for cell counting. *J.Neurotrauma*, **14,** 385-398.

Baranova, A. I., Whiting, M. D. & Hamm, R. J. (2006) Delayed, post-injury treatment with aniracetam improves cognitive performance after traumatic brain injury in rats. *J.Neurotrauma*, **23,** 1233-1240.

Bareyre, F. M., Kerschensteiner, M., Raineteau, O., Mettenleiter, T. C., Weinmann, O. & Schwab, M. E. (2004) The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. *Nat.Neurosci.*, **7,** 269-277.

Becker, S. (2005) A computational principle for hippocampal learning and neurogenesis. *Hippocampus*, **15,** 722-738.

Benes, F. M. & Berretta, S. (2001) GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology*, **25,** 1-27.

Benowitz, L. I., Rodriguez, W. R. & Neve, R. L. (1990) The pattern of GAP-43 immunostaining changes in the rat hippocampal formation during reactive synaptogenesis. *Brain Res.Mol.Brain Res.*, **8,** 17-23.

Benowitz, L. I. & Routtenberg, A. (1997) GAP-43: an intrinsic determinant of neuronal development and plasticity. *Trends Neurosci.*, **20,** 84-91.

Bergsneider, M., Hovda, D. A., McArthur, D. L., Etchepare, M., Huang, S. C., Sehati, N., Satz, P., Phelps, M. E. & Becker, D. P. (2001) Metabolic recovery following human

traumatic brain injury based on FDG-PET: time course and relationship to neurological disability. *J.Head Trauma Rehabil.*, **16,** 135-148.

Bergson, C., Mrzljak, L., Smiley, J. F., Pappy, M., Levenson, R. & Goldman-Rakic, P. S. (1995) Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *J.Neurosci.*, **15,** 7821-7836.

Bernabeu, R., Cammarota, M., Izquierdo, I. & Medina, J. H. (1997) Involvement of hippocampal AMPA glutamate receptor changes and the cAMP/protein kinase A/CREB-P signalling pathway in memory consolidation of an avoidance task in rats. *Braz.J.Med.Biol.Res.*, **30,** 961-965.

Berridge, C. W., Arnsten, A. F. & Foote, S. L. (1993) Noradrenergic modulation of cognitive function: clinical implications of anatomical, electrophysiological and behavioural studies in animal models. *Psychol.Med.*, **23,** 557-564.

Berridge, C. W., Bolen, S. J., Manley, M. S. & Foote, S. L. (1996) Modulation of forebrain electroencephalographic activity in halothane-anesthetized rat via actions of noradrenergic beta-receptors within the medial septal region. *J.Neurosci.*, **16,** 7010-7020.

Biagioni, S., Tata, A. M., De Jaco, A. & Augusti-Tocco, G. (2000) Acetylcholine synthesis and neuron differentiation. *Int.J.Dev.Biol.*, **44,** 689-697.

Biederman, J. & Spencer, T. J. (2000) Genetics of childhood disorders: XIX. ADHD, Part 3: Is ADHD a noradrenergic disorder? *J.Am.Acad.Child Adolesc.Psychiatry*, **39,** 1330-1333.

Binder S, Corrigan JD, Langlois JA. (2005) The public health approach to traumatic brain injury: an overview of CDC's research and programs. *J Head Trauma Rehabil*. 20(3):189-95.

Birthelmer, A., Lazaris, A., Schweizer, T., Jackisch, R. & Cassel, J. C. (2003) Presynaptic regulation of neurotransmitter release in the cortex of aged rats with differential memory impairments. *Pharmacol.Biochem.Behav.*, **75,** 147-162.

Blitz, D. M., Foster, K. A. & Regehr, W. G. (2004) Short-term synaptic plasticity: a comparison of two synapses. *Nat.Rev.Neurosci.*, **5,** 630-640.

Bomze, H. M., Bulsara, K. R., Iskandar, B. J., Caroni, P. & Skene, J. H. (2001) Spinal axon regeneration evoked by replacing two growth cone proteins in adult neurons. *Nat.Neurosci.*, **4,** 38-43.

Boyeson, M. G. & Feeney, D. M. (1990) Intraventricular norepinephrine facilitates motor recovery following sensorimotor cortex injury. *Pharmacol.Biochem.Behav.*, **35,** 497-501.

Boyeson, M. G. & Harmon, R. L. (1993) Effects of trazodone and desipramine on motor recovery in brain-injured rats. *Am.J.Phys.Med.Rehabil.*, **72,** 286-293.

Boyeson, M. G., Harmon, R. L. & Jones, J. L. (1994) Comparative effects of fluoxetine, amitriptyline and serotonin on functional motor recovery after sensorimotor cortex injury. *Am.J.Phys.Med.Rehabil.*, **73,** 76-83.

Brandeis, R., Brandys, Y. & Yehuda, S. (1989) The use of the Morris Water Maze in the study of memory and learning. *Int.J.Neurosci.*, **48,** 29-69.

Brouwer, W., Verzendaal, M., van der, N. J., Smit, J. & van Zomeren, E. (2001) Divided attention years after severe closed head injury: the effect of dependencies between the subtasks. *Brain Cogn*, **46,** 54-56.

Brozoski, T. J., Brown, R. M., Rosvold, H. E. & Goldman, P. S. (1979) Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science*, **205,** 929-932.

Bruns, Jr., J., & Hauser, W.A. (2003). The epidemiology of traumatic brain injury: A review. *Epilepsia, 44*, 2-10.

Buchli, A. D. & Schwab, M. E. (2005) Inhibition of Nogo: a key strategy to increase regeneration, plasticity and functional recovery of the lesioned central nervous system. *Ann.Med.*, **37,** 556-567.

Buffo, A., Holtmaat, A. J., Savio, T., Verbeek, J. S., Oberdick, J., Oestreicher, A. B., Gispen, W. H., Verhaagen, J., Rossi, F. & Strata, P. (1997) Targeted overexpression of the neurite growth-associated protein B-50/GAP-43 in cerebellar Purkinje cells induces sprouting after axotomy but not axon regeneration into growth-permissive transplants. *J.Neurosci.*, **17,** 8778-8791.

Buki, A., Siman, R., Trojanowski, J. Q. & Povlishock, J. T. (1999) The role of calpain-mediated spectrin proteolysis in traumatically induced axonal injury. *J.Neuropathol.Exp.Neurol.*, **58,** 365-375.

Buki, A., Okonkwo, D. O., Wang, K. K. & Povlishock, J. T. (2000) Cytochrome c release and caspase activation in traumatic axonal injury. *J.Neurosci.*, **20,** 2825-2834.

Bunsey, M. D. & Strupp, B. J. (1995) Specific effects of idazoxan in a distraction task: evidence that endogenous norepinephrine plays a role in selective attention in rats. *Behav.Neurosci.*, **109,** 903-911.

Bushnik T, Hanks RA, Kreutzer J, Rosenthal M. (2003) Etiology of traumatic brain injury: characterization of differential outcomes up to 1 year postinjury. *Arch Phys Med Rehabil*. 84(2):255-62.

Butefisch, C. M., Davis, B. C., Sawaki, L., Waldvogel, D., Classen, J., Kopylev, L. & Cohen, L. G. (2002) Modulation of use-dependent plasticity by d-amphetamine. *Ann.Neurol.*, **51,** 59-68.

Bymaster, F. P., Katner, J. S., Nelson, D. L., Hemrick-Luecke, S. K., Threlkeld, P. G., Heiligenstein, J. H., Morin, S. M., Gehlert, D. R. & Perry, K. W. (2002) Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology*, **27,** 699-711.

Cai, D., Shen, Y., De Bellard, M., Tang, S. & Filbin, M. T. (1999) Prior exposure to neurotrophins blocks inhibition of axonal regeneration by MAG and myelin via a cAMP-dependent mechanism. *Neuron*, **22,** 89-101.

Cai, D., Qiu, J., Cao, Z., McAtee, M., Bregman, B. S. & Filbin, M. T. (2001) Neuronal cyclic AMP controls the developmental loss in ability of axons to regenerate. *J.Neurosci.*, **21,** 4731-4739.

Calabrese, F., Molteni, R., Maj, P. F., Cattaneo, A., Gennarelli, M., Racagni, G. & Riva, M. A. (2007) Chronic duloxetine treatment induces specific changes in the expression of BDNF transcripts and in the subcellular localization of the neurotrophin protein. *Neuropsychopharmacology*, **32,** 2351-2359.

Cantallops, I. & Routtenberg, A. (1999) Activity-dependent regulation of axonal growth: posttranscriptional control of the GAP-43 gene by the NMDA receptor in developing hippocampus. *J.Neurobiol.*, **41,** 208-220.

Carli, M., Robbins, T. W., Evenden, J. L. & Everitt, B. J. (1983) Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. *Behav.Brain Res.*, **9,** 361-380.

Cernak, I. (2005) Animal models of head trauma. *NeuroRx.*, **2,** 410-422.

Chapman, E. R., Au, D., Alexander, K. A., Nicolson, T. A. & Storm, D. R. (1991) Characterization of the calmodulin binding domain of neuromodulin. Functional significance of serine 41 and phenylalanine 42. *J.Biol.Chem.*, **266,** 207-213.

Chen, B., Wang, J. F., Sun, X. & Young, L. T. (2003) Regulation of GAP-43 expression by chronic desipramine treatment in rat cultured hippocampal cells. *Biol.Psychiatry*, **53,** 530-537.

Christman, A. K., Fermo, J. D. & Markowitz, J. S. (2004) Atomoxetine, a novel treatment for attention-deficit-hyperactivity disorder. *Pharmacotherapy*, **24,** 1020-1036.

Christman, C. W., Salvant, J. B., Jr., Walker, S. A. & Povlishock, J. T. (1997) Characterization of a prolonged regenerative attempt by diffusely injured axons following traumatic brain injury in adult cat: a light and electron microscopic immunocytochemical study. *Acta Neuropathol.*, **94,** 329-337.

Chytrova, G., Ying, Z. & Gomez-Pinilla, F. (2008) Exercise normalizes levels of MAG and Nogo-A growth inhibitors after brain trauma. *Eur.J.Neurosci.*, **27,** 1-11.

Cicerone KD, Azulay J. (2007). Perceived self-efficacy and life satisfaction after traumatic brain injury. *J Head Trauma Rehabil*. ;22(5):257-66.

Cicerone KD, Mott T, Azulay J, Friel JC. (2004). Community integration and satisfaction with functioning after intensive cognitive rehabilitation for traumatic brain injury. *Arch Phys Med Rehabil*. 85(6):943-50.

Ciliax, B. J., Nash, N., Heilman, C., Sunahara, R., Hartney, A., Tiberi, M., Rye, D. B., Caron, M. G., Niznik, H. B. & Levey, A. I. (2000) Dopamine D(5) receptor immunolocalization in rat and monkey brain. *Synapse*, **37,** 125-145.

Cohen-Cory, S. & Fraser, S. E. (1995) Effects of brain-derived neurotrophic factor on optic axon branching and remodelling in vivo. *Nature*, **378,** 192-196.

Clark R.S, Kochanek P.M. and Watkins SC. *et al.*, (2000). Caspase-3 mediated neuronal death after traumatic brain injury in rats, *J Neurochem* **74** (2), pp. 740–753.

Clifton, G. L., Miller, E. R., Choi, S. C., Levin, H. S., McCauley, S., Smith, K. R., Jr., Muizelaar, J. P., Wagner, F. C., Jr., Marion, D. W., Luerssen, T. G., Chesnut, R. M. & Schwartz, M. (2001) Lack of effect of induction of hypothermia after acute brain injury. *N.Engl.J.Med.*, **344,** 556-563.

Colantonio A, Stamenova V, Abramowitz C, Clarke D, Christensen B. (2007). Brain injury in a forensic psychiatry population. *Brain Injury*. (13-14):1353-60.

Cole, B. J. & Robbins, T. W. (1992) Forebrain norepinephrine: role in controlled information processing in the rat. *Neuropsychopharmacology*, **7,** 129-142.

Collier, T. J., Greene, J. G., Felten, D. L., Stevens, S. Y. & Collier, K. S. (2004) Reduced cortical noradrenergic neurotransmission is associated with increased neophobia and impaired spatial memory in aged rats. *Neurobiol.Aging*, **25,** 209-221.

Connor, B., Young, D., Yan, Q., Faull, R. L., Synek, B. & Dragunow, M. (1997) Brain-derived neurotrophic factor is reduced in Alzheimer's disease. *Brain Res.Mol.Brain Res.*, **49,** 71-81.

Consogno, E., Racagni, G. & Popoli, M. (2001) Modifications in brain CaM kinase II after long-term treatment with desmethylimipramine. *Neuropsychopharmacology*, **24,** 21-30.

Conti, A. C., Cryan, J. F., Dalvi, A., Lucki, I. & Blendy, J. A. (2002) cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. *J.Neurosci.*, **22,** 3262-3268.

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

Costello, B., Meymandi, A. & Freeman, J. A. (1990) Factors influencing GAP-43 gene expression in PC12 pheochromocytoma cells. *J.Neurosci.*, **10,** 1398-1406.

Cui, Q. (2006) Actions of neurotrophic factors and their signaling pathways in neuronal survival and axonal regeneration. *Mol.Neurobiol.*, **33,** 155-179.

Curtiss, G., Vanderploeg, R. D., Spencer, J. & Salazar, A. M. (2001) Patterns of verbal learning and memory in traumatic brain injury. *J.Int.Neuropsychol.Soc.*, **7,** 574-585.

Crepeau, F., Scherzer P. (1993). Predictors and Indicators of work status after traumatic brain injury: a meta-analysis. *Neuropsychological Rehabilitation* 3:5-35.

Dahl, D. & Sarvey, J. M. (1989) Norepinephrine induces pathway-specific long-lasting potentiation and depression in the hippocampal dentate gyrus. *Proc.Natl.Acad.Sci.U.S.A*, **86,** 4776-4780.

Dalby, N. O., Rondouin, G. & Lerner-Natoli, M. (1995) Increase in GAP-43 and GFAP immunoreactivity in the rat hippocampus subsequent to perforant path kindling. *J.Neurosci.Res.*, **41,** 613-619.

Dalley, J. W., Cardinal, R. N. & Robbins, T. W. (2004) Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci.Biobehav.Rev.*, **28,** 771-784.

Dancause, N., Barbay, S., Frost, S. B., Plautz, E. J., Chen, D., Zoubina, E. V., Stowe, A. M. & Nudo, R. J. (2005) Extensive cortical rewiring after brain injury. *J.Neurosci.*, **25,** 10167-10179.

Davis, J. A. & Gould, T. J. (2007) Atomoxetine reverses nicotine withdrawal-associated deficits in contextual fear conditioning. *Neuropsychopharmacology*, **32,** 2011-2019.

Davis, K.L., Joshi, A.V., Tortella, B.J., & Candrilli, S.D. (2007). The direct economic burden of blunt and penetrating trauma in a managed care population. *The Journal of Trauma, 62,* 622-629.

DeKosky, S. T., Goss, J. R., Miller, P. D., Styren, S. D., Kochanek, P. M. & Marion, D. (1994) Upregulation of nerve growth factor following cortical trauma. *Exp.Neurol.*, **130,** 173-177.

Dhillon, H. S., Dose, J. M. & Prasad, R. M. (1998) Amphetamine administration improves neurochemical outcome of lateral fluid percussion brain injury in the rat. *Brain Res.*, **804,** 231-237.

Dinocourt, C., Gallagher, S. E. & Thompson, S. M. (2006) Injury-induced axonal sprouting in the hippocampus is initiated by activation of trkB receptors. *Eur.J.Neurosci.*, **24,** 1857-1866.

Dityatev, A. & Schachner, M. (2003) Extracellular matrix molecules and synaptic plasticity. *Nat.Rev.Neurosci.*, **4,** 456-468.

Dixon, C. E., Lyeth, B. G., Povlishock, J. T., Findling, R. L., Hamm, R. J., Marmarou, A., Young, H. F. & Hayes, R. L. (1987) A fluid percussion model of experimental brain injury in the rat. *J.Neurosurg.* , **67,** 110-119.

Dixon, C. E., Bao, J., Bergmann, J. S. & Johnson, K. M. (1994) Traumatic brain injury reduces hippocampal high-affinity [3H]choline uptake but not extracellular choline levels in rats. *Neurosci.Lett.*, **180,** 127-130.

Dixon, C. E., Flinn, P., Bao, J., Venya, R. & Hayes, R. L. (1997) Nerve growth factor attenuates cholinergic deficits following traumatic brain injury in rats. *Exp.Neurol.*, **146,** 479-490.

Dixon, C. E., Ma, X. & Marion, D. W. (1997) Effects of CDP-choline treatment on neurobehavioral deficits after TBI and on hippocampal and neocortical acetylcholine release. *J.Neurotrauma*, **14,** 161-169

Dixon, C. E., Kraus, M. F., Kline, A. E., Ma, X., Yan, H. Q., Griffith, R. G., Wolfson, B. M. & Marion, D. W. (1999) Amantadine improves water maze performance without affecting motor behavior following traumatic brain injury in rats. *Restor.Neurol.Neurosci.*, **14,** 285-294.

Donnemiller, E., Brenneis, C., Wissel, J., Scherfler, C., Poewe, W., Riccabona, G. & Wenning, G. K. (2000) Impaired dopaminergic neurotransmission in patients with traumatic brain injury: a SPECT study using 123I-beta-CIT and 123I-IBZM. *Eur.J.Nucl.Med.*, **27,** 1410-1414.

Dose, J. M., Dhillon, H. S., Maki, A., Kraemer, P. J. & Prasad, R. M. (1997) Lack of delayed effects of amphetamine, methoxamine, and prazosin (adrenergic drugs) on behavioral outcome after lateral fluid percussion brain injury in the rat. *J.Neurotrauma*, **14,** 327-337.

Duman, R. S., Malberg, J., Nakagawa, S. & D'Sa, C. (2000) Neuronal plasticity and survival in mood disorders. *Biol.Psychiatry*, **48,** 732-739.

Dunn-Meynell, A., Pan, S. & Levin, B. E. (1994) Focal traumatic brain injury causes widespread reductions in rat brain norepinephrine turnover from 6 to 24 h. *Brain Res.*, **660,** 88-95.

Dunn-Meynell, A. A., Yarlagadda, Y. & Levin, B. E. (1997) Alpha 1-adrenoceptor blockade increases behavioral deficits in traumatic brain injury. *J.Neurotrauma*, **14,** 43-52.

Dunn-Meynell, A. A., Hassanain, M. & Levin, B. E. (1998) Norepinephrine and traumatic brain injury: a possible role in post-traumatic edema. *Brain Res.*, **800,** 245-252.

Easton, N., Steward, C., Marshall, F., Fone, K. & Marsden, C. (2007) Effects of amphetamine isomers, methylphenidate and atomoxetine on synaptosomal and synaptic vesicle accumulation and release of dopamine and noradrenaline in vitro in the rat brain. *Neuropharmacology*, **52,** 405-414.

Eichenbaum, H., Stewart, C. & Morris, R. G. (1990) Hippocampal representation in place learning. *J.Neurosci.*, **10,** 3531-3542.

Elenkov, I.J., R.L. Wilder, G.P. Chrousos & E S. Vizi. (2000). The sympathetic nerve–an integrative interface between two supersystems: the brain and the immune system. Pharmacol. Rev. **52**: 595.

Emery, D. L., Raghupathi, R., Saatman, K. E., Fischer, I., Grady, M. S. & McIntosh, T. K. (2000) Bilateral growth-related protein expression suggests a transient increase in regenerative potential following brain trauma. *J.Comp Neurol.*, **424,** 521-531.

Ercan, M., Inci, S., Kilinc, K., Palaoglu, S. & Aypar, U. (2001) Nimodipine attenuates lipid peroxidation during the acute phase of head trauma in rats. *Neurosurg.Rev.*, **24,** 127-130.

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A. & Gage, F. H. (1998) Neurogenesis in the adult human hippocampus. *Nat.Med.*, **4,** 1313-1317.

Eslinger PJ, Grattan LM, Geder L, (1996). Neurologic and neuropsychologic aspects of frontal lobe impairments in postconcussive syndrome, in Head Injury and Postconcussive

Syndrome. Edited by Rizzo M, Traniel D. New York, Churchhill Livingstone, 1996, pp 415-440.

Faden AI, Demediuk P, Panter SS, Vink R. (1989) The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science* 244:798–800.

Falo, M. C., Fillmore, H. L., Reeves, T. M. & Phillips, L. L. (2006) Matrix metalloproteinase-3 expression profile differentiates adaptive and maladaptive synaptic plasticity induced by traumatic brain injury. *J.Neurosci.Res.*, **84,** 768-781.

Fan, L., Young, P. R., Barone, F. C., Feuerstein, G. Z., Smith, D. H. & McIntosh, T. K. (1995) Experimental brain injury induces expression of interleukin-1 beta mRNA in the rat brain. *Brain Res.Mol.Brain Res.*, **30,** 125-130.

Farkas, O. & Povlishock, J. T. (2007) Cellular and subcellular change evoked by diffuse traumatic brain injury: a complex web of change extending far beyond focal damage. *Prog.Brain Res.*, **161,** 43-59.

Fawcett, J. P., Bamji, S. X., Causing, C. G., Aloyz, R., Ase, A. R., Reader, T. A., McLean, J. H. & Miller, F. D. (1998) Functional evidence that BDNF is an anterograde neuronal trophic factor in the CNS. *J.Neurosci.*, **18,** 2808-2821.

Feeney, D.M., Sutton R.L. (1988) Catecholamine and recovery of function after brain damage. In: Stein DG, Sabel BA, eds. Pharmacological approaches to the treatment of brain and spinal cord injuries. New York, Plenum, pp121-142.

Feeney, D.M. (1991). Pharmacologic modulation of recovery after brain injury: reconsideration of diachisis. *J Neurol Rehabil, 5*, 113-28.

Feeney, D. M., Weisend, M. P. & Kline, A. E. (1993) Noradrenergic pharmacotherapy, intracerebral infusion and adrenal transplantation promote functional recovery after cortical damage. *J.Neural Transplant.Plast.*, **4,** 199-213.

Feeney, D. M. (1997) From laboratory to clinic: noradrenergic enhancement of physical therapy for stroke or trauma patients. *Adv.Neurol.*, **73,** 383-394.

Ferrer, I., Marin, C., Rey, M. J., Ribalta, T., Goutan, E., Blanco, R., Tolosa, E. & Marti, E. (1999) BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. *J.Neuropathol.Exp.Neurol.*, **58,** 729-739.

Ferry, B. & McGaugh, J. L. (2000) Role of amygdala norepinephrine in mediating stress hormone regulation of memory storage. *Acta Pharmacol.Sin.*, **21,** 481-493.

Feuerstein, G. Z., Wang, X. & Barone, F. C. (1998) The role of cytokines in the neuropathology of stroke and neurotrauma. *Neuroimmunomodulation.*, **5,** 143-159.

Figurov, A., Pozzo-Miller, L. D., Olafsson, P., Wang, T. & Lu, B. (1996) Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature*, **381,** 706-709.

Finger, S., Koehler, P. J. & Jagella, C. (2004) The Monakow concept of diaschisis: origins and perspectives. *Arch.Neurol.*, **61,** 283-288.

Finkelstein, E., Corso, P., & Miller, T. (2006). *The Incidence and Economic Burden of Injuries in the United States*. New York (NY): Oxford University Press.
Fiorica-Howells, E., Maroteaux, L. & Gershon, M. D. (2000) Serotonin and the 5-HT(2B) receptor in the development of enteric neurons. *J.Neurosci.*, **20,** 294-305.

Follesa, P., Biggio, F., Gorini, G., Caria, S., Talani, G., Dazzi, L., Puligheddu, M., Marrosu, F. & Biggio, G. (2007) Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. *Brain Res.*, **1179,** 28-34.

Foster, D. J., Good, D. C., Fowlkes, A. & Sawaki, L. (2006) Atomoxetine enhances a short-term model of plasticity in humans. *Arch.Phys.Med.Rehabil.*, **87,** 216-221.

Fournier, A. E., Beer, J., Arregui, C. O., Essagian, C., Aguayo, A. J. & McKerracher, L. (1997) Brain-derived neurotrophic factor modulates GAP-43 but not T alpha1 expression in injured retinal ganglion cells of adult rats. *J.Neurosci.Res.*, **47,** 561-572.

Fremeau, R. T., Jr., Duncan, G. E., Fornaretto, M. G., Dearry, A., Gingrich, J. A., Breese, G. R. & Caron, M. G. (1991) Localization of D1 dopamine receptor mRNA in brain supports a role in cognitive, affective, and neuroendocrine aspects of dopaminergic neurotransmission. *Proc.Natl.Acad.Sci.U.S.A*, **88,** 3772-3776.

Friedlander, R. M., Gagliardini, V., Rotello, R. J. & Yuan, J. (1996) Functional role of interleukin 1 beta (IL-1 beta) in IL-1 beta-converting enzyme-mediated apoptosis. *J.Exp.Med.*, **184,** 717-724.

Frohman, E. M., Frohman, T. C., Vayuvegula, B., Gupta, S. & van den, N. S. (1988) Vasoactive intestinal polypeptide inhibits the expression of the MHC class II antigens on astrocytes. *J.Neurol.Sci.*, **88,** 339-346.

Frotscher, M., Deller, T., Heimrich, B., Forster, E., Haas, C. & Naumann, T. (1996) Survival, regeneration and sprouting of central neurons: the rat septohippocampal projection as a model. *Ann.Anat.*, **178,** 311-315.

Fuchs, E., Czeh, B., Kole, M. H., Michaelis, T. & Lucassen, P. J. (2004) Alterations of neuroplasticity in depression: the hippocampus and beyond. *Eur.Neuropsychopharmacol.*, **14 Suppl 5,** S481-S490.

Fujinaka, T., Kohmura, E., Yuguchi, T. & Yoshimine, T. (2003) The morphological and neurochemical effects of diffuse brain injury on rat central noradrenergic system. *Neurol.Res.*, **25,** 35-41.

Fukuchi, M., Tabuchi, A. & Tsuda, M. (2005) Transcriptional regulation of neuronal genes and its effect on neural functions: cumulative mRNA expression of PACAP and BDNF genes controlled by calcium and cAMP signals in neurons. *J.Pharmacol.Sci.*, **98,** 212-218.

Furlow, B. (2006). Diagnostic imaging of traumatic brain injury. *Radiologic Technology, 78,* 145-156.

Furuya, K., Kawai, K., Asai, A. & Tamura, A. (1997) Growth-associated protein GAP-43 detection in the neuronal somata following middle cerebral artery occlusion in the rat. *Neurol.Res.*, **19,** 160-164.

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

Galli, R., Gritti, A. & Vescovi, A. L. (2008) Adult neural stem cells. *Methods Mol.Biol.*, **438,** 67-84.

Gao, Y., Nikulina, E., Mellado, W. & Filbin, M. T. (2003) Neurotrophins elevate cAMP to reach a threshold required to overcome inhibition by MAG through extracellular signal-regulated kinase-dependent inhibition of phosphodiesterase. *J.Neurosci.*, **23,** 11770-11777.

Garcia, C., Chen, M. J., Garza, A. A., Cotman, C. W. & Russo-Neustadt, A. (2003) The influence of specific noradrenergic and serotonergic lesions on the expression of hippocampal brain-derived neurotrophic factor transcripts following voluntary physical activity. *Neuroscience*, **119,** 721-732.

Gasbarri, A., Sulli, A. & Packard, M. G. (1997) The dopaminergic mesencephalic projections to the hippocampal formation in the rat. *Prog.Neuropsychopharmacol.Biol.Psychiatry*, **21,** 1-22.

Gehlert, D. R., Gackenheimer, S. L. & Robertson, D. W. (1993) Localization of rat brain binding sites for [3H]tomoxetine, an enantiomerically pure ligand for norepinephrine reuptake sites. *Neurosci.Lett.*, **157,** 203-206.

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

Gentry, L. R., Godersky, J. C. & Thompson, B. (1988) MR imaging of head trauma: review of the distribution and radiopathologic features of traumatic lesions. *AJR Am.J.Roentgenol.*, **150,** 663-672.

Ghosh, A., Carnahan, J. & Greenberg, M. E. (1994) Requirement for BDNF in activity-dependent survival of cortical neurons. *Science*, **263,** 1618-1623.

Giacino, J. T. & Trott, C. T. (2004) Rehabilitative management of patients with disorders of consciousness: grand rounds. *J.Head Trauma Rehabil.*, **19,** 254-265.

Gillespie, L. N., Clark, G. M., Bartlett, P. F. & Marzella, P. L. (2003) BDNF-induced survival of auditory neurons in vivo: Cessation of treatment leads to accelerated loss of survival effects. *J.Neurosci.Res.*, **71,** 785-790.

Gladstone, D. J. & Black, S. E. (2000) Enhancing recovery after stroke with noradrenergic pharmacotherapy: a new frontier? *Can.J.Neurol.Sci.*, **27,** 97-105.

Globus, M. Y., Alonso, O., Dietrich, W. D., Busto, R. & Ginsberg, M. D. (1995) Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. *J.Neurochem.*, **65,** 1704-1711.

Goldenberg, G., Steiner, M., Podreka, I. & Deecke, L. (1992) Regional cerebral blood flow patterns related to verification of low- and high-imagery sentences. *Neuropsychologia*, **30,** 581-586.

Goldenberg, G., Oder, W., Spatt, J. & Podreka, I. (1992) Cerebral correlates of disturbed executive function and memory in survivors of severe closed head injury: a SPECT study. *J.Neurol.Neurosurg.Psychiatry*, **55,** 362-368.

Goldstein, L. B. (1999) Pharmacological approach to functional reorganization: the role of norepinephrine. *Rev.Neurol.(Paris)*, **155,** 731-736.

Goldstein, L. B. (2003) Amphetamines and related drugs in motor recovery after stroke. *Phys.Med.Rehabil.Clin.N.Am.*, **14,** S125-34, x.

Goldstein, R. Z., Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Depue, R. A. & Gur, R. C. (2002) The orbitofrontal cortex in methamphetamine addiction: involvement in fear. *Neuroreport*, **13,** 2253-2257.

Grady, M. S., McLaughlin, M. R., Christman, C. W., Valadka, A. B., Fligner, C. L. & Povlishock, J. T. (1993) The use of antibodies targeted against the neurofilament subunits for the detection of diffuse axonal injury in humans. *J.Neuropathol.Exp.Neurol.*, **52,** 143-152.

Grady, M. S., Charleston, J. S., Maris, D., Witgen, B. M. & Lifshitz, J. (2003) Neuronal and glial cell number in the hippocampus after experimental traumatic brain injury: analysis by stereological estimation. *J.Neurotrauma*, **20,** 929-941.

Griesbach, G. S., Hovda, D. A., Molteni, R., Wu, A. & Gomez-Pinilla, F. (2004) Voluntary exercise following traumatic brain injury: brain-derived neurotrophic factor upregulation and recovery of function. *Neuroscience*, **125,** 129-139.

Groenewegen, H. J. & Uylings, H. B. (2000) The prefrontal cortex and the integration of sensory, limbic and autonomic information. *Prog.Brain Res.*, **126,** 3-28.

Gromwall D (1987). Advances in the assessment of attention and information processing after head injury, in Neurobehavioral Recovery from Head Injury. Edited by Levin HS et al. New York, Oxford University Press, pp 355-371.

Gronwall D (1991). Minor head injury. *Neuropsychology* 5:253-265.

Gross, C. G. (2000) Neurogenesis in the adult brain: death of a dogma. *Nat.Rev.Neurosci.*, **1,** 67-73.

Grunwald, T. & Kurthen, M. (2006) Novelty detection and encoding for declarative memory within the human hippocampus. *Clin.EEG.Neurosci.*, **37,** 309-314.

Gualtieri, C. T. & Evans, R. W. (1988) Stimulant treatment for the neurobehavioural sequelae of traumatic brain injury. *Brain Inj.*, **2,** 273-290.

Guo, N. N. & Li, B. M. (2007) Cellular and subcellular distributions of beta1- and beta2-adrenoceptors in the CA1 and CA3 regions of the rat hippocampus. *Neuroscience*, **146,** 298-305.

Hall, E. D. (1985) High-dose glucocorticoid treatment improves neurological recovery in head-injured mice. *J.Neurosurg.*, **62,** 882-887.

Hallam, T. M., Floyd, C. L., Folkerts, M. M., Lee, L. L., Gong, Q. Z., Lyeth, B. G., Muizelaar, J. P. & Berman, R. F. (2004) Comparison of behavioral deficits and acute neuronal degeneration in rat lateral fluid percussion and weight-drop brain injury models. *J.Neurotrauma*, **21,** 521-539.

Hamill, R. W., Woolf, P. D., McDonald, J. V., Lee, L. A. & Kelly, M. (1987) Catecholamines predict outcome in traumatic brain injury. *Ann.Neurol.*, **21,** 438-443.

Hamm, R. J., O'Dell, D. M., Pike, B. R. & Lyeth, B. G. (1993) Cognitive impairment following traumatic brain injury: the effect of pre- and post-injury administration of scopolamine and MK-801. *Brain Res.Cogn Brain Res.*, **1,** 223-226.

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. *Behav.Brain Res.*, **59,** 169-173.

Harley C., (1991) Noradrenergic and locus coeruleus modulation of the perforant path-evoked potential in rat dentate gyrus supports a role for the locus coeruleus in attentional and memorial processes. *Prog. Brain Res.* **88**, pp. 307–321.

Harley, C. W. (1998) Noradrenergic long-term potentiation in the dentate gyrus. *Adv.Pharmacol.*, **42,** 952-956.

Harley, C. W. (2004) Norepinephrine and dopamine as learning signals. *Neural Plast.*, **11,** 191-204.

Harley, C. W. (2007) Norepinephrine and the dentate gyrus. *Prog.Brain Res.*, **163,** 299-318.

Harrell, L. E., Parsons, D. S. & Kolasa, K. (2005) The effect of central cholinergic and noradrenergic denervation on hippocampal sympathetic ingrowth and apoptosis-like reactivity in the rat. *Brain Res.*, **1033,** 68-77.

Haydon, P. G. (2001) GLIA: listening and talking to the synapse. *Nat.Rev.Neurosci.*, **2,** 185-193.

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

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

Hayes, R. L., Yang, K., Raghupathi, R. & McIntosh, T. K. (1995) Changes in gene expression following traumatic brain injury in the rat. *J.Neurotrauma*, **12,** 779-790.

Heneka M.T., Gavrilyuk V., Landreth GE, O'Banion M.K, Weinberg G and Feinstein D. (2003). Noradrenergic depletion increases inflammatory responses in brain: effects on IkappaB and HSP70 expression. *J. Neurochem.* **85**, pp. 387–398.

Heneka MT., E. Galea, V. Gavriluyk, L. Dumitrescu-Ozimek, J. Daeschner, M.K. O'Banion, G. Weinberg, T. Klockgether and D.L. Feinstein. (2002). Noradrenergic depletion potentiates beta-amyloid-induced cortical inflammation: implications for Alzheimer's disease. *J. Neurosci.* **22**, pp. 2434–2442.

Hicks, R., Soares, H., Smith, D. & McIntosh, T. (1996) Temporal and spatial characterization of neuronal injury following lateral fluid-percussion brain injury in the rat. *Acta Neuropathol.*, **91,** 236-246.

Hicks, R. R., Baldwin, S. A. & Scheff, S. W. (1997) Serum extravasation and cytoskeletal alterations following traumatic brain injury in rats. Comparison of lateral fluid percussion and cortical impact models. *Mol.Chem.Neuropathol.*, **32,** 1-16.

Hicks, R. R., Martin, V. B., Zhang, L. & Seroogy, K. B. (1999) Mild experimental brain injury differentially alters the expression of neurotrophin and neurotrophin receptor mRNAs in the hippocampus. *Exp.Neurol.*, **160,** 469-478.

Hoffman, A. N., Malena, R. R., Westergom, B. P., Luthra, P., Cheng, J. P., Aslam, H. A., Zafonte, R. D. & Kline, A. E. (2008) Environmental enrichment-mediated functional improvement after experimental traumatic brain injury is contingent on task-specific neurobehavioral experience. *Neurosci.Lett.*, **431,** 226-230.

Hollingworth, W., Relvea-Chew, A., Comstock, B.A., Overstreet, J.K., & Jarvik, J.G. (2007). The risk of bankruptcy before and after brain or spinal cord injury: A glimpse of the iceberg's tip. *Medical Care, 45*, 702-711.

Horner, P. J. & Palmer, T. D. (2003) New roles for astrocytes: the nightlife of an 'astrocyte'. La vida loca! *Trends Neurosci.*, **26,** 597-603.

Hornstein, A., Lennihan, L., Seliger, G., Lichtman, S. & Schroeder, K. (1996) Amphetamine in recovery from brain injury. *Brain Inj.*, **10,** 145-148.

Hovda, D. A., Sutton, R. L. & Feeney, D. M. (1989) Amphetamine-induced recovery of visual cliff performance after bilateral visual cortex ablation in cats: measurements of depth perception thresholds. *Behav.Neurosci.*, **103,** 574-584.

Hu, X. X., Goldmuntz, E. A. & Brosnan, C. F. (1991) The effect of norepinephrine on endotoxin-mediated macrophage activation. *J.Neuroimmunol.*, **31,** 35-42.

Huang, E. J. & Reichardt, L. F. (2001) Neurotrophins: roles in neuronal development and function. *Annu.Rev.Neurosci.*, **24,** 677-736.

Hulsebosch, C. E., DeWitt, D. S., Jenkins, L. W. & Prough, D. S. (1998) Traumatic brain injury in rats results in increased expression of Gap-43 that correlates with behavioral recovery. *Neurosci.Lett.*, **255,** 83-86.

Ikeda, Y. & Long, D. M. (1990) The molecular basis of brain injury and brain edema: the role of oxygen free radicals. *Neurosurgery*, **27,** 1-11.

Inazu, M., Takeda, H. & Matsumiya, T. (2003) Functional expression of the norepinephrine transporter in cultured rat astrocytes. *J.Neurochem.*, **84,** 136-144.

Ingelsson, M., Fukumoto, H., Newell, K. L., Growdon, J. H., Hedley-Whyte, E. T., Frosch, M. P., Albert, M. S., Hyman, B. T. & Irizarry, M. C. (2004) Early Abeta accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. *Neurology*, **62,** 925-931.

Inoue, S., Susukida, M., Ikeda, K., Murase, K. & Hayashi, K. (1997) Dopaminergic transmitter up-regulation of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) synthesis in mouse astrocytes in culture. *Biochem.Biophys.Res.Commun.*, **238,** 468-472.

Irwin, N. & Madsen, J. R. (1997) Molecular biology of axonal outgrowth. 1. Growth cones and GAP-43. *Pediatr.Neurosurg.*, **27,** 113-120.

Ivy, A. S., Rodriguez, F. G., Garcia, C., Chen, M. J. & Russo-Neustadt, A. A. (2003) Noradrenergic and serotonergic blockade inhibits BDNF mRNA activation following exercise and antidepressant. *Pharmacol.Biochem.Behav.*, **75,** 81-88.

Izumi, Y. & Zorumski, C. F. (1999) Norepinephrine promotes long-term potentiation in the adult rat hippocampus in vitro. *Synapse*, **31,** 196-202.

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 Res.*, **651,** 123-128.

Jiang, X.B., Ohno, K., Qian, L., Tominaga, B., Kuroiwa, T., Nariai, T., & Hirakawa, K. (2000). Changes in local cerebral blood flow, glucose utilization, and mitochondrial function following traumatic brain injury in rats. *Neurologia Medico Chirurgica (Tokyo), 40,* 16-29.

Juric, D. M., Miklic, S. & Carman-Krzan, M. (2006) Monoaminergic neuronal activity up-regulates BDNF synthesis in cultured neonatal rat astrocytes. *Brain Res.*, **1108,** 54-62.

Kannan, K. & Jain, S. K. (2000) Oxidative stress and apoptosis. *Pathophysiology.*, **7,** 153-163.

Kaplan, S., Heiligenstein, J., West, S., Busner, J., Harder, D., Dittmann, R., Casat, C. & Wernicke, J. F. (2004) Efficacy and safety of atomoxetine in childhood attention-deficit/hyperactivity disorder with comorbid oppositional defiant disorder. *J.Atten.Disord.*, **8,** 45-52.

Kapur S, Craik FI, Jones C, Brown GM, Houle S, Tulving E. (1995). Functional role of the prefrontal cortex in retrieval of memories: a PET study. Neuroreport. Oct 2;6(14):1880-4.

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. *J.Neurosurg.*, **73,** 889-900.

Katz, D., & Alexander, M. (1994). Traumatic brain injury: Predicting course of recovery and outcome for patients admitted to rehabilitation. *Archives of Neurology, 51,* 661-670.

Katz, R.T., & DeLuca, J. (1992). Sequelae of mild traumatic brain injury. *American Family Physician, 46,* 1491-1498.

Kelley, B. J., Farkas, O., Lifshitz, J. & Povlishock, J. T. (2006) Traumatic axonal injury in the perisomatic domain triggers ultrarapid secondary axotomy and Wallerian degeneration. *Exp.Neurol.*, **198,** 350-360.

Kernie, S. G., Erwin, T. M. & Parada, L. F. (2001) Brain remodeling due to neuronal and astrocytic proliferation after controlled cortical injury in mice. *J.Neurosci.Res.*, **66,** 317-326.

Kieburtz, K., McDermott, M., Como, P., Growdon, J., Brady, J., Carter, J., Huber, S., Kanigan, B., Landow, E., Rudolph, A. & . (1994) The effect of deprenyl and tocopherol on cognitive performance in early untreated Parkinson's disease. Parkinson Study Group. *Neurology*, **44,** 1756-1759.

Kikuchi, K., Nishino, K. & Ohyu, H. (2000) Increasing CNS norepinephrine levels by the precursor L-DOPS facilitates beam-walking recovery after sensorimotor cortex ablation in rats. *Brain Res.*, **860,** 130-135.

Kim, D. H. & Zhao, X. (2005) BDNF protects neurons following injury by modulation of caspase activity. *Neurocrit.Care*, **3,** 71-76.

King, C. E., Canty, A. J. & Vickers, J. C. (2001) Alterations in neurofilaments associated with reactive brain changes and axonal sprouting following acute physical injury to the rat neocortex. *Neuropathol.Appl.Neurobiol.*, **27,** 115-126.

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. *Neurosci.Lett.*, **280,** 163-166.

Kline, A. E., Massucci, J. L., Marion, D. W. & Dixon, C. E. (2002) Attenuation of working memory and spatial acquisition deficits after a delayed and chronic bromocriptine

treatment regimen in rats subjected to traumatic brain injury by controlled cortical impact. *J.Neurotrauma*, **19,** 415-425.

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. *J.Neurotrauma,* **21,** 1712-1722.

Klocker, N., Jung, M., Struemer, C.A., Bahr, M. (2001) BDNF increases the number of axotomized rat retinal ganglion cells expressing GAP-43, L1, and TAG-1 mRNA-a supportive role for nitric oxide? *Neurobiology Discussions,* **8,** 203-113

Knoblach, S. M., Nikolaeva, M., Huang, X., Fan, L., Krajewski, S., Reed, J. C. & Faden, A. I. (2002) Multiple caspases are activated after traumatic brain injury: evidence for involvement in functional outcome. *J.Neurotrauma*, **19,** 1155-1170.

Knoll, J. (1993) The pharmacological basis of the beneficial effects of (-)deprenyl (selegiline) in Parkinson's and Alzheimer's diseases. *J.Neural Transm.Suppl*, **40,** 69-91.

Kobayashi, N. R., Fan, D. P., Giehl, K. M., Bedard, A. M., Wiegand, S. J. & Tetzlaff, W. (1997) BDNF and NT-4/5 prevent atrophy of rat rubrospinal neurons after cervical axotomy, stimulate GAP-43 and Talpha1-tubulin mRNA expression, and promote axonal regeneration. *J.Neurosci.,* **17,** 9583-9595.

Kobori, N., Clifton, G. L. & Dash, P. K. (2006) Enhanced catecholamine synthesis in the prefrontal cortex after traumatic brain injury: implications for prefrontal dysfunction. *J.Neurotrauma*, **23,** 1094-1102.

Korz, V. & Frey, J. U. (2007) Hormonal and monoamine signaling during reinforcement of hippocampal long-term potentiation and memory retrieval. *Learn.Mem.,* **14,** 160-166.

Kotapka M.J, Graham D., and Adams J.H., *et al*., (1992). Hippocampal pathology in fatal non-missile human head injury, *Acta Neuropathol (Berl)* **83** (5), pp. 530–534.

Kraus, M. F. & Maki, P. (1997) The combined use of amantadine and l-dopa/carbidopa in the treatment of chronic brain injury. *Brain Inj.,* **11,** 455-460.

Kulkarni, V. A., Jha, S. & Vaidya, V. A. (2002) Depletion of norepinephrine decreases the proliferation, but does not influence the survival and differentiation, of granule cell progenitors in the adult rat hippocampus. *Eur.J.Neurosci.*, **16,** 2008-2012.

Laifenfeld, D., Klein, E. & Ben Shachar, D. (2002) Norepinephrine alters the expression of genes involved in neuronal sprouting and differentiation: relevance for major depression and antidepressant mechanisms. *J.Neurochem.,* **83,** 1054-1064.

Laifenfeld, D., Karry, R., Grauer, E., Klein, E. & Ben Shachar, D. (2005) Antidepressants and prolonged stress in rats modulate CAM-L1, laminin, and pCREB, implicated in neuronal plasticity. *Neurobiol.Dis.*, **20,** 432-441.

Langham, J., Goldfrad, C., Teasdale, G., Shaw, D. & Rowan, K. (2003) Calcium channel blockers for acute traumatic brain injury. *Cochrane.Database.Syst.Rev.*, CD000565.

Langlois JA, Sattin RW. (2005). Traumatic brain injury in the United States: research and programs of the Centers for Disease Control and Prevention (CDC). J Head Trauma Rehabil. 20(3):187-8. No abstract available.

Langlois JA, Kegler SR, Butler JA, Gotsch KE, Johnson RL, Reichard AA, Webb KW, Coronado VG, Selassie AW, Thurman DJ. (2003) Traumatic brain injury-related hospital discharges. Results from a 14-state surveillance system, 1997. *MMWR Surveill Summ.* 2(4):1-20.

Langlois JA, Rutland-Brown W, Wald MM. (2006) The epidemiology and impact of traumatic brain injury: a brief overview. *J Head Trauma Rehabil*. 21(5):375-8.

Lauri, S. E., Palmer, M., Segerstrale, M., Vesikansa, A., Taira, T. & Collingridge, G. L. (2007) Presynaptic mechanisms involved in the expression of STP and LTP at CA1 synapses in the hippocampus. *Neuropharmacology,* **52,** 1-11.

Leon-Carrion, J., Alarcon, J. C., Revuelta, M., Murillo-Cabezas, F., Dominguez-Roldan, J. M., Dominguez-Morales, M. R., Machuca-Murga, F. & Forastero, P. (1998) Executive functioning as outcome in patients after traumatic brain injury. *Int.J.Neurosci.*, **94,** 75-83.

Leslie, F. M., Loughlin, S. E., Sternberg, D. B., McGaugh, J. L., Young, L. E. & Zornetzer, S. F. (1985) Noradrenergic changes and memory loss in aged mice. *Brain Res.*, **359,** 292-299.

Lessmann, V., Gottmann, K. & Malcangio, M. (2003) Neurotrophin secretion: current facts and future prospects. *Prog.Neurobiol.*, **69,** 341-374.

Levey, A. I., Hersch, S. M., Rye, D. B., Sunahara, R. K., Niznik, H. B., Kitt, C. A., Price, D. L., Maggio, R., Brann, M. R., Ciliax, B. J. & . (1993) Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. *Proc.Natl.Acad.Sci.U.S.A*, **90,** 8861-8865.

Levin, H. S. & Goldstein, F. C. (1986) Organization of verbal memory after severe closed-head injury. *J.Clin.Exp.Neuropsychol.*, **8,** 643-656.

Levin, H. S. (1991) Treatment of postconcussional symptoms with CDP-choline. *J.Neurol.Sci.*, **103 Suppl,** S39-S42.

Levin, H. S., Williams, D. H., Eisenberg, H. M., High, W. M., Jr. & Guinto, F. C., Jr. (1992) Serial MRI and neurobehavioural findings after mild to moderate closed head injury. *J.Neurol.Neurosurg.Psychiatry*, **55,** 255-262.

Levine, B., Dawson, D., Boutet, I., Schwartz, M. L. & Stuss, D. T. (2000) Assessment of strategic self-regulation in traumatic brain injury: its relationship to injury severity and psychosocial outcome. *Neuropsychology.*, **14,** 491-500.

Lewis, J., Westerberg, V. & Corcoran, M. E. (1987) Monoaminergic correlates of kindling. *Brain Res.*, **403,** 205-212.

Lezak M. (1995). Neuropsychological assessment. New York, Oxford University Press, pp 176-193; 429-465.

Li, Y., Bickel, K. D., Im, M. J., Hu, L., Dellon, A. L., Vander Kolk, C. A. & Manson, P. N. (1996) Effects of deferoxamine on ischemia/reperfusion injury after peripheral nerve compression. *Ann.Plast.Surg.*, **36,** 365-369.

Lin, L. H., Bock, S., Carpenter, K., Rose, M. & Norden, J. J. (1992) Synthesis and transport of GAP-43 in entorhinal cortex neurons and perforant pathway during lesion-induced sprouting and reactive synaptogenesis. *Brain Res.Mol.Brain Res.*, **14,** 147-153.

Luque, J. M., Puig, N., Martinez, J. M., Gonzalez-Garcia, C. & Cena, V. (2001) Glutamate N-methyl-D-aspartate receptor blockade prevents induction of GAP-43 after focal ischemia in rats. *Neurosci.Lett.*, **305,** 87-90.

Malberg, J. E., Eisch, A. J., Nestler, E. J. & Duman, R. S. (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J.Neurosci.*, **20,** 9104-9110.

Malone, M. A., Kershner, J. R. & Swanson, J. M. (1994) Hemispheric processing and methylphenidate effects in attention-deficit hyperactivity disorder. *J.Child Neurol.*, **9,** 181-189.

Marien, M. R., Colpaert, F. C. & Rosenquist, A. C. (2004) Noradrenergic mechanisms in neurodegenerative diseases: a theory. *Brain Res.Brain Res.Rev.*, **45,** 38-78.

Marklund, N., Clausen, F., McIntosh, T. K. & Hillered, L. (2001) Free radical scavenger posttreatment improves functional and morphological outcome after fluid percussion injury in the rat. *J.Neurotrauma*, **18,** 821-832.

Marklund, N., Bareyre, F. M., Royo, N. C., Thompson, H. J., Mir, A. K., Grady, M. S., Schwab, M. E. & McIntosh, T. K. (2007) Cognitive outcome following brain injury and

treatment with an inhibitor of Nogo-A in association with an attenuated downregulation of hippocampal growth-associated protein-43 expression. *J.Neurosurg.*, **107,** 844-853.

Markowitsch HJ. (2000). Memory and amnesia, in Principles of Behavior and Cognitive Neurology, 2nd edition. Edited by Mesulam MM. New York, Oxford University Press, 2000, pp257-293.

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

Martin, S. J. & Clark, R. E. (2007) The rodent hippocampus and spatial memory: from synapses to systems. *Cell Mol.Life Sci.*, **64,** 401-431.

Masliah, E., Terry, R. D., Alford, M. & DeTeresa, R. (1990) Quantitative immunohistochemistry of synaptophysin in human neocortex: an alternative method to estimate density of presynaptic terminals in paraffin sections. *J.Histochem.Cytochem.*, **38,** 837-844.

Masliah, E., Fagan, A. M., Terry, R. D., DeTeresa, R., Mallory, M. & Gage, F. H. (1991) Reactive synaptogenesis assessed by synaptophysin immunoreactivity is associated with GAP-43 in the dentate gyrus of the adult rat. *Exp.Neurol.*, **113,** 131-142.

Massey, P. V. & Bashir, Z. I. (2007) Long-term depression: multiple forms and implications for brain function. *Trends Neurosci.*, **30,** 176-184.

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. *Neurosci.Lett.*, **372,** 127-131.

Mattiuz, E. L., Ponsler, G. D., Barbuch, R. J., Wood, P. G., Mullen, J. H., Shugert, R. L., Li, Q., Wheeler, W. J., Kuo, F., Conrad, P. C. & Sauer, J. M. (2003) Disposition and metabolic fate of atomoxetine hydrochloride: pharmacokinetics, metabolism, and excretion in the Fischer 344 rat and beagle dog. *Drug Metab Dispos.*, **31,** 88-97.

McAllister, A. K., Katz, L. C. & Lo, D. C. (1999) Neurotrophins and synaptic plasticity. *Annu.Rev.Neurosci.*, **22,** 295-318.

McAllister, T. W., Flashman, L. A., Sparling, M. B. & Saykin, A. J. (2004) Working memory deficits after traumatic brain injury: catecholaminergic mechanisms and prospects for treatment -- a review. *Brain Inj.*, **18,** 331-350.

McAllister TW. (2008). Neurobehavioral sequelae of traumatic brain injury: evaluation and management. *World Psychiatry*. 7(1):3-10.

McDermott, K. L., Raghupathi, R., Fernandez, S. C., Saatman, K. E., Protter, A. A., Finklestein, S. P., Sinson, G., Smith, D. H. & McIntosh, T. K. (1997) Delayed administration of basic fibroblast growth factor (bFGF) attenuates cognitive dysfunction following parasagittal fluid percussion brain injury in the rat. *J.Neurotrauma*, **14,** 191-200.

McDowell, S., Whyte, J. & D'Esposito, M. (1998) Differential effect of a dopaminergic agonist on prefrontal function in traumatic brain injury patients. *Brain*, **121 (Pt 6),** 1155-1164.

McGregor, A., Hayward, A. J., Pearce, J. M. & Good, M. A. (2004) Hippocampal lesions disrupt navigation based on the shape of the environment. *Behav.Neurosci.*, **118,** 1011-1021.

McIntosh, T. K., Vink, R., Soares, H., Hayes, R. & Simon, R. (1989) Effects of the N-methyl-D-aspartate receptor blocker MK-801 on neurologic function after experimental brain injury. *J.Neurotrauma*, **6,** 247-259.

McIntosh TK, Noble L, Andrews B, Faden AI. Traumatic brain injury in the rat: characterization of a midline fluid-percussion model. *Cent Nerv Syst Trauma* 4:119 –134, 1987.

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

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. *Lab Invest*, **74,** 315-342.

McKinney, R. A., Debanne, D., Gahwiler, B. H. & Thompson, S. M. (1997) Lesion-induced axonal sprouting and hyperexcitability in the hippocampus in vitro: implications for the genesis of posttraumatic epilepsy. *Nat.Med.*, **3,** 990-996.

McKinney, R. A., Luthi, A., Bandtlow, C. E., Gahwiler, B. H. & Thompson, S. M. (1999) Selective glutamate receptor antagonists can induce or prevent axonal sprouting in rat hippocampal slice cultures. *Proc.Natl.Acad.Sci.U.S.A*, **96,** 11631-11636.

McMahon, H. T., Bolshakov, V. Y., Janz, R., Hammer, R. E., Siegelbaum, S. A. & Sudhof, T. C. (1996) Synaptophysin, a major synaptic vesicle protein, is not essential for neurotransmitter release. *Proc.Natl.Acad.Sci.U.S.A*, **93,** 4760-4764.

McNamara, R. K., Namgung, U. & Routtenberg, A. (1996) Distinctions between hippocampus of mouse and rat: protein F1/GAP-43 gene expression, promoter activity, and spatial memory. *Brain Res.Mol.Brain Res.*, **40,** 177-187.

Mearow, K. M. (1998) The effects of NGF and sensory nerve stimulation on collateral sprouting and gene expression in adult sensory neurons. *Exp.Neurol.*, **151,** 14-25.

Meberg, P. J., Gall, C. M. & Routtenberg, A. (1993) Induction of F1/GAP-43 gene expression in hippocampal granule cells after seizures [corrected]. *Brain Res.Mol.Brain Res.*, **17,** 295-299.

Messaoudi, E., Ying, S. W., Kanhema, T., Croll, S. D. & Bramham, C. R. (2002) Brain-derived neurotrophic factor triggers transcription-dependent, late phase long-term potentiation in vivo. *J.Neurosci.*, **22,** 7453-7461.

Mesulam MM (2000). Attentional networks, confusional states, and neglect syndromes, in Principles of Behavioral and Cognitive Neurology, 2nd edition. Edited by Mesulam MM. New York, Oxford University Press, pp174-256.

Meyer, J. S., Kondo, A., Nomura, F., Sakamoto, K. & Teraura, T. (1970) Cerebral hemodynamics and metabolism following experimental head injury. *J.Neurosurg.*, **32,** 304-319.

Meyer, J. S., Shinohara, Y., Kanda, T., Fukuuchi, Y., Ericsson, A. D. & Kok, N. K. (1970) Diaschisis resulting from acute unilateral cerebral infarction. Quantitative evidence for man. *Arch.Neurol.*, **23,** 241-247.

Meythaler, J. M., Brunner, R. C., Johnson, A. & Novack, T. A. (2002) Amantadine to improve neurorecovery in traumatic brain injury-associated diffuse axonal injury: a pilot double-blind randomized trial. *J.Head Trauma Rehabil.*, **17,** 300-313.

Miklic, S., Juric, D. M. & Carman-Krzan, M. (2004) Differences in the regulation of BDNF and NGF synthesis in cultured neonatal rat astrocytes. *Int.J.Dev.Neurosci.*, **22,** 119-130.

Millan, M. J. (2004) The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. *Eur.J.Pharmacol.*, **500,** 371-384.

Miyazaki, S., Katayama, Y., Lyeth, B. G., Jenkins, L. W., DeWitt, D. S., Goldberg, S. J., Newlon, P. G. & Hayes, R. L. (1992) Enduring suppression of hippocampal long-term potentiation following traumatic brain injury in rat. *Brain Res.*, **585,** 335-339.

Moran-Gates, T., Zhang, K., Baldessarini, R. J. & Tarazi, F. I. (2005) Atomoxetine blocks motor hyperactivity in neonatal 6-hydroxydopamine-lesioned rats: implications for treatment of attention-deficit hyperactivity disorder. *Int.J.Neuropsychopharmacol.*, **8,** 439-444.

Morishita, S. & Aoki, S. (2002) Effects of tricyclic antidepressants on protein kinase C activity in rabbit and human platelets in vivo. *J.Affect.Disord.*, **70,** 329-332.

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

Morris, R. G., Moser, E. I., Riedel, G., Martin, S. J., Sandin, J., Day, M. & O'Carroll, C. (2003) Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. *Philos.Trans.R.Soc.Lond B Biol.Sci.*, **358,** 773-786.

Moudy, A. M., Kunkel, D. D. & Schwartzkroin, P. A. (1993) Development of dopamine-beta-hydroxylase-positive fiber innervation of the rat hippocampus. *Synapse*, **15,** 307-318.

Munro, C. A., Walling, S. G., Evans, J. H. & Harley, C. W. (2001) Beta-adrenergic blockade in the dentate gyrus in vivo prevents high frequency-induced long-term potentiation of EPSP slope, but not long-term potentiation of population spike amplitude. *Hippocampus*, **11,** 322-328.

Murchison, C. F., Zhang, X. Y., Zhang, W. P., Ouyang, M., Lee, A. & Thomas, S. A. (2004) A distinct role for norepinephrine in memory retrieval. *Cell*, **117,** 131-143.

Murdoch, I., Perry, E. K., Court JA, Graham, D. I. & Dewar, D. (1998) Cortical cholinergic dysfunction after human head injury. *J.Neurotrauma*, **15,** 295-305.

Murer, M. G., Yan, Q. & Raisman-Vozari, R. (2001) Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Prog.Neurobiol.*, **63,** 71-124.

Nakamura, H., Uzura, M., Uchida, K., Nakayama, H., Furuya, Y., Hayashi, T., Sekino, H., Ominato, M. & Owada, S. (2003) Effects of edaravone on experimental brain injury in view of free radical reaction. *Acta Neurochir.Suppl*, **86,** 309-311.

Napolitano, E., Elovic, E. P. & Qureshi, A. I. (2005) Pharmacological stimulant treatment of neurocognitive and functional deficits after traumatic and non-traumatic brain injury. *Med.Sci.Monit.*, **11,** RA212-RA220.

Natale, J. E., Ahmed, F., Cernak, I., Stoica, B. & Faden, A. I. (2003) Gene expression profile changes are commonly modulated across models and species after traumatic brain injury. *J.Neurotrauma*, **20,** 907-927.

Navarra, R., Graf, R., Huang, Y., Logue, S., Comery, T., Hughes, Z. & Day, M. (2008) Effects of atomoxetine and methylphenidate on attention and impulsivity in the 5-choice serial reaction time test. *Prog.Neuropsychopharmacol.Biol.Psychiatry*, **32,** 34-41.

Nestler, E. J., Terwilliger, R. Z. & Duman, R. S. (1989) Chronic antidepressant administration alters the subcellular distribution of cyclic AMP-dependent protein kinase in rat frontal cortex. *J.Neurochem.*, **53,** 1644-1647.

Newburn, G. & Newburn, D. (2005) Selegiline in the management of apathy following traumatic brain injury. *Brain Inj.*, **19,** 149-154.

Newcomb, J. K., Zhao, X., Pike, B. R. & Hayes, R. L. (1999) Temporal profile of apoptotic-like changes in neurons and astrocytes following controlled cortical impact injury in the rat. *Exp.Neurol.*, **158,** 76-88.

Nibuya, M., Morinobu, S. & Duman, R. S. (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J.Neurosci.*, **15,** 7539-7547.

Nibuya, M., Nestler, E. J. & Duman, R. S. (1996) Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J.Neurosci.*, **16,** 2365-2372.

Nickels, J. L., Schneider, W. N., Dombovy, M. L. & Wong, T. M. (1994) Clinical use of amantadine in brain injury rehabilitation. *Brain Inj.*, **8,** 709-718.

Nyberg G, Andersson J, Antoni G, Lilja A, Pellettieri L, Valind S, Långström B. (1996). Activation PET scanning in pretreatment evaluation of patients with cerebral tumours or vascular lesions in or close to the sensorimotor cortex. *Acta Neurochir* (Wien).138(6):684-94.

O'Carroll, C. M. & Morris, R. G. (2004) Heterosynaptic co-activation of glutamatergic and dopaminergic afferents is required to induce persistent long-term potentiation. *Neuropharmacology*, **47,** 324-332.

O'Dell, D. M. & Hamm, R. J. (1995) Chronic postinjury administration of MDL 26,479 (Suritozole), a negative modulator at the GABAA receptor, and cognitive impairment in rats following traumatic brain injury. *J.Neurosurg.*, **83,** 878-883.

O'Dell, D. M., Gibson, C. J., Wilson, M. S., DeFord, S. M. & Hamm, R. J. (2000) Positive and negative modulation of the GABA(A) receptor and outcome after traumatic brain injury in rats. *Brain Res.*, **861,** 325-332.

O'Keefe, J., and Nadel, L. (1978) The Hippocampus as a Cognitive Map, p 570. Oxford University Press, Oxford

Olivier, B., Soudijn, W. & van, W., I (2000) Serotonin, dopamine and norepinephrine transporters in the central nervous system and their inhibitors. *Prog.Drug Res.*, **54,** 59-119.

Orgogozo, J. M. & Loiseau, P. (1977) [Teratogenic and oncogenic effects of central nervous system drugs]. *Rev.Prat.*, **27,** 2225-2233.

Otmakhova, N. A. & Lisman, J. E. (1996) D1/D5 dopamine receptor activation increases the magnitude of early long-term potentiation at CA1 hippocampal synapses. *J.Neurosci.*, **16,** 7478-7486.

Page, G., Peeters, M., Maloteaux, J. M. & Hermans, E. (2000) Increased dopamine uptake in striatal synaptosomes after treatment of rats with amantadine. *Eur.J.Pharmacol.*, **403,** 75-80.

Palmer, A. M., Marion, D. W., Botscheller, M. L., Bowen, D. M. & DeKosky, S. T. (1994) Increased transmitter amino acid concentration in human ventricular CSF after brain trauma. *Neuroreport*, **6,** 153-156.

Pang, P. T. & Lu, B. (2004) Regulation of late-phase LTP and long-term memory in normal and aging hippocampus: role of secreted proteins tPA and BDNF. *Ageing Res.Rev.*, **3,** 407-430.

Park, N. W., Moscovitch, M. & Robertson, I. H. (1999) Divided attention impairments after traumatic brain injury. *Neuropsychologia*, **37,** 1119-1133.

Pataki, C. S., Feinberg, D. T. & McGough, J. J. (2004) New drugs for the treatment of attention-deficit/hyperactivity disorder. *Expert.Opin.Emerg.Drugs*, **9,** 293-302.

Patanow, C. M., Day, J. R. & Billingsley, M. L. (1997) Alterations in hippocampal expression of SNAP-25, GAP-43, stannin and glial fibrillary acidic protein following mechanical and trimethyltin-induced injury in the rat. *Neuroscience*, **76,** 187-202.

Patterson, S. L., Abel, T., Deuel, T. A., Martin, K. C., Rose, J. C. & Kandel, E. R. (1996) Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron*, **16,** 1137-1145.

Peeters, M., Maloteaux, J. M. & Hermans, E. (2003) Distinct effects of amantadine and memantine on dopaminergic transmission in the rat striatum. *Neurosci.Lett.*, **343,** 205-209.

Peeters, M., Romieu, P., Maurice, T., Su, T. P., Maloteaux, J. M. & Hermans, E. (2004) Involvement of the sigma 1 receptor in the modulation of dopaminergic transmission by amantadine. *Eur.J.Neurosci.*, **19,** 2212-2220.

Peterson, G. M. (1994) Sprouting of central noradrenergic fibers in the dentate gyrus following combined lesions of its entorhinal and septal afferents. *Hippocampus*, **4,** 635-648.

Pfenninger, E. G., Reith, A., Breitig, D., Grunert, A. & Ahnefeld, F. W. (1989) Early changes of intracranial pressure, perfusion pressure, and blood flow after acute head injury. Part 1: An experimental study of the underlying pathophysiology. *J.Neurosurg.*, **70,** 774-779.

Phillips, H. S., Hains, J. M., Laramee, G. R., Rosenthal, A. & Winslow, J. W. (1990) Widespread expression of BDNF but not NT3 by target areas of basal forebrain cholinergic neurons. *Science*, **250,** 290-294.

Phillips, L. L., Lyeth, B. G., Hamm, R. J., Reeves, T. M. & Povlishock, J. T. (1998) Glutamate antagonism during secondary deafferentation enhances cognition and axo-dendritic integrity after traumatic brain injury. *Hippocampus*, **8,** 390-401.

Piehl, F., Hammarberg, H., Hokfelt, T. & Cullheim, S. (1998) Regulatory effects of trophic factors on expression and distribution of CGRP and GAP-43 in rat motoneurons. *J.Neurosci.Res.*, **51,** 1-14.

Pierce, J. E., Smith, D. H., Trojanowski, J. Q. & McIntosh, T. K. (1998) Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. *Neuroscience*, **87,** 359-369.

Pike, B. R. & Hamm, R. J. (1995) Post-injury administration of BIBN 99, a selective muscarinic M2 receptor antagonist, improves cognitive performance following traumatic brain injury in rats. *Brain Res.*, **686,** 37-43.

Plenger, P. M., Dixon, C. E., Castillo, R. M., Frankowski, R. F., Yablon, S. A. & Levin, H. S. (1996) Subacute methylphenidate treatment for moderate to moderately severe traumatic brain injury: a preliminary double-blind placebo-controlled study. *Arch.Phys.Med.Rehabil.*, **77,** 536-540.

Poo, M. M. (2001) Neurotrophins as synaptic modulators. *Nat.Rev.Neurosci.*, **2,** 24-32.

Porter, A. G. & Janicke, R. U. (1999) Emerging roles of caspase-3 in apoptosis. *Cell Death.Differ.*, **6,** 99-104.

Povlishock, J. T., Becker, D. P., Sullivan, H. G. & Miller, J. D. (1978) Vascular permeability alterations to horseradish peroxidase in experimental brain injury. *Brain Res.*, **153,** 223-239.

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

Povlishock, J. T. & Kontos, H. A. (1992) The role of oxygen radicals in the pathobiology of traumatic brain injury. *Hum.Cell* , **5,** 345-353.

Povlishock, J. T., Hayes, R. L., Michel, M. E. & McIntosh, T. K. (1994) Workshop on animal models of traumatic brain injury. *J.Neurotrauma*, **11,** 723-732.

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

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

Prasad, M. R., Tzigaret, C. M., Smith, D., Soares, H. & McIntosh, T. K. (1992) Decreased alpha 1-adrenergic receptors after experimental brain injury. *J.Neurotrauma,* **9,** 269-279.

Prasad, M. R., Ramaiah, C., McIntosh, T. K., Dempsey, R. J., Hipkens, S. & Yurek, D. (1994) Regional levels of lactate and norepinephrine after experimental brain injury. *J.Neurochem.,* **63,** 1086-1094.

Prins, M. L., Povlishock, J. T. & Phillips, L. L. (2003) The effects of combined fluid percussion traumatic brain injury and unilateral entorhinal deafferentation on the juvenile rat brain. *Brain Res.Dev.Brain Res.*, **140,** 93-104.

Pupo, A. S. & Minneman, K. P. (2001) Adrenergic pharmacology: focus on the central nervous system. *CNS.Spectr.,* **6,** 656-662.

Queen, S. A., Chen, M. J. & Feeney, D. M. (1997) d-Amphetamine attenuates decreased cerebral glucose utilization after unilateral sensorimotor cortex contusion in rats. *Brain Res.,* **777,** 42-50.

Rall, J. M., Matzilevich, D. A. & Dash, P. K. (2003) Comparative analysis of mRNA levels in the frontal cortex and the hippocampus in the basal state and in response to experimental brain injury. *Neuropathol.Appl.Neurobiol.*, **29,** 118-131.

Ramos, B. P. & Arnsten, A. F. (2007) Adrenergic pharmacology and cognition: focus on the prefrontal cortex. *Pharmacol.Ther.*, **113,** 523-536.

Reddy, P. H., Mani, G., Park, B. S., Jacques, J., Murdoch, G., Whetsell, W., Jr., Kaye, J. & Manczak, M. (2005) Differential loss of synaptic proteins in Alzheimer's disease: implications for synaptic dysfunction. *J.Alzheimers.Dis.*, **7,** 103-117.

Reeves, T. M., Lyeth, B. G. & Povlishock, J. T. (1995) Long-term potentiation deficits and excitability changes following traumatic brain injury. *Exp.Brain Res.*, **106,** 248-256.

Reid, W. M. & Hamm, R. J. (2008) Post-injury atomoxetine treatment improves cognition following experimental traumatic brain injury. *J.Neurotrauma,* **25,** 248-256.

Reinhard, D. L., Whyte, J. & Sandel, M. E. (1996) Improved arousal and initiation following tricyclic antidepressant use in severe brain injury. *Arch.Phys.Med.Rehabil.*, **77,** 80-83.

Richardson, R. M., Sun, D. & Bullock, M. R. (2007) Neurogenesis after traumatic brain injury. *Neurosurg.Clin.N.Am.*, **18,** 169-81, xi.

Richardson JTE. (2000). Clinical and Neuropsyphological Aspects of Closed Head Injury. East Suxxex, UK, Psychology Press, pp 97-124.

Ricker, J. H., Hillary, F. G. & DeLuca, J. (2001) Functionally activated brain imaging (O-15 PET and fMRI) in the study of learning and memory after traumatic brain injury. *J.Head Trauma Rehabil.*, **16,** 191-205.

Rimel, R.W., Giordani, B., Barth, J.T., Boll, T.J., & Jane, J.A. (1981). Disability caused by minor head injury. *Neurosurgery, 9*, 221-228.

Rimel, R.W., Giordani, B., Barth, J.T., & Jane, J.A. (1982). Moderate head injury: Completing the clinical spectrum of brain trauma. *Neurosurgery, 11*, 344-351.

Rios, M., Perianez, J. A. & Munoz-Cespedes, J. M. (2004) Attentional control and slowness of information processing after severe traumatic brain injury. *Brain Inj.*, **18,** 257-272.

Ripley, D. L. (2006) Atomoxetine for individuals with traumatic brain injury. *J.Head Trauma Rehabil.*, **21,** 85-88.

Rita, P. & Bjelke, B. (1991) Lasting recovery of motor function, following brain damage, with a single dose of amphetamine combined with physical therapy; changes in gene expression? *Scand.J.Rehabil.Med.*, **23,** 219-220.

Rizk, P., Salazar, J., Raisman-Vozari, R., Marien, M., Ruberg, M., Colpaert, F. & Debeir, T. (2006) The alpha2-adrenoceptor antagonist dexefaroxan enhances hippocampal neurogenesis by increasing the survival and differentiation of new granule cells. *Neuropsychopharmacology*, **31,** 1146-1157.

Rodriguez-Paez, A. C., Brunschwig, J. P. & Bramlett, H. M. (2005) Light and electron microscopic assessment of progressive atrophy following moderate traumatic brain injury in the rat. *Acta Neuropathol.*, **109,** 603-616.

Rutland-Brown W, Langlois JA, Thomas KE, Xi YL. (2006). Incidence of traumatic brain injury in the United States, 2003. *J Head Trauma Rehabil*. 21(6):544-8.

Saatman, K. E., Contreras, P. C., Smith, D. H., Raghupathi, R., McDermott, K. L., Fernandez, S. C., Sanderson, K. L., Voddi, M. & McIntosh, T. K. (1997) Insulin-like growth factor-1 (IGF-1) improves both neurological motor and cognitive outcome following experimental brain injury. *Exp.Neurol.*, **147,** 418-427.

Sagvolden, T. & Xu, T. (2008) l-Amphetamine improves poor sustained attention while d-amphetamine reduces overactivity and impulsiveness as well as improves sustained attention in an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). *Behav.Brain Funct.*, **4,** 3.

Sairanen, M., Lucas, G., Ernfors, P., Castren, M. & Castren, E. (2005) Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J.Neurosci.*, **25,** 1089-1094.

Sanders, J. D., Happe, H. K., Bylund, D. B. & Murrin, L. C. (2005) Development of the norepinephrine transporter in the rat CNS. *Neuroscience*, **130,** 107-117.

Sanders, M. J., Sick, T. J., Perez-Pinzon, M. A., Dietrich, W. D. & Green, E. J. (2000) Chronic failure in the maintenance of long-term potentiation following fluid percussion injury in the rat. *Brain Res.*, **861,** 69-76.

Sara, S. J. (1989) Noradrenergic-cholinergic interaction: its possible role in memory dysfunction associated with senile dementia. *Arch.Gerontol.Geriatr.Suppl*, **1,** 99-108.

Sawaguchi, T. & Goldman-Rakic, P. S. (1994) The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. *J.Neurophysiol.*, **71,** 515-528.

Schallert, T., Leasure, J. L. & Kolb, B. (2000) Experience-associated structural events, subependymal cellular proliferative activity, and functional recovery after injury to the central nervous system. *J.Cereb.Blood Flow Metab*, **20,** 1513-1528.

Schauwecker, P. E., Cheng, H. W., Serquinia, R. M., Mori, N. & McNeill, T. H. (1995) Lesion-induced sprouting of commissural/associational axons and induction of GAP-43 mRNA in hilar and CA3 pyramidal neurons in the hippocampus are diminished in aged rats. *J.Neurosci.*, **15,** 2462-2470.

Schauwecker, P. E., Ramirez, J. J. & Steward, O. (2000) Genetic dissection of the signals that induce synaptic reorganization. *Exp.Neurol.*, **161,** 139-152.

Scheff, S. W., Price, D. A., Hicks, R. R., Baldwin, S. A., Robinson, S. & Brackney, C. (2005) Synaptogenesis in the hippocampal CA1 field following traumatic brain injury. *J.Neurotrauma*, **22,** 719-732.

Scheff, S. W., Price, D. A., Hicks, R. R., Baldwin, S. A., Robinson, S. & Brackney, C. (2005) Synaptogenesis in the hippocampal CA1 field following traumatic brain injury. *J.Neurotrauma*, **22,** 719-732.

Schicho, R., Schuligoi, R., Sirinathsinghji, D. J. & Donnerer, J. (1999) Increased expression of GAP-43 in small sensory neurons after stimulation by NGF indicative of neuroregeneration in capsaicin-treated rats. *Regul.Pept.*, **83,** 87-95.

Schimanski, L. A., Ali, D. W., Baker, G. B. & Nguyen, P. V. (2007) Impaired hippocampal LTP in inbred mouse strains can be rescued by beta-adrenergic receptor activation. *Eur.J.Neurosci.*, **25,** 1589-1598.

Schmidt-Kastner, R., Bedard, A. & Hakim, A. (1997) Transient expression of GAP-43 within the hippocampus after global brain ischemia in rat. *Cell Tissue Res.*, **288,** 225-238.

Schmidt, H. D. & Duman, R. S. (2007) The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. *Behav.Pharmacol.*, **18,** 391-418.

Schmidt, U., Pilgrim, C. & Beyer, C. (1998) Differentiative effects of dopamine on striatal neurons involve stimulation of the cAMP/PKA pathway. *Mol.Cell Neurosci.*, **11,** 9-18.

Schmitter-Edgecombe, M. & Kibby, M. K. (1998) Visual selective attention after severe closed head injury. *J.Int.Neuropsychol.Soc.*, **4,** 144-159.

Schoettle, R. J., Kochanek, P. M., Magargee, M. J., Uhl, M. W. & Nemoto, E. M. (1990) Early polymorphonuclear leukocyte accumulation correlates with the development of posttraumatic cerebral edema in rats. *J.Neurotrauma*, **7,** 207-217.

Schultz, W. (2001) Reward signaling by dopamine neurons. *Neuroscientist.*, **7,** 293-302.

Schultz, W. (2006) Behavioral theories and the neurophysiology of reward. *Annu.Rev.Psychol.*, **57,** 87-115.

Schwartz, J. P. & Nishiyama, N. (1994) Neurotrophic factor gene expression in astrocytes during development and following injury. *Brain Res.Bull.*, **35,** 403-407.

Schwenkreis, P., Witscher, K., Janssen, F., Addo, A., Dertwinkel, R., Zenz, M., Malin, J. P. & Tegenthoff, M. (1999) Influence of the N-methyl-D-aspartate antagonist memantine on human motor cortex excitability. *Neurosci.Lett.*, **270,** 137-140.

Selassie AW, Zaloshnja E, Langlois JA, Miller T, Jones P, Steiner C. (2008) Incidence of long-term disability following traumatic brain injury hospitalization, United States, 2003. *J Head Trauma Rehabil*. 23(2):123-31.

Selmeczy, Z., Szelenyi, J. & Vizi, E. S. (2003) Intact noradrenaline transporter is needed for the sympathetic fine-tuning of cytokine balance. *Eur.J.Pharmacol.*, **469,** 175-181.

Selzer, M. E. (2003) Promotion of axonal regeneration in the injured CNS. *Lancet Neurol.*, **2,** 157-166.

Seneca, N., Gulyas, B., Varrone, A., Schou, M., Airaksinen, A., Tauscher, J., Vandenhende, F., Kielbasa, W., Farde, L., Innis, R. B. & Halldin, C. (2006) Atomoxetine occupies the norepinephrine transporter in a dose-dependent fashion: a PET study in nonhuman primate brain using (S,S)-[18F]FMeNER-D2. *Psychopharmacology (Berl)*, **188,** 119-127.

Shearer, M. C., Niclou, S. P., Brown, D., Asher, R. A., Holtmaat, A. J., Levine, J. M., Verhaagen, J. & Fawcett, J. W. (2003) The astrocyte/meningeal cell interface is a barrier to neurite outgrowth which can be overcome by manipulation of inhibitory molecules or axonal signalling pathways. *Mol.Cell Neurosci.*, **24,** 913-925.

Shein, N. A., Grigoriadis, N., Alexandrovich, A. G., Simeonidou, C., Spandou, E., Tsenter, J., Yatsiv, I., Horowitz, M. & Shohami, E. (2008) Differential neuroprotective properties of endogenous and exogenous erythropoietin in a mouse model of traumatic brain injury. *J.Neurotrauma*, **25,** 112-123.

Shohami, E., Gallily, R., Mechoulam, R., Bass, R. & Ben Hur, T. (1997) Cytokine production in the brain following closed head injury: dexanabinol (HU-211) is a novel TNF-alpha inhibitor and an effective neuroprotectant. *J.Neuroimmunol.*, **72,** 169-177.

Shojo, H. & Kibayashi, K. (2006) Changes in localization of synaptophysin following fluid percussion injury in the rat brain. *Brain Res.*, **1078,** 198-211.

Shum D, Sweeper S, Murray R (1996). Performance on verbal and implicit and explicit memory tasks following traumatic brain injury. J Head Trauma Rehabilitation 11: 43-53.

Siesjo, B. K. & Wieloch, T. (1985) Cerebral metabolism in ischaemia: neurochemical basis for therapy. *Br.J.Anaesth.*, **57,** 47-62.

Simon, H. (1981) [Dopaminergic A10 neurons and frontal system (author's transl)]. *J.Physiol (Paris)*, **77,** 81-95.

Skene, J. H., Jacobson, R. D., Snipes, G. J., McGuire, C. B., Norden, J. J. & Freeman, J. A. (1986) A protein induced during nerve growth (GAP-43) is a major component of growth-cone membranes. *Science*, **233,** 783-786.

Slavik, R. S. & Rhoney, D. H. (1999) Indomethacin: a review of its cerebral blood flow effects and potential use for controlling intracranial pressure in traumatic brain injury patients. *Neurol.Res.*, **21,** 491-499.

Smith, F. M., Raghupathi, R., MacKinnon, M. A., McIntosh, T. K., Saatman, K. E., Meaney, D. F. & Graham, D. I. (2000) TUNEL-positive staining of surface contusions after fatal head injury in man. *Acta Neuropathol.*, **100,** 537-545.

Soares, H. D., Thomas, M., Cloherty, K. & McIntosh, T. K. (1992) Development of prolonged focal cerebral edema and regional cation changes following experimental brain injury in the rat. *J.Neurochem.*, **58,** 1845-1852.

Song, H. J., Stevens, C. F. & Gage, F. H. (2002) Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nat.Neurosci.*, **5,** 438-445.

Spencer, T. & Filbin, M. T. (2004) A role for cAMP in regeneration of the adult mammalian CNS. *J.Anat.*, **204,** 49-55.

Steward, O. (1992) Signals that induce sprouting in the central nervous system: sprouting is delayed in a strain of mouse exhibiting delayed axonal degeneration. *Exp.Neurol.*, **118,** 340-351.

Stewart, C. A. & Reid, I. C. (2000) Repeated ECS and fluoxetine administration have equivalent effects on hippocampal synaptic plasticity. *Psychopharmacology (Berl)*, **148,** 217-223.

Stroemer, R. P., Kent, T. A. & Hulsebosch, C. E. (1995) Neocortical neural sprouting, synaptogenesis, and behavioral recovery after neocortical infarction in rats. *Stroke*, **26,** 2135-2144.

Stroemer, R. P., Kent, T. A. & Hulsebosch, C. E. (1998) Enhanced neocortical neural sprouting, synaptogenesis, and behavioral recovery with D-amphetamine therapy after neocortical infarction in rats. *Stroke*, **29,** 2381-2393.

Suehiro, E., Ueda, Y., Wei, E. P., Kontos, H. A. & Povlishock, J. T. (2003) Posttraumatic hypothermia followed by slow rewarming protects the cerebral microcirculation. *J.Neurotrauma*, **20,** 381-390.

Sun, D., Colello, R. J., Daugherty, W. P., Kwon, T. H., McGinn, M. J., Harvey, H. B. & Bullock, M. R. (2005) Cell proliferation and neuronal differentiation in the dentate gyrus in juvenile and adult rats following traumatic brain injury. *J.Neurotrauma*, **22,** 95-105.

Surmeier, D. J. & Kitai, S. T. (1994) Dopaminergic regulation of striatal efferent pathways. *Curr.Opin.Neurobiol.*, **4,** 915-919.

Surmeier, D. J., Bargas, J., Hemmings, H. C., Jr., Nairn, A. C. & Greengard, P. (1995) Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron*, **14,** 385-397.

Sutherland, R. J., Whishaw, I. Q. & Kolb, B. (1983) A behavioural analysis of spatial localization following electrolytic, kainate- or colchicine-induced damage to the hippocampal formation in the rat. *Behav.Brain Res.*, **7,** 133-153.

Sutton, M. A. & Schuman, E. M. (2005) Local translational control in dendrites and its role in long-term synaptic plasticity. *J.Neurobiol.*, **64,** 116-131.

Sutton, R. L., Hovda, D. A., Chen, M. J. & Feeney, D. M. (2000) Alleviation of brain injury-induced cerebral metabolic depression by amphetamine: a cytochrome oxidase histochemistry study. *Neural Plast.*, **7,** 109-125.

Swanson, C. J., Perry, K. W., Koch-Krueger, S., Katner, J., Svensson, K. A. & Bymaster, F. P. (2006) Effect of the attention deficit/hyperactivity disorder drug atomoxetine on extracellular concentrations of norepinephrine and dopamine in several brain regions of the rat. *Neuropharmacology*, **50,** 755-760.

Tabaddor, K., Mattis, S., & Zazula, T. (1984). Cognitive sequelae and recovery course after moderate and severe head injury. *Neurosurgery, 14*, 701-708.

Tan, C. H., He, X., Yang, J. & Ong, W. Y. (2006) Changes in AMPA subunit expression in the mouse brain after chronic treatment with the antidepressant maprotiline: a link between noradrenergic and glutamatergic function? *Exp.Brain Res.*, **170,** 448-456.

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. *Eur.J.Neurosci.*, **9,** 1720-1727.

Tardito, D., Perez, J., Tiraboschi, E., Musazzi, L., Racagni, G. & Popoli, M. (2006) Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. *Pharmacol.Rev.*, **58,** 115-134.

Temple, M. D., Delahunty, T. M., Hamm, R. J., Phillips, L. L., Lyeth, B. G. & Povlishock, J. T. (2001) Subtle alterations in NMDA-stimulated cyclic GMP levels following lateral fluid percussion brain injury. *J.Neurotrauma*, **18,** 47-55.

Tenovuo, O. (2006) Pharmacological enhancement of cognitive and behavioral deficits after traumatic brain injury. *Curr.Opin.Neurol.*, **19,** 528-533.

Thoenen, H. (1995) Neurotrophins and neuronal plasticity. *Science*, **270,** 593-598.

Thomas, S., Prins, M.L., Samii, M., & Hovda, D.A. (2000). Cerebral metabolic response to traumatic brain injury sustained early in development: a 2-deoxy-D-glucose autoradiographic study. *Journal of Neurotrauma, 17*, 649-665.

Thome, J., Sakai, N., Shin, K., Steffen, C., Zhang, Y. J., Impey, S., Storm, D. & Duman, R. S. (2000) cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *J.Neurosci.*, **20,** 4030-4036.

Thompson, H. J., Lifshitz, J., Marklund, N., Grady, M. S., Graham, D. I., Hovda, D. A. & McIntosh, T. K. (2005) Lateral fluid percussion brain injury: a 15-year review and evaluation. *J.Neurotrauma*, **22,** 42-75.

Thompson, S. N., Gibson, T. R., Thompson, B. M., Deng, Y. & Hall, E. D. (2006) Relationship of calpain-mediated proteolysis to the expression of axonal and synaptic plasticity markers following traumatic brain injury in mice. *Exp.Neurol.*, **201,** 253-265.

Thurman DJ, Alverson C, Dunn KA, Guerrero J, Sniezek JE. Traumatic brain injury in the United States: A public health perspective. J Head Trauma Rehabil. 1999 Dec;14(6):602-15.

Thurman D, Guerrero J. Trends in hospitalization associated with traumatic brain injury. JAMA. 1999 Sep 8;282(10):954-7.

Tolias, C. M. & Bullock, M. R. (2004) Critical appraisal of neuroprotection trials in head injury: what have we learned? *NeuroRx.*, **1,** 71-79.

Tsacopoulos, M. & Magistretti, P. J. (1996) Metabolic coupling between glia and neurons. *J.Neurosci.*, **16,** 877-885.

Tyler, W. J. & Pozzo-Miller, L. D. (2001) BDNF enhances quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses. *J.Neurosci.*, **21,** 4249-4258.

Tzavara, E. T., Bymaster, F. P., Overshiner, C. D., Davis, R. J., Perry, K. W., Wolff, M., McKinzie, D. L., Witkin, J. M. & Nomikos, G. G. (2006) Procholinergic and memory enhancing properties of the selective norepinephrine uptake inhibitor atomoxetine. *Mol.Psychiatry*, **11,** 187-195.

Urrea, C., Castellanos, D. A., Sagen, J., Tsoulfas, P., Bramlett, H. M. & Dietrich, W. D. (2007) Widespread cellular proliferation and focal neurogenesis after traumatic brain injury in the rat. *Restor.Neurol.Neurosci.*, **25,** 65-76.

Van Eden, C. G., Hoorneman, E. M., Buijs, R. M., Matthijssen, M. A., Geffard, M. & Uylings, H. B. (1987) Immunocytochemical localization of dopamine in the prefrontal cortex of the rat at the light and electron microscopical level. *Neuroscience*, **22,** 849-862.

von Monakow C. (1969). Diaschisis, the localization in the cerebrum and functional impairment by cortical loci. In: K. Pribram, editor. Brand and behavior: moods, states, and mind. Vol. 1. Baltimore: Penguin Books. p. 27-36 (translated by G. Harris).

van Zomeren, A. H. & van den, B. W. (1985) Residual complaints of patients two years after severe head injury. *J.Neurol.Neurosurg.Psychiatry*, **48,** 21-28.

Van Zomeren, AH, Brouwer, WH. (1994). Clinical Neuropsychology of Attention. New York, Oxford University Press, pp 63-94.

Vanderploeg, R. D., Crowell, T. A. & Curtiss, G. (2001) Verbal learning and memory deficits in traumatic brain injury: encoding, consolidation, and retrieval. *J.Clin.Exp.Neuropsychol.*, **23,** 185-195.

Veyrac, A., Didier, A., Colpaert, F., Jourdan, F. & Marien, M. (2005) Activation of noradrenergic transmission by alpha2-adrenoceptor antagonists counteracts deafferentation-induced neuronal death and cell proliferation in the adult mouse olfactory bulb. *Exp.Neurol.*, **194,** 444-456.

Wall PM, Messier C. (2001). The hippocampal formation--orbitomedial prefrontal cortex circuit in the attentional control of active memory. *Behav Brain Res*. (1-2):99-117.

Wagner, A. K., Sokoloski, J. E., Ren, D., Chen, X., Khan, A. S., Zafonte, R. D., Michael, A. C. & Dixon, C. E. (2005) Controlled cortical impact injury affects dopaminergic transmission in the rat striatum. *J.Neurochem.*, **95,** 457-465.

Walker-Batson, D., Smith, P., Curtis, S., Unwin, H. & Greenlee, R. (1995) Amphetamine paired with physical therapy accelerates motor recovery after stroke. Further evidence. *Stroke*, **26,** 2254-2259.

Wallace, T. L., Stellitano, K. E., Neve, R. L. & Duman, R. S. (2004) Effects of cyclic adenosine monophosphate response element binding protein overexpression in the basolateral amygdala on behavioral models of depression and anxiety. *Biol.Psychiatry*, **56,** 151-160.

Warden, D. L., Gordon, B., McAllister, T. W., Silver, J. M., Barth, J. T., Bruns, J., Drake, A., Gentry, T., Jagoda, A., Katz, D. I., Kraus, J., Labbate, L. A., Ryan, L. M., Sparling, M. B., Walters, B., Whyte, J., Zapata, A. & Zitnay, G. (2006) Guidelines for the pharmacologic treatment of neurobehavioral sequelae of traumatic brain injury. *J.Neurotrauma*, **23,** 1468-1501.

Wehman, P., Sherron, P., Kregel, J., Kreutzer, J., Tran, S., & Cifu, D. (1993). Return to work for persons following severe traumatic brain injury. Supported employment outcomes after five years. *American Journal of Physical Medicine and Rehabilitation, 72*, 355-363.

Werner, C. & Engelhard, K. (2007) Pathophysiology of traumatic brain injury. *Br.J.Anaesth.*, **99,** 4-9.

Whiting, M.D., & Hamm, R.J. (2004). Treating chronic cognitive impairment after traumatic brain injury: A review of post-traumatic neurotransmitter-based interventions. *Crit Rev Phys Rehabil Med, 16*, 273-90.

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. *Am.J.Phys.Med.Rehabil.*, **76,** 440-450.

Whyte, J., Schuster, K., Polansky, M., Adams, J. & Coslett, H. B. (2000) Frequency and duration of inattentive behavior after traumatic brain injury: effects of distraction, task, and practice. *J.Int.Neuropsychol.Soc.*, **6,** 1-11.

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

Wiedenmann, B. & Franke, W. W. (1985) Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38,000 characteristic of presynaptic vesicles. *Cell*, **41,** 1017-1028.

Willis, S. A. & Nisen, P. D. (1995) Inhibition of lipopolysaccharide-induced IL-1 beta transcription by cyclic adenosine monophosphate in human astrocytic cells. *J.Immunol.*, **154,** 1399-1406.

Williams, S., Raghupathi, R., MacKinnon, M. A., McIntosh, T. K., Saatman, K. E. & Graham, D. I. (2001) In situ DNA fragmentation occurs in white matter up to 12 months after head injury in man. *Acta Neuropathol.*, **102,** 581-590.

Wilson, M. S., Gibson, C. J. & Hamm, R. J. (2003) Haloperidol, but not olanzapine, impairs cognitive performance after traumatic brain injury in rats. *Am.J.Phys.Med.Rehabil.*, **82,** 871-879.

Winzer-Serhan, U. H. & Leslie, F. M. (1997) Alpha2B adrenoceptor mRNA expression during rat brain development. *Brain Res.Dev.Brain Res.*, **100,** 90-100.

Witter, M., Amaral, D.G. (2004) Hippocampal Formation. In: The rat Nervous System, 3rd edition, pp637-703, Academic Press, San Diego.

Wong, E. H., Sonders, M. S., Amara, S. G., Tinholt, P. M., Piercey, M. F., Hoffmann, W. P., Hyslop, D. K., Franklin, S., Porsolt, R. D., Bonsignori, A., Carfagna, N. & McArthur, R. A. (2000) Reboxetine: a pharmacologically potent, selective, and specific norepinephrine reuptake inhibitor. *Biol.Psychiatry*, **47,** 818-829.

Woodward, D. J., Moises, H. C., Waterhouse, B. D., Yeh, H. H. & Cheun, J. E. (1991) Modulatory actions of norepinephrine on neural circuits. *Adv.Exp.Med.Biol.*, **287,** 193-208.

Wu, H., Lu, D., Jiang, H., Xiong, Y., Qu, C., Li, B., Mahmood, A., Zhou, D. & Chopp, M. (2008) Simvastatin-mediated upregulation of VEGF and BDNF, activation of the PI3K/Akt pathway, and increase of neurogenesis are associated with therapeutic improvement after traumatic brain injury. *J.Neurotrauma*, **25,** 130-139.

Wu, H. M., Huang, S. C., Hattori, N., Glenn, T. C., Vespa, P. M., Yu, C. L., Hovda, D. A., Phelps, M. E. & Bergsneider, M. (2004) Selective metabolic reduction in gray matter acutely following human traumatic brain injury. *J.Neurotrauma*, **21,** 149-161

Xiong Y, Gu Q, Peterson PL, Muizelaar JP, et al. (1997). Mitochondrial dysfunction and calcium perturbation induced by traumatic brain injury. *J Neurotrauma*;14:23–34.

Xu, F., Gainetdinov, R. R., Wetsel, W. C., Jones, S. R., Bohn, L. M., Miller, G. W., Wang, Y. M. & Caron, M. G. (2000) Mice lacking the norepinephrine transporter are supersensitive to psychostimulants. *Nat.Neurosci.*, **3,** 465-471.

Yan, H. Q., Kline, A. E., Ma, X., Hooghe-Peters, E. L., Marion, D. W. & Dixon, C. E. (2001) Tyrosine hydroxylase, but not dopamine beta-hydroxylase, is increased in rat frontal cortex after traumatic brain injury. *Neuroreport*, **12,** 2323-2327.

Yao, Y., Yin, D., Jas, G. S., Kuczer, K., Williams, T. D., Schoneich, C. & Squier, T. C. (1996) Oxidative modification of a carboxyl-terminal vicinal methionine in calmodulin by hydrogen peroxide inhibits calmodulin-dependent activation of the plasma membrane Ca-ATPase. *Biochemistry*, **35,** 2767-2787.

Yoshimura, S., Teramoto, T., Whalen, M. J., Irizarry, M. C., Takagi, Y., Qiu, J., Harada, J., Waeber, C., Breakefield, X. O. & Moskowitz, M. A. (2003) FGF-2 regulates neurogenesis and degeneration in the dentate gyrus after traumatic brain injury in mice. *J.Clin.Invest*, **112,** 1202-1210.

Yoshino, A., Hovda, D. A., Kawamata, T., Katayama, Y. & Becker, D. P. (1991) Dynamic changes in local cerebral glucose utilization following cerebral conclusion in rats: evidence of a hyper- and subsequent hypometabolic state. *Brain Res.*, **561,** 106-119.

Zafonte, R. D., Watanabe, T. & Mann, N. R. (1998) Amantadine: a potential treatment for the minimally conscious state. *Brain Inj.*, **12,** 617-621.

Zafra, F., Lindholm, D., Castren, E., Hartikka, J. & Thoenen, H. (1992) Regulation of brain-derived neurotrophic factor and nerve growth factor mRNA in primary cultures of hippocampal neurons and astrocytes. *J.Neurosci.*, **12,** 4793-4799.

Zhou, J., Klass, T., Zhang, A., Johnson, K. M., Wang, C. Z., Ye, Y. & Kozikowski, A. P. (2003) Synthesis and pharmacological evaluation of (Z)-9-(heteroarylmethylene)-7-azatricyclo[4.3.1.0(3,7)]decanes: thiophene analogues as potent norepinephrine transporter inhibitors. *Bioorg.Med.Chem.Lett.*, **13,** 3565-3569.

Zhou, J. (2004) Norepinephrine transporter inhibitors and their therapeutic potential. *Drugs Future.*, **29,** 1235-1244.

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

Zhuang, J., Shackford, S. R., Schmoker, J. D. & Anderson, M. L. (1993) The association of leukocytes with secondary brain injury. *J.Trauma*, **35,** 415-422.

Zsiros, V. & Maccaferri, G. (2008) Noradrenergic modulation of electrical coupling in GABAergic networks of the hippocampus. *J.Neurosci.*, **28,** 1804-1815.

VITA

Wendy Murdock Reid was born in Roanoke, Virginia on June 11, 1978. She later attended North Carolina State University in Raleigh, N.C. After switching majors several times, she finally graduated with a B.S in Biology. Following graduation, she worked in RTP, N.C. at a small start-up biotechnology company called Paradigm Genetics. After working for several years, she returned to academia to pursue an interest in Medicine. Shortly after, she discovered Cognitive Neuroscience and TBI and became dedicated to research in these areas. Other non-scientific interests include: Art history, Anthropology (Physical and Cultural), Evolution and Paleontology, History of Civilization, and Political Activism.

Education

Bachelor of Science Degree in Biological Sciences **Dec 1999**
North Carolina State University, Raleigh, North Carolina

Certificate in Basic Medical Sciences **May 2003**
Medical College of Virginia, Richmond, VA

Doctorate of Philosophy in Neurobiology (Neuroscience) **May 2008**
Medical College of Virginia, Richmond, VA

Career History

Graduate Student/ Research and Teaching Assistant/ 2004-2008
Department of Anatomy and Neurobiology-VCU

Clinical Scientist 2003- 2004
Pharmaceutical Product Development, Inc.

Paradigm Genetics, RTP, North Carolina (Sold to Monsanto) 2000-2002
Assistant Scientist

Volunteer

Friends of the James River, Tree of Life, Habitat for Humanity