The Dissertation Committee for Uylissa Ann Rodriguez Certifies that this is the approved version of the following dissertation:

EFFECTS OF MILD BLAST TRAUMATIC BRAIN INJURY ON CEREBRAL VASCULAR FUNCTION, HISTOPATHOLOGICAL AND BEHAVIORAL OUTCOMES IN RATS

Committee:

Douglas S. DeWitt, Ph.D., Supervisor

Claire E. Hulsebosch, Ph.D., Chair

J. Regino Perez-Polo, Ph.D.

Darrell H. Carney, Ph.D.

Joseph B. Long, Ph.D.

Dean, Graduate School of Biomedical Sciences

EFFECTS OF MILD BLAST TRAUMATIC BRAIN INJURY ON CEREBRAL VASCULAR FUNCTION, HISTOPATHOLOGICAL AND BEHAVIORAL OUTCOMES IN RATS

by

Uylissa Ann Rodriguez, M.S., B.S.

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas Medical Branch

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas Medical Branch

March, 2017

Dedication

Dedicated to my wonderful and devoted parents, Armando and Lydia Rodriguez, whose unwavering love, steadfast support and unrelenting faith in me provided the cornerstones that anchored this entire journey of my pursuit to attain as much of a formal education as was enduringly possible. Thank you Mom and Dad, for each and every sacrifice you made for me, for inspiring me to "want a better life", for dedicating yourselves to our family and for reassuring me that it was okay to spread my wings and fly; you undisputably provided every opportunity that allowed me to pursue my dreams and make them come true. I love you both.

Acknowledgements

I'd like to first and foremost express my sincerest gratitude and thanks to my mentor and PI, Dr. Douglas S. DeWitt, for taking me in as his graduate student despite his immensely hectic schedule, his infinite amounts of patience with me and for allowing me the chance and opportunity to join and work in his lab. Regardless of the multitude of other responsibilities and priorities that required his attention, he always made sure to make time for me whether it was responding to every email I sent him at all hours with questions and concerns about the latest set of experiments, an abstract, poster, presentation, manuscript and everything else in between to keeping our weekly meetings when I knew he was already stretched thin that week to discussing how my Cowboys and his Steelers were doing during the season. I'd also like to thank our lab supervisor and general manager, Margaret O. Parsley, for patiently and meticulously training, showing and walking me through every experimental procedure, technique and protocol that required mastery in order for the completion of all my study experiments to have even been made possible. I'd also like to especially thank Dr. Donald J. Deyo for being our unofficial ABS device doctor, technician and mechanic who always made sure the device was functioning properly and troubleshooting any issues that came up, ensuring we always had a working machine ready for blasting. I thank Dr. Claire Hulsebosch for accepting the responsibility of chairperson of my dissertation committee and the rest of my committee members, Drs. J. Regino Perez-Polo, Darrell H. Carney, and Joseph B. Long for their constant encouragement, invaluable guidance and advice and for ultimately believing in me. Special thanks to Yaping Zeng who was vital with the MCA experiments, Dr. Maria Adelaide Micci, Jutatip 'June' Guptarak and Debbie Kennedy who advised me with the FJC experiments, Kathia M. Johnson who showed me how to do the behavior experiments and Ian J. Bolding for being our IT specialist that made sure my lab computers, programs and applications always ran

smoothly. My sincerest thanks to Dr. Bridget E. Hawkins for becoming my secondary editor on everything I wrote and for patiently walking me through those exciting manuscript submissions even though she had more than enough duties necessitating her time. Many, many thanks to Ms. Aurora Galvan of the Cell Biology Program department for tirelessly keeping me updated on every deadline and due date and for always fixing everything I often contacted her in a panic about and to Dr. Pomila Singh for being available to me anytime I contacted her since my very first day of grad school. I would also like to convey my heartfelt appreciation and gratitude to Dr. Scott Gunn, my senior mentor during all my years as an undergrad and Master's student, who was my sounding board on every little thing that entered my thoughts and who always kept his door open for me, whether it was in person, through email, or text message; Mr. Jose F. Alvarado, my favorite undergrad professor who had a gift for making Algebra, Trigonometry, and Calculus fun; Dr. Anita Davelos-Baines, my PI and mentor during my Master's research and thesis, whose nurturing wisdom never failed me and whose complete trust and faith in me basically allowed my running of our lab as I saw fit; Drs. Kristine Lowe, Jonathan Lieman and Chris Little for serving on my thesis committee even though they each had more than enough students to keep them occupied; and Dr. Mohammed Farooqui for always granting me permission to enroll in more classes than the 17-hour maximum allowed. Each of these unforgettable individuals was instrumental in my success during my undergrad and Master's years at the University of Texas-Pan American. Their support, inspiration and the trajectory they set me on for graduate school unquestionably influenced where I am today. My indebtedness for each of them extends far beyond words. I would like to acknowledge the amazing members of the DeWitt lab as a whole for warmly welcoming me into the team when I first arrived, for providing an educational environment full of constant learning and for ultimately being a source of entertainment that made coming to work every day a real pleasure. Last but certainly not least in the ultimate scheme of things, I want to thank my family: Mom, Dad, Bee and A.J., thank you all for your unconditional love,

continuous motivation, unrelenting encouragement and limitless amounts of support throughout this entire voyage as well as throughout my life. I could not have possibly seen it all through without each of you in my corner. I love you all more than I will ever be able to put into words. And to Sean, thank you for coming into my life when you did, for your unlimited patience with me during the chaos that was my last 2 years of graduate school, for making me come alive again, and most of all, for completely accepting me and loving me for who I am. I love you now and forever.

EFFECTS OF MILD BLAST TRAUMATIC BRAIN INJURY ON CEREBRAL VASCULAR FUNCTION, HISTOPATHOLOGICAL AND BEHAVIORAL OUTCOMES IN RATS

Publication No._____

Uylissa Ann Rodriguez, Ph.D.

The University of Texas Medical Branch, 2017

Supervisor: Douglas S. DeWitt

Impact traumatic brain injury (i.e. non-blast TBI) is often associated with reduced cerebral perfusion and impaired cerebrovascular function due, in part, to the generation of reactive oxygen (ROS) and reactive nitrogen species (RNS) (e.g. peroxynitrite, $ONOO^-$). Although blast TBI (bTBI) also reduces cerebral perfusion, less is known about other cerebral vascular effects of bTBI. To determine the effects of mild bTBI on cerebral vascular function with and without the administration of the $ONOO^-$ scavenger penicillamine methyl ester (PenME), we subjected isoflurane-anesthetized rats to sham bTBI, bTBI alone or bTBI + PenME in five experiments using a compressed air shock tube. Dilator responses to reduced intravascular pressure were assessed in isolated middle cerebral arterial (MCA) segments collected 30 or 60 minutes post-blast in one study. Mean arterial blood pressure (MAP), relative cerebral perfusion (laser Doppler flowmetry, LDF) and cerebral vascular resistance (CVR) were monitored for two hours post-blast in a second study. To assess the effects of bTBI on neuronal injury, numbers of FluoroJade-C (FJC) positive cells were

counted in brain sections obtained 24 or 48 hours post-bTBI in a third study. Vestibulomotor (beam balance/beam walk) and working memory (Morris water maze, MWM) function were evaluated up to two weeks post-bTBI in the fourth study. Lastly, MAP, relative cerebral perfusion and CVR were monitored in rats treated with PenME or a vehicle five minutes after mild bTBI. Shock wave overpressures of 20.9 psi ± 1.14 (138 kPa ± 7.9) produced mild bTBI, based on righting reflex (RR) suppression for 5.2 minutes (Sham bTBI = 4.3 minutes). Mild bTBI resulted in reductions in cerebral perfusion and MCA dilator responses, increases in CVR and numbers of FJC-positive cells and significantly impaired working memory. Treatment with PenME resulted in significant reductions in CVR and a trend towards increased cerebral perfusion. These results that bTBI increased CVR, reduced cerebral perfusion and impaired cerebral dilator responses to reduced intravascular pressure indicate that mild bTBI is associated with significant cerebral arterial dysfunction. Furthermore, the significant reduction of CVR after treatment with PenME indicated that blast-induced cerebral vascular dysfunction may be due, in part, to the generation of $ONOO^{-}$.

TABLE OF CONTENTS

List of Figures

List of Abbreviations

3-NT	3-nitrotyrosine
4-HNE	4-hydroxynonenal
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
ABS	Advanced Blast Simulator
ANOVA	Analysis of variance
BA	Basilar artery
β-APP	Beta-amyloid precursor protein
BBB	Blood brain barrier
bTBI	blast/blast-induced TBI
CA1	*Cornu ammonis* hippocampal region 1
CA3	*Cornu ammonis* hippocampal region 3
$CaCl_2$	Calcium chloride
CBF	Cerebral blood flow
CBV	Cerebral blood volume
CCA	Common carotid artery/ies
CCI	Controlled cortical impact
$CMRO_2$	Cerebral oxygen consumption
CNS	Central nervous system
CO_2	Carbon dioxide
CVR	Cerebral vascular resistance
DAI	Diffuse axonal injury

dcBI	Direct cranial blast injury
ECA	External carotid artery/ies
EDRF	Endothelium-derived relaxing factor
EEG	Electroencephalogram
eNOS	Endothelial nitric oxide synthase
FITC	Fluorescein isothiocyanate
FJ	FluoroJade
FJC	FluoroJade-C
FPI	Fluid percussion injury
FRSS	Forward Resuscitation Surgical System
GFAP	Glial fibrillary acidic protein
GSHpx	Glutathione peroxidase
H&E	Hematoxylin and eosin
H_2	Hydrogen
H_2O	Water
H_2O_2	Hydrogen peroxide
HH	Hemorrhagic hypotension
IA	Impact acceleration
IACUC	Institutional Animal Care and Use Committee
ICA	Internal carotid artery/ies
IED	Improvised explosive device
IFN-ɣ	Interferon-gamma
IgG	Immunoglobulin G

iNOS	Inducible nitric oxide synthase
KCl	Potassium chloride
kPa	Kilopascal
LDF	Laser Doppler flowmetry
LPS	Lipopolysaccharides
MAC	Minimum alveolar concentration
MAP	Mean arterial pressure
MCA	Middle cerebral artery/ies
$MgSO_4$	Magnesium sulfate
MilliQ	Millipore quality
mM	millimolar
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MWM	Morris water maze
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	Sodium bicarbonate
NeuN	Neuronal nuclear antigen
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase/s
O_2	Oxygen
$\bullet O_2^-$	Superoxide anion

OEF	Operation Enduring Freedom
OIF	Operation Iraqi Freedom
OND	Operation New Dawn
$ONOO^-$	Peroxynitrite
$PaCO_2$	Partial pressure of carbon dioxide
PaO_2	Partial pressure of oxygen
PCA	Posterior cerebral artery/ies
PE	Polyethylene
Pen	Penicillamine
PenME	Penicillamine methyl ester
PSI	Pounds per square inch
PSS	Physiologic salt solution
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RR	Righting reflex
SAH	Subarachnoid hemorrhage
SEM	Standard error of mean
SOD	Superoxide dismutase
TBI	Traumatic brain injury/ies
VSMC	Vascular smooth muscle cells

INTRODUCTION

Chapter 1: Background and Significance

Traumatic brain injuries (TBI) caused by blast exposures have been called the "signature wound" of the latest conflicts in the Middle East (DePalma et al., 2005; Okie, 2005; Warden, 2006; Hanley, 2007; Shanker, 2007; Long et al., 2009; Snell et al., 2010; Garman et al., 2011; Nakagawa et al., 2011). TBI's are one of the most common types of injuries among combatants in Operations Iraqi Freedom (OIF), Enduring Freedom (OEF) and New Dawn (OND) (McCrea, 2007; Elder and Cristian, 2009; Cernak and Noble-Haeusslein, 2010; MacGregor et al., 2011; Eskridge et al., 2012) due, in part, to the high incidence of blast-induced TBI (bTBI). Although estimates of the prevalence of bTBI vary widely, some are as high as 19% - 23% (Tanielian and Jaycox, 2008; Terrio et al., 2009; Polusny et al., 2011). As of January 2015, the Department of Defense reported that over 73% of all U.S. military casualties were caused by explosive weaponry such as improvised explosive devices (IED) or mortars (Kocsis and Tessler, 2009; Risling et al., 2011) making blast the most widespread cause of combat-related mortality and morbidity (Hanley, 2007; Shanker, 2007; Long et al., 2009).

CLASSIFICATION OF bTBI

There are currently four accepted classifications of injury due to blast exposure. Primary blast injury results from exposure to blast wave over/underpressures which consist of a front of high positive pressure followed by a negative pressure wave of lower magnitude but longer duration (Courtney and Courtney, 2009; Nakagawa et al., 2011). Secondary injury is produced by small fragments traveling at high speed (Clemedson,

1956; Born, 2005; Nakagawa et al., 2011) and impacting the body. Tertiary blast injury is blunt impact injury that occurs when the body is thrown against a hard, stationary object or when a large fragment impacts the body (Born, 2005; Courtney and Courtney, 2009; Singh et al., 2016). Quaternary blast injuries include any other injuries caused by the explosions like burns and toxic inhalations (Born, 2005; Nakagawa et al., 2011; Singh et al., 2016). The central nervous system (CNS) is susceptible to damage from all four blast injury mechanisms (Koliatsos et al., 2011; Nakagawa et al., 2011) however the severity and characteristics of the resulting injuries are determined by proximity to the blast center and the size, mass and velocity of objects that strike the person (Cernak, 2015). Of these four classifications, primary blast injury is the least understood (Nakagawa et al., 2011). Blast and non-blast induced TBI typically result in damage to both white and gray matter with secondary cascades of cellular, molecular and biochemical abnormalities, all of which may contribute to neuronal and/or glial injury (Owen-Smith, 1981; Werner and Engelhard, 2007; Cernak and Noble-Haeusslein, 2010).

PRIMARY BLAST TBI

A blast wave in an open space consists of a near-instantaneous pressure rise followed by an exponential decay of the overpressure (Friedlander, 1946). The high rate of energy release is responsible for the injury and property destruction that follows detonation; the detonation properties of the explosive define the properties of the blast wave (Cullis, 2001; Ngo et al., 2007). Though body armor protects against some projectiles, it does not fully eliminate exposure to blast positive and negative pressures.

Several factors affect the degree of primary blast injury. The principal components that contribute to blast injury are impulse, proximity to the blast center, the peak

overpressure and duration of the positive phase, the overpressure waveform, orientation of the body to the blast wave, and environmental factors which can influence primary blast effects (Clemedson, 1956; Elsayed, 1997; Cullis, 2001; DePalma et al., 2005; Ngo et al., 2007; Leung et al., 2008; Champion et al., 2009; Chavko et al., 2011; Cernak, 2015). Blasts in enclosed spaces or close proximity to walls can be intensified by shockwave reflection, causing greater injury than open space exposure (Cullis, 2001; Ngo et al., 2007; Cernak, 2015).

Incident (static) pressure is measured when a pressure gauge is parallel to the blast wave direction of travel. Dynamic (reflected) pressure is recorded when the pressure gauge is perpendicular to the direction of blast wave front (Bass et al., 2012).

MECHANISM OF INJURY

The manner in which blast energy is transmitted to the brain remains controversial. When a blast wave strikes the body, a portion is reflected or deflected while the majority of the energy is absorbed and transmitted throughout the body. The result is high frequency stress waves and low-frequency shear-waves (Chavko et al., 2011; Leonardi et al., 2011, Leonardi et al., 2012). These stress and shear waves are thought to contribute to the pathogenesis of primary blast injuries. Putative mechanisms include inertial effects, implosion, pressure differentials and spallation (Cernak and Noble-Haeusslein, 2010; Alley et al., 2011). One of the hypothesized mechanisms of primary blast injury is head rotation resulting from the combination of rotational and translational accelerations ensuing from the shock wave interaction with the head (Bolander et al., 2011; Courtney and Courtney, 2011). A second is the direct transmission of the blast energy by explicit compression of the skull material, or transosteal wave propagation (Taylor and Ford, 2009;

Chafi et al., 2010; Bolander et al., 2011). A third proposed mechanism is the transfer of kinetic blast energy to the cerebral vasculature and brain via compression of the great vessels of the thorax (Rossle, 1950; Courtney and Courtney, 2009; Courtney and Courtney, 2011; Nakagawa et al., 2011). A fourth mechanism is air emboli (Wolf et al., 2009) potentially interrupting cerebral perfusion leading to tissue infarction. Transmission of the blast wave through the orbital sockets and nasal sinuses producing damage to the orbitofrontal cortex has also been suggested (Elder et al., 2010).

PATHOPHYSIOLOGICAL EFFECTS OF SHOCK WAVE EXPOSURE

The pathobiology of primary bTBI consists of a complex set of systemic, cerebral and cerebral vascular events that begin at blast exposure and likely continue for minutes to days or months afterward (Cernak and Noble-Haeusslein, 2010). The rate of incidence, strength, and extent of the characteristics of explosive blast injury to the brain can be considered unique (Bauman et al., 2009). After blast exposure, survivors may exhibit injuries such as concussion, bradycardia, hemorrhage, edema, hypotension and loss of consciousness and/or confusion, disorientation, dizziness, nausea/vomiting and/or headaches and tinnitus (DePalma et al., 2005; Okie, 2005; Anderson, 2008; Martin et al., 2008). In contrast to concussive and/or penetrating wounds where the nature of the injury is focal, blast injuries often are multifocal. Primary blast injury alone is characterized by injuries to numerous internal organs without any signs of external penetrating injury (Hamit, 1973; Phillips, 1986; Mellor, 1988). Tissues and organs most susceptible to primary blast effects are those containing fluid-air interfaces: the lungs (blast lung), tympanic membranes (Avidan et al., 2005), gastrointestinal tract and abdominal viscera (Cernak et al., 2001b; Bass et al., 2008; Cernak and Noble-Haeusslein, 2010). All are

especially vulnerable to the compacting and shearing effects of the swift pressure change (Cernak et al., 2001b; Cernak and Noble-Haeusslein, 2010; Cernak, 2015). Fortunately, pulmonary and gastrointestinal injury is less common in recent warfare, likely due to the use and efficacy of personal protective equipment (Bauman et al., 2009).

Experimental blast studies in rodents have demonstrated several outcomes resulting from various combinations of torso/body shielding and/or cranial protection. Whole body and head-only repeated primary blast exposures in mice led to compromised cell membrane integrity and a significant initial decrease followed by an increase in the levels of glial fibrillary acidic protein (GFAP) and total *Tau* protein biomarkers of TBI in the brain and plasma (Arun et al., 2013) as well as brain DNA fragmentation (Wang et al., 2014). Protein analysis of sera and brain regions in whole body, single blast overpressure-exposed mice showed significantly elevated inflammatory, vascular, neuronal and glial biomarker levels (Kovesdi et al., 2012). Long et al. (2009) observed that protecting the torso virtually eliminated axonopathy and fiber degeneration. Koliatsos et al. (2011) observed that a plexiglass covering around the torso of blast-injured mice abolished axonal nerve cell damage compared to non-shielded mice who suffered up to 80% axonal damage. Conversely, a moderately severe blast injury model that was associated with survival in the majority of rats with body shielding was characterized primarily by diffuse axonal injury (DAI), degeneration of other neuronal processes and increased blood brain barrier (BBB) permeability (Garman et al., 2011). Bauman et al. (2009) studied a swine model in which all animals wore a lead-and-foam-lined vest that outfitted the chest and upper abdomen of the animal. Despite the torso protection, vasospasm, disruptions of electroencephalogram (EEG) waveforms, white matter fiber degeneration and astrocytosis, among other

perturbations, were observed after blast. Cernak et al. (2001b) utilized a whole-body blast injury rat model where the head was protected and observed myelin debris in the hippocampus, neuronal swelling, microglial reactivity, oxidative stress development and cognitive impairment. Therefore, while there is evidence that torso protection reduces some of the effects of bTBI, there is evidence to the contrary. To avoid controversial effects of torso protection in whole body blast exposure, our studies utilized an experimental protocol in which only the head is exposed to the shock wave.

CEREBRAL VASCULAR EFFECTS OF bTBI

Recent evidence of level-dependent reductions in relative blood flow in the cortex and hippocampus of rats exposed to several shock wave intensities in an air-driven shock tube indicates that bTBI is associated with some degree of cerebral vascular injury and alterations in cerebral perfusion (Bir et al., 2012). However, the degree to which blast-related cerebral vascular dysfunction contributes to the pathophysiology of bTBI is unknown.

Normal cerebral vascular responses

The cerebral circulation responds to variations in systemic arterial pressure in order to maintain a constant supply of oxygen and nutrients provided to the metabolically active brain (for review, see Mchedlishvili, 1980; Kontos, 1981; Golding et al., 1999a; DeWitt & Prough 2003). Autoregulation is a particular type of homeostasis (Paulson, 1990; Lang et al., 2001; Soehle et al., 2004) in which "*an organ maintains a constant blood flow despite changes in blood (perfusion) pressure or other physiologic or pathologic stimuli"* (Roy and Sherrington, 1890). Cerebral arteries constrict or relax in response to alterations in blood pressure, the partial pressures of carbon dioxide ($PaCO_2$) and oxygen (PaO_2), nitric

oxide (NO), blood viscosity, etc. (Wei et al., 1980b; DeWitt et al., 1986; Mathew et al., 1999; DeWitt and Prough, 2003). Arterial myogenic response refers to an inherent ability of contractile tissues (such as vascular smooth muscle cells, VSMC's) to respond to stretch and/or changes in wall tension (Johnson and Henrich, 1975; Johnson, 1977; Atkinson et al., 1985; Johnson, 1989; Allen et al., 1997; Owens et al., 2004; Ahn et al., 2007). When arteries are stretched (e.g. during intravascular pressure increases), VSMC's constrict (Johnson, 1977; Atkinson et al., 1985; Johnson, 1989; Owens et al., 2004). The coupled organization of arteries modulates the response of neighboring arterial networks so that arteries and arterioles closer to a pressure increase will reduce the increase in pressure transferred to the distal vessels (Johnson and Henrich, 1975; Johnson, 1977; Johnson, 1989). This property is responsible, in part, for the maintenance of constant cerebral perfusion during systemic arterial blood pressure fluctuations (Johnson and Henrich, 1975; Johnson, 1977; Johnson, 1989; Ping and Johnson, 1994; Owens et al., 2004). Microcirculatory studies revealed that the myogenic response tends to maintain a constant capillary pressure (Intaglietta and Zweifach, 1974; Zweifach, 1974; Chien and Lipowsky, 1982). Other aspects of cerebral vascular reactivity, in addition to pressure autoregulation, such as the close association between functional activity in the brain and regional cerebral blood flow (CBF) (Roy and Sherrington, 1890; Raichle et al.,1976) and their dependence on the production of vasodilator metabolites by neurons and glia (Kontos, 1981; Edvinson et al., 1993; Hawkins and Davis, 2005; Koehler et al., 2009; Attwell et al., 2010; Itoh et al., 2012), are beyond the scope of our research (for review, see Lenzi et al., 1999; Murkin, 2007; Østergaard et al., 2014).

Cerebral vascular dysfunction after impact (non-blast) TBI

Failure of CBF autoregulation is a common observation in the different types of cerebral injury and may contribute to cerebral injury in the case of secondary arterial hypotension (DeWitt and Prough, 2003). Studies assessing cerebral autoregulation after impact (non-blast) TBI resulted in a pathology associated with cerebral ischemia and secondary insults (Miller and Becker, 1982; Steiner et al., 2003). In the laboratory setting, hypotension after TBI (Ishige et al., 1988; Kroppenstedt et al., 1999; Lammie et al., 1999; Matsushita et al., 2001) was associated with reduced CBF (Giri et al., 2000; Matsushita et al., 2001; Elder et al., 2015) and brain tissue oxygen levels (Giri et al., 2000), impaired ion homeostasis (Stiefel et al., 2005), compromised metabolism (Ishige et al., 1988), lessened neuroprotective gene expression (Hellmich et al., 2005b) and increased neuronal injury/loss (Jenkins et al., 1989; Cherian et al., 1996; Kroppenstedt et al., 1999; Lammie et al., 1999; Matsushita et al., 2001). Clinical evidence indicates that hypotension (systolic blood pressure < 90 mmHg) or hypoxemia ($PaO_2 \leq 60$ mmHg) or a combination of the two were associated with doubled mortality and reduced recovery rates in TBI patients (Miller et al., 1992; Chesnut et al., 1993b) and that autoregulation was impaired to some degree during the initial days after TBI (Overgaard and Tweed, 1974; Bouma and Muizelaar, 1990). Bouma and Muizelaar (1990) tested autoregulation by both increasing and decreasing blood pressure and observed that in 41% of patients studied, autoregulation was impaired in response to increased blood pressure while in 78% of patients studied decreased blood pressure resulted in impaired autoregulation. These results suggest that after TBI, increases in arterial blood pressure lead to less sensitive vascular constrictor responses compared to the vessel's extra sensitive dilatory responses to lowered arterial

blood pressure (DeWitt and Prough, 2009). In addition to the varied elements of cerebral vascular dysfunction investigated above, the inflammatory response is known to be invoked in models of moderate-to-severe TBI. However, Perez-Polo et al. (2013) observed acute inflammatory biomarkers, macrophage and microglial (astrocyte) activation and BBB dysfunction at 3 and 6 hours and at 18 days post mild-TBI, indicating that even mild injuries are capable of activating a detrimental inflammatory-surge response that affects cellular and glial activation as well as BBB integrity, among others.

Cerebral vascular dysfunction after bTBI

Both single and repeated blast overpressures resulted in impaired cerebral vascular endothelium-dependent dilation (Toklu et al., 2015), a vascular pathology associated with extracellular matrix alterations, an increase in inflammatory cytokines for sustained periods post-blast and cerebral arterial vasospasm (Armonda et al., 2006; Bauman et al., 2009).

Furthermore, blast exposure in animals caused BBB breakdown (Yeoh et al., 2013; Wang and Cheng, 2016) leading to increased vascular permeability (Readnower et al., 2010; Garman et al., 2011) and decreased CBF (Elder et al., 2015). The intensity of blast overpressures also was positively correlated with increased vascular leakage due to disturbance in BBB integrity (Elder et al., 2015), an increase in brain reactive oxygen species (ROS) levels, astrocytosis and cell apoptosis at several time points after blast exposure (Kabu et al., 2015). Gama Sosa et al. (2014) reported a selective vascular pathology that was present 24 hours after injury and persisted for months post-blast in brain regions with a seemingly undamaged neuropil while Ahmed et al. (2013) detected vascular abnormalities. Blast injury can also result in mRNA expression pattern changes, mild

venous hemorrhage in the subarachnoid space and thrombosis (accompanied by inflammatory markers) (Balaban et al., 2016). Sosa et al. (20013) detected shear-injury type focal lesions and microhemorrhages observed 24 hours after blast injury while cerebral infarction due to gas emboli from blast lung (Elsayed, 1997; Cernak et al., 2001b; Wolf et al., 2009) has also been observed.

CELLULAR INJURY

Direct insult to the brain after impact (non-blast) TBI induces cell degeneration and/or death (for review, see Kovesdi et al., 2007; Werner and Engelhard, 2007; Loane and Faden, 2010; Giunta et al., 2012; Lozano et al., 2015). In mice and rats, the hippocampus has been widely studied as part of the brain responsible for behavior, spatial memory, and navigation. Hippocampal neurons, specifically those found in the *Cornu ammonis* region 3 (CA3), have typically demonstrated a greater vulnerability to injury (Clark et al., 2005; Leung et al., 2008; Moser and Kropf, 2008) in rodent models of experimental TBI while pyramidal neurons in the CA1 sector of the hippocampus have generally been vulnerable to hypoxemia or ischemia (Clark et al., 2005; Moser and Kropf, 2008). Post-mortem examinations of TBI patients revealed neuronal injury/death in the CA1 and CA3 regions (Leung et al., 2008).

MEMORY AND COGNITIVE FUNCTION

Cognitive dysfunction is one of the most prevalent sequelae of TBI (Gronwall and Wrightson, 1974; Rimel et al., 1981; Levin et al., 1982; Rimel et al., 1982; Stuss et al., 1985; Binder, 1986; Hamm et al., 1996), with disruption of memory (TBI patients had trouble recalling lists of numbers) (Brooks, 1972), retrograde and anterograde amnesias, attention deficits (difficulty executing matching tasks and concentrating) (McLean et al.,

1983), problems with speech, the appearance of seizures and loss of executive functions appearing as the most common deficits after closed head injury (bTBI) at all severity levels (Levin et al., 1988; Capruso and Levin, 1992; Arciniegas et al., 2002). Post concussive symptoms can occur immediately post-blast or months to years later (DePalma et al., 2005; Okie, 2005; Anderson, 2008; Martin et al., 2008). Disturbances in memory and attention can be principally problematic, as interruption of these cognitive functions may lead to or worsen additional disruptions in communication and other more intricate cognitive functions (Hamm et al., 1993; Hamm et al., 1996; Scheff et al., 1997; Cernak et al., 2001a; Wolf and Koch, 2016).

FREE RADICALS/PEROXYNITRITE

Increased production of ROS and reactive nitrogen species (RNS) is a feature of experimental, impact brain injury models such as fluid percussion injury (FPI) TBI (Kontos and Povlishock, 1986; Kontos and Wei, 1986; Povlischock and Kontos, 1992; Hall and Braughler, 1993; Globus et al., 1995; Hall, 1995a). Like impact TBI, bTBI resulted in the generation of ROS (Readnower et al., 2010; Abdul-Muneer et al., 2013; Heibert et al., 2015; Kabu et al., 2015) and RNS (Abdul-Muneer et al., 2013) that can individually or collectively produce oxidative/nitrosative stress that damages cells. Increased NO levels due to TBI may result in the production of elevated concentrations of peroxynitrite ($ONOO^-$), a harmful nitrogen/oxygen anion (Beckman et al., 1990). Although $ONOO^-$ is not a free radical, it is a potent oxidant. Because increased ROS/RNS are a consequence of blast injury (Elsayed, 1997; Elsayed et al., 1997; Cernak et al., 2001a; DeWitt and Prough, 2009), it is probable that blast-induced impairment of compensatory cerebral vascular responses is due, in part, to the actions of $ONOO^-$.

STUDY FOCUS

Although the effects of TBI on the morphopathology of the cerebral vasculature have been conducted in non-blast TBI models (fluid percussion, impact acceleration, controlled cortical impact injury, etc.), to date, most experimental studies on the effects of blast exposure have focused on the neuronal/glial components of the CNS, making the effects of primary blast TBI on cerebral vascular reactivity still basically unidentified. Since the consequences of bTBI on the cerebral circulation and on vascular reactivity have been far less well studied than concussive effects on the brain and only scattered information exists about the effects of blast on the cerebral vasculature, this research is focused on the effects of primary blast injury on cerebral perfusion and cerebral vascular reactivity.

I tested the hypothesis that bTBI, like non-blast TBI, results in cerebral vascular injury, impaired cerebral arterial compensatory responses and cerebral hypoperfusion. Specific Aim 1 was to measure cerebral dilatory responses to reduced intravascular pressure in harvested middle cerebral arterial (MCA) segments.

Specific Aim 2 was to measure relative cerebral perfusion *in vivo* (laser Doppler flowmetry, LDF) in rats subjected to mild bTBI using a compressed air driven shock tube.

Specific Aim 3 was to count the numbers of FluoroJade-C (FJC)-positive neurons throughout the brain to determine whether levels of bTBI that produced cerebral vascular injury were also associated with neuronal injury.

Specific Aim 4 was to explore the effects of bTBI on behavioral/cognitive function by assessing vestibulomotor and working memory function in rats after mild bTBI or Sham bTBI.

Specific Aim 5 was to test the hypothesis that blast-induced cerebral vascular dysfunction is mediated, in part, by $ONOO^-$ by assessing the effects of $ONOO^-$ scavenging after bTBI by measuring relative cerebral perfusion, mean arterial blood pressure (MAP) and cerebral vascular resistance (CVR) in rats treated with penicillamine methyl ester (PenME) after bTBI.

METHODS

Chapter 2: Materials and Methods

ADVANCED BLAST SIMULATOR

The shock wave from an idealized blast can be assumed to be spherically symmetric so that any solid angle cut from the flow field as subtended from the source will replicate the basic physics of spherical blast decay with distance (Stahl, 2003; Banchoff and Lovett, 2015). The distinctive blast-wave characteristics of an exponential-like decay profile, negative phase and secondary shock are direct artifacts of blast propagation in spherical geometry (Meserve, 1983; Stahl, 2003). A shock-tube having a constantly diverging cross-section from the driver chamber would best serve the purpose of blast simulator (Dressler, 1954; Martin, 1958). For simulation of shock wave exposures (<100 psi) (Courant and Friedrichs, 1948), a duct having a diverging area with rectangular cross-section (a 'pyramidal horn') will have straight walls well-suited for wall-mounting of diagnostics including optical windows. The Advanced Blast Simulator (ABS) was designed and constructed to have a diverging rectangular section with dimensions that accurately replicate the properties of a conical tube (Dowling, 1917) while allowing for the mounting of pressure sensors, optical windows and a removable tray that supports the experimental animal. Another unique aspect of the ABS is the reflected wave suppressor. Open- or closed-ended shock tubes produce reflected waves that travel back through the tube. These reflected waves are artifacts, that is, they are not present in an open-field blast. The reflected wave suppressor eliminates these negative waves and, therefore, produces a more accurate primary blast wave (Henshall, 1957; Martin, 1958; Duff and Blackwell, 1966;

Cullis, 2001; Segars and Carboni, 2008; Reneer et al., 2011; Stewart and Pecora, 2015).

bTBI was produced by an ABS shock tube designed by David Ritzel (Dyn-FX Consulting, Ltd., Ontario, Canada) and produced by Steven Parks (ORA, Inc., Fredericksburg, VA). The ABS device is 14-feet long, 90 square-inches wide (**Figure 1**) and generates Freidlander-like over/underpressures (Friedlander, 1946) (**Figure 2**) using a compressed air driver. The ABS consists of a 10-inch driver chamber, a 3.4-foot expansion section with diverging side walls, a 5-foot specimen section and a cylindrical reflected wave suppressor. The driver chamber is separated from the expansion section by Mylar membranes that rupture without loose debris. The specimen chamber has a removable specimen tray for the experimental animal. Five piezoelectric pressure probe transducers (Piezotronics Inc., Buffalo, NY) located flush along the inside roof of the ABS (**Figure 3**) were used to measure blast shock wave pressures (**Figure 4**): one transducer in the driver chamber and four transducers in the specimen chamber – one right above the specimen tray, one 12 inches to the left of the tray, one 12 inches to the right of the tray and one adjacent to the animal's head. Additional details about the ABS device parameters are available in **Appendix A**.

ANIMAL INJURY

All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Texas Medical Branch, an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility. All animals were housed under controlled environmental conditions and allowed food and water *ad libitum*. In order to ensure a fully formed/fused skull, adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) ranging in weight from 350 – 480

grams were anesthetized (4% isoflurane), intubated, and ventilated (2.0% isoflurane) in O_2/room air (80:20) using a volume ventilator (Small Animal Ventilator, Harvard Apparatus, Inc., Holliston, MA). Core body temperature was monitored using a rectal telethermometer (Thermalert Monitoring Thermometer, Physitemp Instruments, Inc., Clifton, NJ) and maintained using a thermostatically controlled water blanket (Mul-T-Pad Temperature Therapy Pad, Gaymar Industries, Inc., Orchard Park, NY). After intubation, the scalp was shaved, foam plugs were placed into each ear and the animal was secured on the specimen tray with Velcro® straps in a transverse prone position with the head supported at right angles to the direction of the shock wave by a leather sling connected between two posts. When the specimen tray was placed in the ABS, only the head of the rat was exposed to the shock wave (**Figure 5**). After the rat was secured to the specimen tray, the isoflurane was temporarily discontinued, the ventilator hoses were detached but the rat remained intubated, the specimen tray was locked into the ABS and, at the return of a withdrawal reflex to paw pinch, was subjected to bTBI (20.9 psi, ± 1.14, 138 kPa ± 7.9) or Sham bTBI. Shock wave overpressures had a mean rise time of 0.37 milliseconds ± 0.006 and a duration of 3.50 milliseconds ± 0.063, calculated using the slope and y-intercept formula. After injury, the animal was removed from the ABS, the duration of suppression of the righting reflex (RR) was recorded (only animals in Experiments 1, 3 and 4), the animal was reconnected to the ventilator and anesthesia with isoflurane was resumed. For all Sham animals, the preparatory procedure stated above was followed but the rat was not subjected to bTBI.

MIDDLE CEREBRAL ARTERIAL MEASUREMENTS

Cerebral vascular responses to dilator or constrictor stimuli were measured in MCA segments (**Figure 6**) harvested from rats subjected to mild, ABS shock wave exposure *in vivo*. One of the difficulties associated with studying the effects of TBI on the cerebral circulation is separating the direct effects of TBI on the cerebral vasculature from the indirect vascular effects of substances produced by the injured brain (DeWitt and Prough, 2012). This potential confound can be circumvented, in part, by studying the vasodilator/vasoconstrictor responses of isolated, pressurized and/or perfused cerebral arteries *ex vivo*. Admittedly, cerebral arteries would be exposed to substances produced/due to traumatic injury to the brain parenchyma prior to harvest but the magnitude of this effect can be minimized by harvesting cerebral arteries immediately after TBI, thereby limiting the time available for vasoactive substances to be released locally and the duration of exposure of the arteries *in vivo*. Studies of isolated cerebral arteries *in vitro* also provide the opportunity to test mechanisms of traumatic vascular injury using specific receptor agonists and antagonists or putative agents of vascular injury that cannot be examined as effectively or selectively *in vivo*. The effects of different types of impact (i.e. non-blast) TBI on cerebral vascular responses have been studied on cerebral arterial segments (Golding et al., 1998; Golding et al., 1999c; Mathew et al., 1999; Yu et al., 2014).

Experiment 1: Effects of ABS bTBI on dilator responses to reduced intravascular pressure in isolated MCA segments (Figure 7)

Animals were anesthetized (4% isoflurane), intubated, and ventilated with 2.0% isoflurane in O_2/room air (80:20). After intubation, the scalp was shaved, foam plugs were placed into each ear and the animal was secured on the specimen tray in a transverse prone

position with the head supported at right angles to the direction of the shock wave. The anesthesia was temporarily discontinued, the ventilator hoses were detached but the animal remained intubated and after securing the specimen tray in the ABS and upon the return of a withdrawal reflex to paw pinch, the animal was subjected to bTBI (20.9 psi ± 1.14, 138 kPa ± 7.9) or Sham bTBI. For all Sham animals, the preparatory procedure stated above was followed but the rat was not subjected to bTBI. Immediately after injury, the animal was removed from the ABS, the duration of suppression of the RR measured, reconnected to the ventilator and anesthesia resumed (2.0% isoflurane). The animal was survived for thirty or sixty minutes after ABS bTBI (n=6 for 30 and 60 minute groups) or Sham (n=6 for 30 and 60 minute groups) injury after which the isoflurane level was increased to 4%, the rat was decapitated, the brain removed, MCA segments collected and mounted in an Arteriograph (Living Systems Instrumentation, Inc., Burlington, VT) (Bryan et al., 1996; DeWitt et al., 2001) within 15 – 20 minutes of harvest by an investigator blinded to the injury type. The mounted arterial segment was bathed in physiologic salt solution (PSS) of the following composition (mM): NaCl, 130; KCl, 4.7; $MgSO_4$, 7; H_2O, 1.17; glucose, 5; $CaCl_2$, 1.50; $NaHCO_3$, 15, warmed from room temperature to 37°C and equilibrated for 60 minutes with intravascular pressure set at 50 mmHg. The MCA segments were viewed with an inverted microscope equipped with a video camera and video scaler. Dilator responses were tested by decreasing intravascular pressure in 20 mmHg increments with a 10 minute equilibration period at each pressure level before diameter measurements were made.

LASER DOPPLER FLOWMETRY

Laser Doppler flowmetry provides a continuous measurement of relative cerebral perfusion. The laser Doppler flow probe emits monochromatic red light (632.8 nm) which is reflected by moving erythrocytes (Stern et al., 1977). The power and frequency of the reflected signal, detected by optodes in the needle probe head, are proportional to the blood volume and blood velocity, respectively. Blood velocity is calculated from the Doppler shift created by erythrocytes (red blood cells) moving in the area perfused by the probe laser and reflected back to the receiver in the same probe (**Figure 8**). Perfusion was calculated as the product of blood volume and velocity in a 1 mm^3 tissue volume under the probe (Stern et al., 1977; Haberl et al., 1989). Relative changes in CBF determined using LDF correlated well with microsphere CBF in rabbits (Eyre et al., 1988) and with CBF measured using the H_2-clearance method in cats (Haberl et al., 1989).

Experiment 2: Effects of ABS bTBI on MAP, relative cerebral perfusion and CVR (Figure 9)

Animals were anesthetized (4% isoflurane), intubated, and ventilated with 2.0% isoflurane in O_2/room air (80:20). After intubation, the scalp was shaved, the tail artery was cannulated with polyethylene (PE) 10 tubing and the animal placed in a stereotaxic frame. The midline scalp was incised, reflected and a ¼ inch portion of the skull lateral to the midline over the frontal-parietal cortex was thinned using an air-cooled dental drill. A fiber-optic needle probe shielded from light was positioned over the thinned area away from large blood vessels. Baseline cerebral perfusion and mean arterial pressure were measured, the LDF probe removed, the edges of the scalp sutured, foam plugs were placed into each ear and the animal was removed from the stereotaxic frame and secured on the

ABS specimen tray in a transverse prone position with the head supported at right angles to the direction of the shock wave. The isoflurane was temporarily discontinued, the ventilator hoses were detached but the animal remained intubated and after securing the specimen tray in the ABS and upon the return of a withdrawal reflex to paw pinch, the animal was subjected to bTBI (20.9 psi ± 1.14, 138 kPa ± 7.9) (n=12) or Sham bTBI (n=10). For all Sham animals, the preparatory procedure stated above was followed but the rat was not subjected to bTBI. Immediately after injury, the animal was removed from the ABS, reconnected to the ventilator and anesthesia resumed (2.0% isoflurane), re-secured on the stereotaxic frame, the scalp reflected and the LDF needle probe repositioned over the thinned skull. A temperature probe was then placed deep to the temporalis muscle. Measurements of cerebral perfusion and arterial blood pressure were continued for two hours post-injury: every 5 minutes for the first 30 minutes after injury followed by every 15 minutes for the remaining hour and a half. Relative cerebral perfusion was calculated and expressed as a percent of pre-bTBI baseline. CVR was calculated from MAP and cerebral perfusion (CVR = MAP/LDF).

In order to compensate for misplaced probes in both the Sham and bTBI rats, I excluded all animals in which the first measurement after the probe was replaced (5-minute post-blast time-point) yielded LDF values 20% higher or lower than baseline in the absence of comparable changes in MAP (five excluded Sham rats and three bTBI rats).

FLUOROJADE

Studies that have examined multiple types of brain injury have utilized FluoroJade (FJ) staining for successful identification of neuronal injury and degeneration (see: Schmued et al., 1997; Schmued and Hopkins, 2000; Butler et al., 2003). FJ is a

fluorochrome derived from fluorescein and is popularly employed to label degenerating neurons in *ex vivo* tissue (Schmued et al., 1997; Schmued and Hopkins, 2000) of the CNS. It has similar excitation and emission profiles as fluorescein (excitation: 495 nm; emission: 521 nm) and can be viewed using a fluorescein isothiocyanate (FITC) filter. Neuronal cell bodies, dendrites, axons and axon terminals can be visualized using FJ, or more specifically, FJC (Shmued et al., 2005). When viewed under a fluorescence microscope, injured or dead neurons will fluoresce brightly in contrast to neighboring neurons (**Figure 10A**). FJ appears to be more a responsive, definitive indicator of neuronal injury than hematoxylin and eosin (H&E) or Nissl stains and is significantly simpler to undertake than silver staining techniques. It exhibits the greatest signal to background ratio, highest resolution, maximum contrast and affinity for neurons making it ideal for localizing degenerating nerve cell bodies (Shmued et al., 1997; Schmued and Hopkins, 2000; Schmued et al., 2005; Schmuck et al., 2009). However, though the exact mechanism by which FJ stains injured and/or dying neurons is currently not known, some inferences can be made concerning the histochemical specificity of FJ based on its chemical properties. An injured/dying neuron presumably expresses a strongly basic molecule since it has an affinity for the powerfully acidic FJ but a repulsion towards the intensely basic dyes used for Nissl counterstaining purposes (Shmued et al., 1997). This putative "degeneration molecule" may be generated during the progression of degeneration and is not merely a normally occurring molecule that labels only when FJ comes into contact with the intercellular environment by way of a disrupted plasma membrane (Shmued et al., 1997). This is inferred from the observation that treatments which can permeabilize membranes (freezing, solvent extraction, and detergent exposure) fail to alter the characteristic staining

patterns (Shmued et al., 1997; Schmuck et al., 2000; Schmuck and Kahl, 2009). It should also be noted that, although FJ is widely used to stain injured and/or dying neurons (Schmued et al., 1997; Larsson et al., 2001; Sato et al., 2001; Hellmich et al., 2005a; Hellmich et al., 2005b) there is evidence that FJ-positive cells may be injured but not necessarily dying (Wang et al., 2015) and FJ may stain non-neural cells (e.g. activated microglia, astrocytes) under circumstances when combined with specific markers for detection of GFAP or activated CD68 microglia (Damjanaca et al., 2007).

Experiment 3: Effects of ABS bTBI on cell injury in the brain (Figure 11)

Animals were anesthetized (4% isoflurane), intubated, and ventilated with 2.0% isoflurane in O_2/room air (80:20). After intubation, the scalp was shaved, foam plugs were placed into each ear and the animal was secured on the specimen tray in a transverse prone position with the head supported at right angles to the direction of the shock wave. The anesthesia was temporarily discontinued, the ventilator hoses were detached but the animal remained intubated and after securing the specimen tray in the ABS and upon the return of a withdrawal reflex to paw pinch, the animal was subjected to bTBI (20.9 psi ± 1.14, 138 kPa ± 7.9) (n=6 for 24 and 48 hour groups) or Sham bTBI (n=6 for 24 and 48 hour groups). For all Sham animals, the preparatory procedure stated above was followed but the rat was not subjected to bTBI. Immediately after injury, the animal was removed from the ABS, the duration of suppression of the RR measured and 24 or 48 hours later, anesthetized with 4.0% isoflurane, decapitated and the brains were removed, frozen on dry ice and stored at -80°C. Brains were sectioned (10 μm) (**Figure 10B**) on a cryostat (Leica CM1860, Leica Biosystems, Inc., Buffalo Grove, IL) and every 25th section was mounted on Superfrost™ Plus microscope slides (Fisherbrand™ , Fisher Scientific Co., Pittsburgh, PA). Sections

were immersed in 75% ethanol for 1 minute, Millipore quality (MilliQ) H_2O for 1 minute and cresyl violet for 15 – 20 seconds at room temperature. Sections were then washed in MilliQ H_2O two times for 30 seconds each and then immersed in FJC (0.0001% in MilliQ H_2O with 0.1% acetic acid vehicle) for 4 minutes. Sections were removed from the FJC and washed in MilliQ H_2O three separate times for 1 minute each, in 95% ethanol for 30 seconds and in 100% ethanol for 30 seconds. Lastly, sections were immersed in xylene twice for 3 minutes each, then allowed to air dry overnight in a darkened fume hood. Two investigators, blinded to experimental groups, counted 30 slides with two sections mounted on each slide (60 brain sections for each animal). Ten sections (5 slides) were taken from the region corresponding to the frontal lobe region; 40 sections (20 slides) were taken from the parietal/temporal lobe region/s; and ten sections (5 slides) were taken from the occipital lobe/cerebellum region. FJC-positive cells in each section were viewed using an imaging system monitor connected to an Olympus BX51 research system microscope (Olympus Corporation, Tokyo, Japan) using a filter system suitable for visualizing fluorescein or FITC. FJC-positive cells were summed across all sections. The mean of the two investigators' counts was then calculated to get a total mean count for each whole brain.

BEHAVIOR AND CONGNITIVE FUNCTION

Beam balance/walking tasks are used to assess vestibulomotor function through measurements of fine motor coordination and balance (Hicks and D'Amato, 1975; Goldstein and Davis, 1990). Originally described by Hicks and D'Amato (1975), these tasks examine the ability of the animal to remain upright and/or walk on an elevated and relatively narrow beam. Both the beam walk and beam balance tasks provide a means of preventing the animals from masking motor deficits through other compensatory

mechanisms that may present the animal as having no motor dysfunction at all (Hicks and D'Amato, 1975; Goldstein and Davis, 1990; Sedy et al., 2008; Sweis et al., 2016).

Beam walk

The beam walk task involved training rats to traverse a 3 foot long solid pine beam (1-inch wide) with a darkened goal box attached at the far end and a lamp and white noise generator as an aversive stimulus at the starting end (**Figure 12**). Dulled nails (1¾-inch length) acting as distracting obstacle pegs were equidistantly spaced along the beam. Once trained, the time (in seconds) required for the animal to reach the goal box from the starting point was recorded for three successive trials.

Beam balance

For the beam balance task, rats are trained to balance for 60 seconds on a plywood beam approximately 1 foot in length and ½ inch in width that is open-ended on one side with a whiteboard barrier on the other end (**Figure 13**). Animals underwent two training sessions and one pre-injury assessment. Once rats were able to remain on the beam, they were evaluated on their ability to balance and scored for three consecutive trials. These trials are scored using an ordinal 0 – 2 scoring system based on foot placement, body balance and tail movement, with a lower score indicating better vestibulomotor function.

Morris water maze

The Morris water maze (MWM) task (Morris, 1981; Morris et al., 1982; Hamm et al., 1993; Hamm et al., 1996) is a widely used method for assessing cognitive processes (D'Hooge and De Deyn, 2001). The test examines spatial learning and working memory in laboratory rodents (Morris, 1984; Hamm et al., 1996; D'Hooge and De Deyn, 2001). MWM performance is sensitive to lesions of the hippocampus and pre-frontal cortex

(Schenk and Morris, 1985), making it suitable for detecting TBI-induced memory and learning deficits. Place navigation is often used as a general assay of cognitive function (Black et al., 1977; O'Keefe and Conway, 1978; Morris et al., 1982). The water maze consists of a circular pool, 6 feet in diameter filled to a depth of approximately 2.5 feet (**Figure 14A**). The animals escape from the water by locating and climbing onto a transparent Plexiglass goal platform 4.5 inches in diameter located just beneath the surface of the water. The location of the platform is determined relative to distal visual cues (**Figure 14B**) or landmarks surrounding the pool (placement of objects at different locations or on the walls of the testing room) (Morris, 1984; D'Hooge and De Deyn, 2001; Vorhees and Williams, 2014). The swim path of the animal was tracked using a ceiling-mounted videocamera connected to a computer with tracking software (Anymaze, Stoelting Co., Wood Dale, IL). The task consists of multiple sets of measurements of the time required for the rat to find the goal platform. Rats that fail to locate the platform within 120 seconds were placed on the platform for 30 seconds (D'Hooge and De Deyn, 2001; Vorhees and Williams, 2014). The animals were placed into the water facing the pool wall at fixed starting positions. Since working memory is particularly sensitive to hippocampal injury, a working MWM protocol was used for my studies (see below) (Hamm et al., 1992; Hamm et al., 1996). For assessments of working memory, the platform position and the entry position changed between pairs of trials but remained constant between the trials in each pair. Although the time to the goal platform on both trials of each pair was measured, the difference between the first and second trials was used to assess working memory function.

Experiment 4: Effects of ABS bTBI on vestibulomotor and cognitive function (Figure 15)

To ensure that the assessment of vestibulomotor and working memory function were not confounded by motor deficits following ABS blast injury, animals were trained on beam walk (Dixon et al., 1987; Dixon et al., 1999; Hamm, 2001) and beam balance (Feeney et al., 1982; Dixon et al., 1987; Dixon et al., 1999) tasks the day before bTBI or Sham bTBI and on the day of blasting prior to injury. On the day of injury, animals were anesthetized (4% isoflurane), intubated, and ventilated with 2.0% isoflurane in O_2/room air (80:20). After intubation, the scalp was shaved, foam plugs were placed into each ear and the animal was secured on the specimen tray in a transverse prone position with the head supported at right angles to the direction of the shock wave. The anesthesia was temporarily discontinued, the ventilator hoses were detached but the animal remained intubated and after securing the specimen tray in the ABS and upon the return of a withdrawal reflex to paw pinch, the animal was subjected to bTBI (20.9 psi ± 1.14, 138 kPa ± 7.9) (n=10) or Sham bTBI (n=10). For all Sham animals, the preparatory procedure stated above was followed but the rat was not subjected to bTBI. Immediately after injury, the animal was removed from the ABS, the duration of suppression of the RR measured then were placed back in their cage after fully recovering from the anesthesia. Animals were subsequently tested on the beam walk and beam balance tasks on post-injury days 1 – 5 and on the MWM task on post-injury days 11 – 15. For the working memory MWM protocol employed, each animal received four pairs of timed trials per day, for five consecutive days. For each pair of trials, the entry point and platform locations were randomized while visual cues located on the walls of the testing chamber remained constant throughout each day. For each trial,

the animal was placed in the maze facing the pool wall and given 120 seconds to locate and climb onto the hidden platform. If the animal was not successful in locating the platform by the allotted time frame at the end of the first trial, it was placed on the platform for 30 seconds before being returned to the start position for the second trial. Between the pairs of timed trials the animals were kept in a lamp-heated warming box. During the 4-minute interval between pairs of trials, both the start position and the goal location were changed. Movement within the maze was recorded with a video camera, video scanning unit and the SMART tracking computer software (San Diego Instruments, Inc., San Diego, CA).

PEROXYNITRITE SCAVENGERS

Increased production of ROS and RNS is a feature of FPI TBI and bTBI (Kontos and Povlishock, 1986; Kontos and Wei, 1986; Povlischock and Kontos, 1992; Hall and Braughler, 1993; Globus et al., 1995; Hall, 1995a; Readnower et al., 2010; Heibert et al., 2015; Kabu et al., 2015). Scavenging of free radicals, ROS/RNS and $ONOO^-$ (**Figure 16**), have been reported to reduce the effects of TBI on the brain and cerebral vasculature (Kontos and Povlishock, 1986; Kontos and Wei, 1986; Althaus et al., 1994; Hall, 1995b; Hall et al., 1999). I tested the effects of the $ONOO^-$ scavenger PenME on cerebral perfusion after bTBI.

Experiment 5: Effects of ABS bTBI on MAP, relative cerebral perfusion and CVR after PenME administration (Figure 17)

Animals were anesthetized (4% isoflurane), intubated, and ventilated with 2.0% isoflurane in O_2/room air (80:20). After intubation, the scalp was shaved, the tail artery was cannulated with polyethylene (PE) 10 tubing and the animal placed in a stereotaxic frame.

The midline scalp was incised, reflected and a ¼ inch portion of the skull lateral to the midline over the frontal-parietal cortex was thinned using an air-cooled dental drill. A fiber-optic needle probe shielded from light was positioned over the thinned area away from large blood vessels. Baseline cerebral perfusion and mean arterial pressure were measured, the LDF probe removed, the edges of the scalp sutured, foam plugs were placed into each ear and the animal was removed from the stereotaxic frame and secured on the ABS specimen tray in a transverse prone position with the head supported at right angles to the direction of the shock wave. The isoflurane was temporarily discontinued, the ventilator hoses were detached but the animal remained intubated and after securing the specimen tray in the ABS and upon the return of a withdrawal reflex to paw pinch, the animal was subjected to bTBI (20.9 psi ± 1.14, 138 kPa ± 7.9) (n=8), bTBI + PenME treatment (n=8) or Sham bTBI (n=8). For all Sham animals, the preparatory procedure stated above was followed but the rat was not subjected to bTBI. Immediately after injury, the animal was removed from the ABS, reconnected to the ventilator and anesthesia resumed (2.0% isoflurane), re-secured on the stereotaxic frame, the scalp reflected and the LDF needle probe repositioned over the thinned skull. A temperature probe was then placed deep to the temporalis muscle. Five minutes post-bTBI, 10 mg/kg of PenME was administered in 0.1 ml saline vehicle through the cannulated tail artery. Measurements of cerebral perfusion and arterial blood pressure were continued for two hours post-injury: every 5 minutes for the first 30 minutes after injury followed by every 15 minutes for the remaining hour and a half. Relative cerebral perfusion was calculated and expressed as a percent of pre-bTBI baseline. CVR was calculated from MAP and cerebral perfusion (CVR = MAP/LDF).

DATA ANALYSIS

Statistical analyses were performed with the use of GraphPad Prism 5 software, (GraphPad software version 5.00, San Diego, CA). The myogenic response to changes in intravascular pressure was assessed by calculating percent change from baseline (100 mmHg) for each level of intra-arterial pressure (80, 60, 40, and 20 mmHg). Unpaired Student's t-tests were used to evaluate differences between the bTBI and Sham group baselines. Differences in MCA dilator responses between bTBI and Sham groups were assessed using a repeated one-way analysis of variance (ANOVA) Dunnett's multiple comparisons test and a Bartlett's test for equal variance. Relative cerebral perfusion, MAP and CVR data in both the untreated blood flow study (Experiment 2) and PenME-treated study (Experiment 5) were analyzed using unpaired Student's t-tests and two-way ANOVA after calculating percent change from baseline for each respective parameter. FJC-stained cell counts were averaged for each of the four groups (24 and 48-hour Sham, 24 and 48-hour bTBI) and for each lobe region (frontal, parietal/temporal and occipital) in each of the four groups. Unpaired Student's t-test analyses were performed between the 24 and 48-hour Sham groups versus the 24 and 48-hour bTBI groups as well as between each time point's respective bTBI and Sham group's lobe region in an effort to evaluate differences between the Sham and bTBI animals. A one-way ANOVA was performed between all three lobe regions in the 24-hour Sham group versus the 24-hour bTBI group and between all three lobe regions in the 48-hour Sham group versus the 48-hour bTBI group. A repeated measure two-way ANOVA was performed on the differences in the MWM latencies to the goal platform between the first and second trials of each successive day between the two groups as a whole. Since beam balance scores were ordinal, those data was analyzed using

the Mann-Whitney test.

Due to the reduction in statistical power that results from repeated testing, comparisons at each specific pressure point in the MCA experiments (e.g. between 100 and 80 mmHg or between 60 and 40 mmHg, etc.) or between individual days between the two groups in the beam walk, beam balance and MWM trials (e.g. between days 1 and 5 or between days 12 and 14) were not conducted.

Significance was accepted at the $P \leq 0.05$ level. All data in the text, table, and figures are expressed as means ± standard errors of the means (SEM).

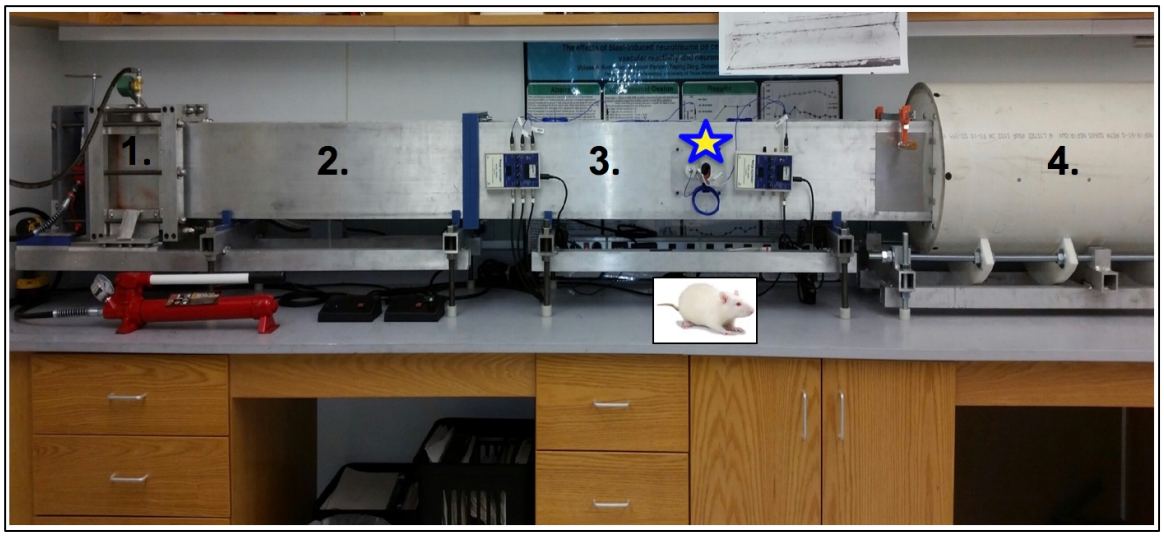

Figure 1: Advanced Blast Simulator (ABS) shock tube device

The ABS used to produce primary blast injury in all study animals. 1 = driver chamber; 2 = expansion chamber; 3 = specimen chamber; 4 = reflected wave suppressor ☆ = specimen tray.

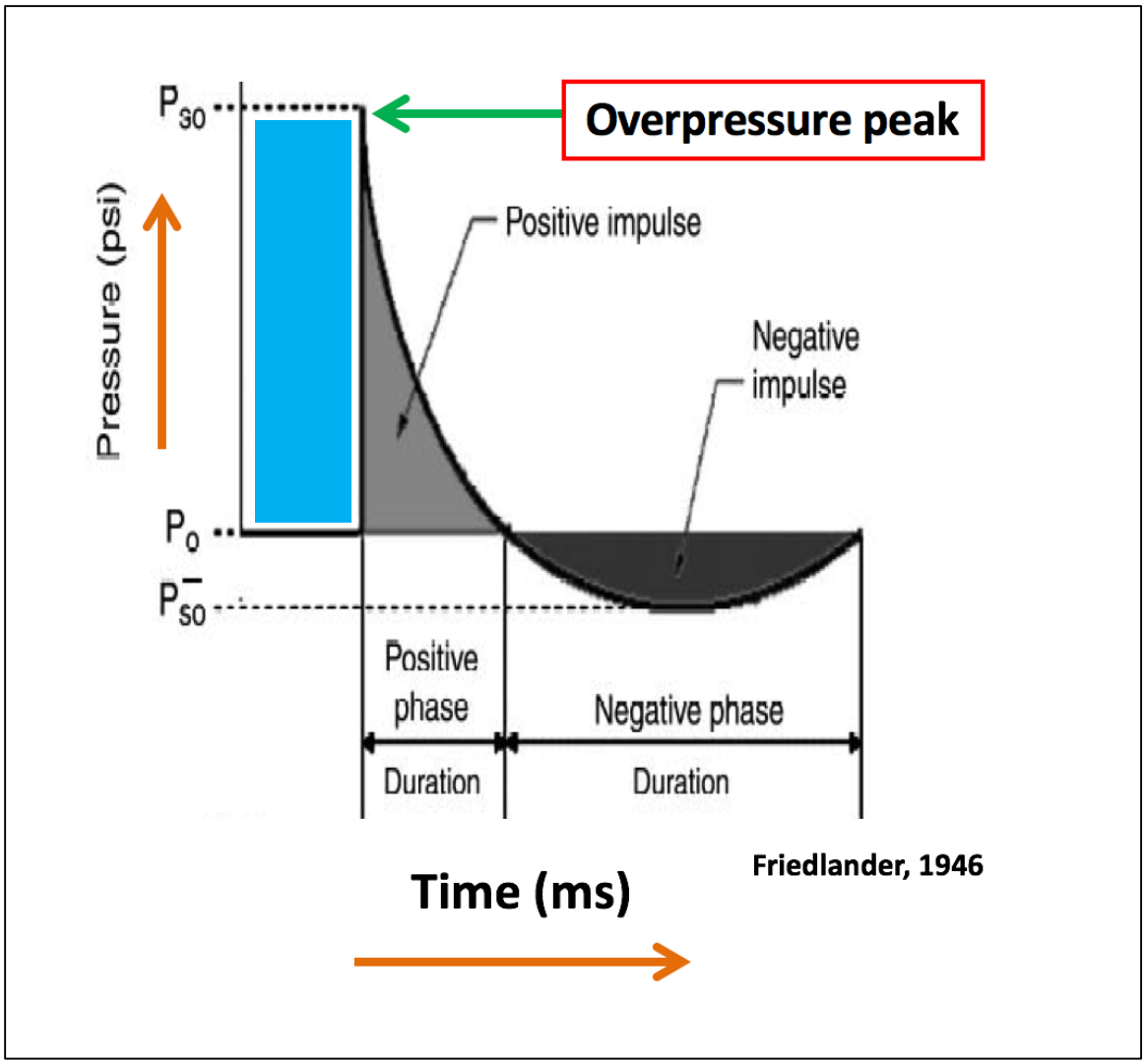

Figure 2: Ideal Friedlander curve

A schematic of a classical, ideal blast waveform (Friedlander curve, Friedlander, 1946) from an open field explosion, characterized by an instantaneous rise in pressure immediately followed by a decaying curve. Prior to detonation, pressure is normal (P_O, the blue-shaded area before and below the overpressure peak). With the passage of a shock front, the blast forces are maximal, resulting in a peak overpressure and positive impulse. As the overpressure wave passes, the drop in atmospheric pressure to below normal results in a reversed blast wind which allows for the negative impulse and negative phase duration. Atmospheric pressure then returns to normal after the blast wave subsides.

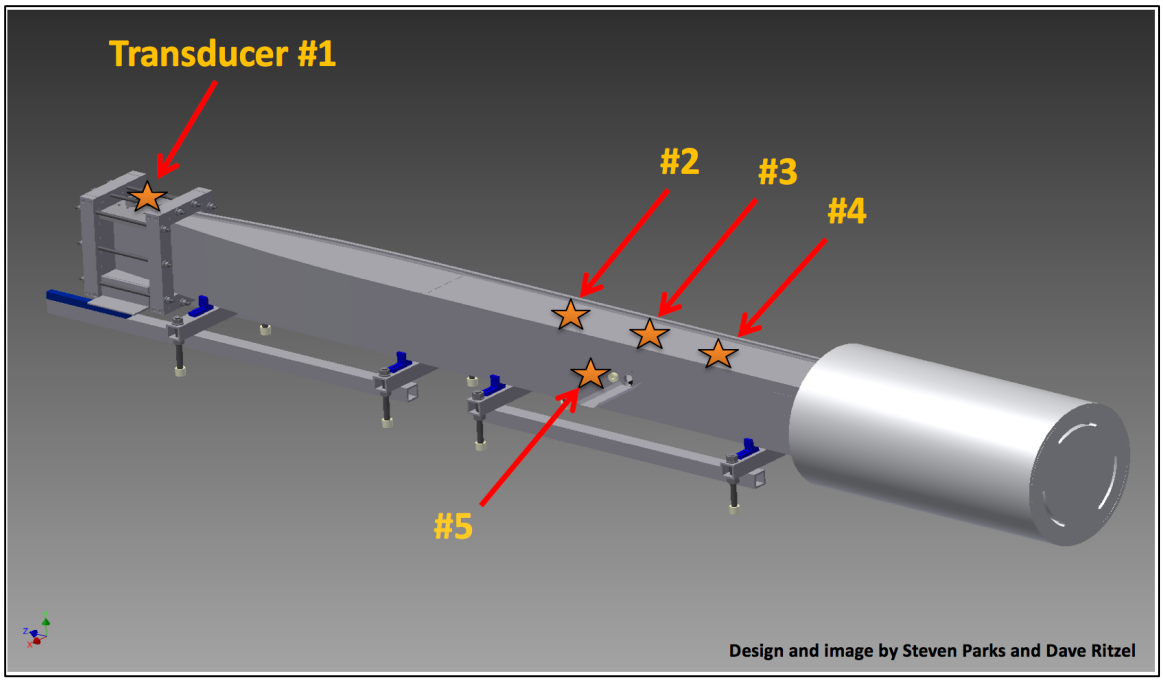

Figure 3: Location of transducers in ABS

Placement of the five transducers located within the ABS used to measure blast shock wave pressures. The driver chamber contains one transducer while the specimen chamber contains four transducers – one right above the specimen tray, one 12 inches to the left of the tray, one 12 inches to the right of the tray and one on the specimen tray, adjacent to the animal's head.

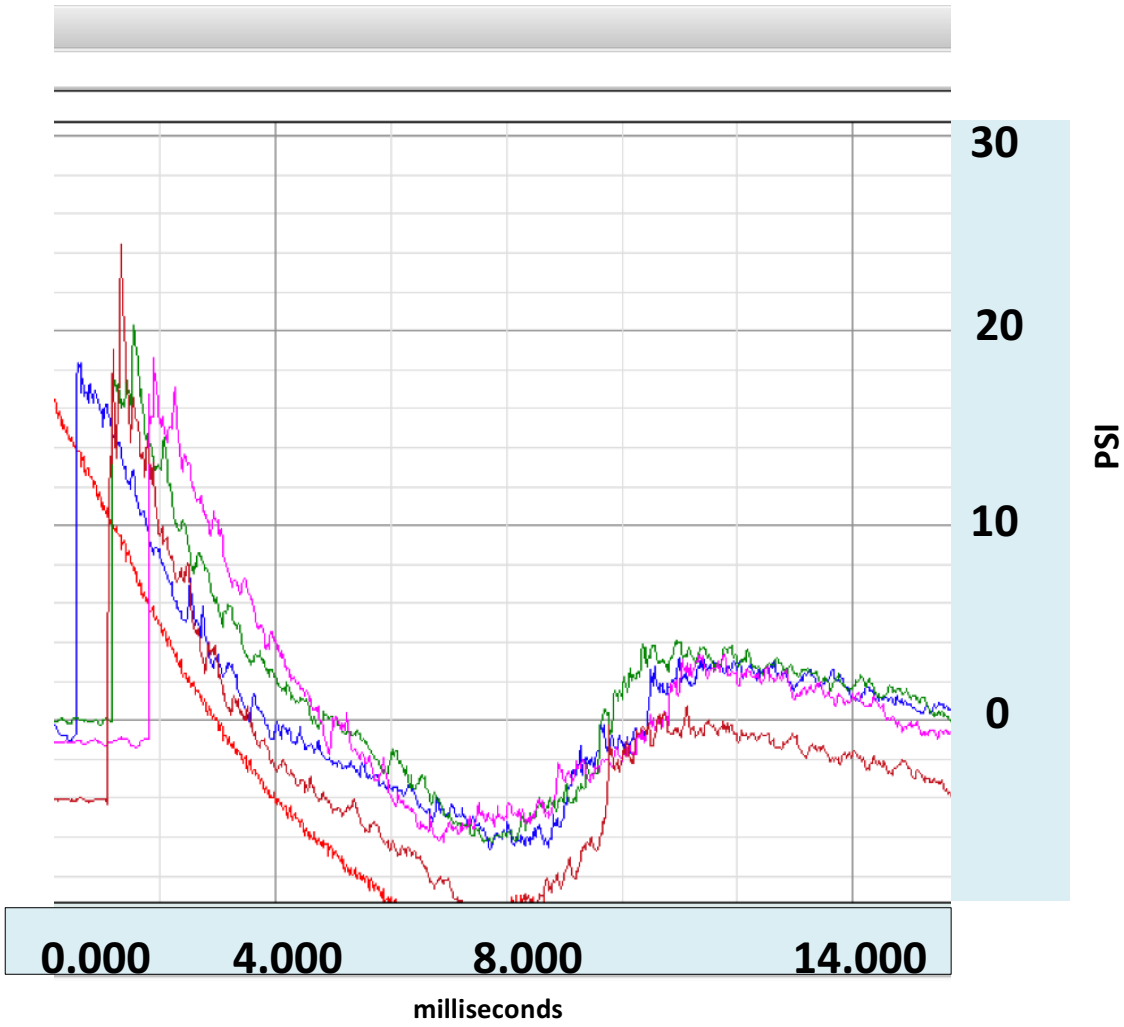

Figure 4: Blast wave over- and underpressures (in pounds/inch^2, PSI) produced by the ABS versus time.

The y-axis represents shock wave pressures recorded by each of the five transducers located throughout the ABS (each colored line represents the recorded pressure collected by a different transducer) while the x-axis represents the time in milliseconds of phase duration.

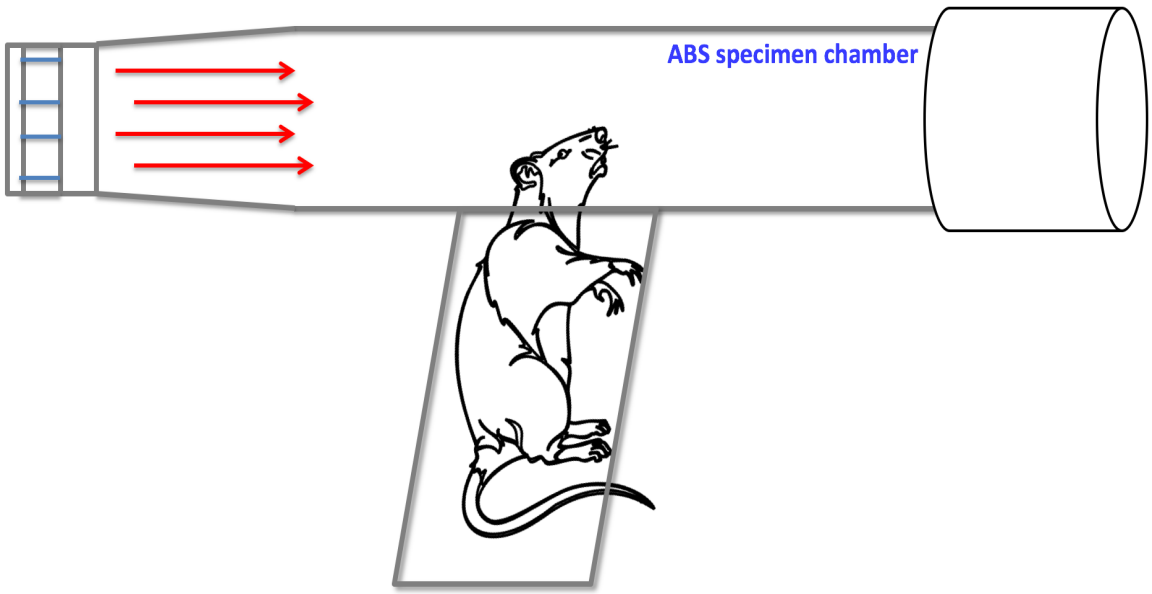

Figure 5: Animal placement inside ABS

Direction and orientation of the experimental animal inside the ABS. When placed in the ABS, the animal is in a transverse prone position with the dorsal surface of the head perpendicular to the shock wave direction (red arrows).

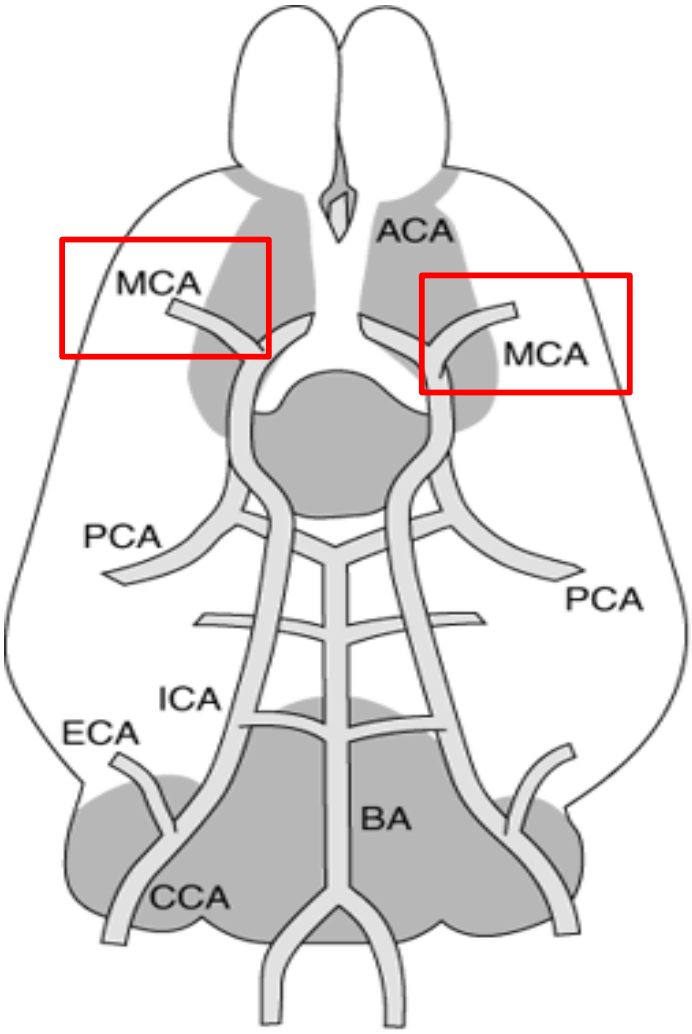

Figure 6: Location of middle cerebral arteries (MCA)

Ventral view of the rat brain highlighting the location of the MCA's relative to the posterior cerebral arteries (PCA), internal carotid arteries (ICA), external carotid arteries (ECA), basilar artery (BA) and common carotid arteries (CCA).

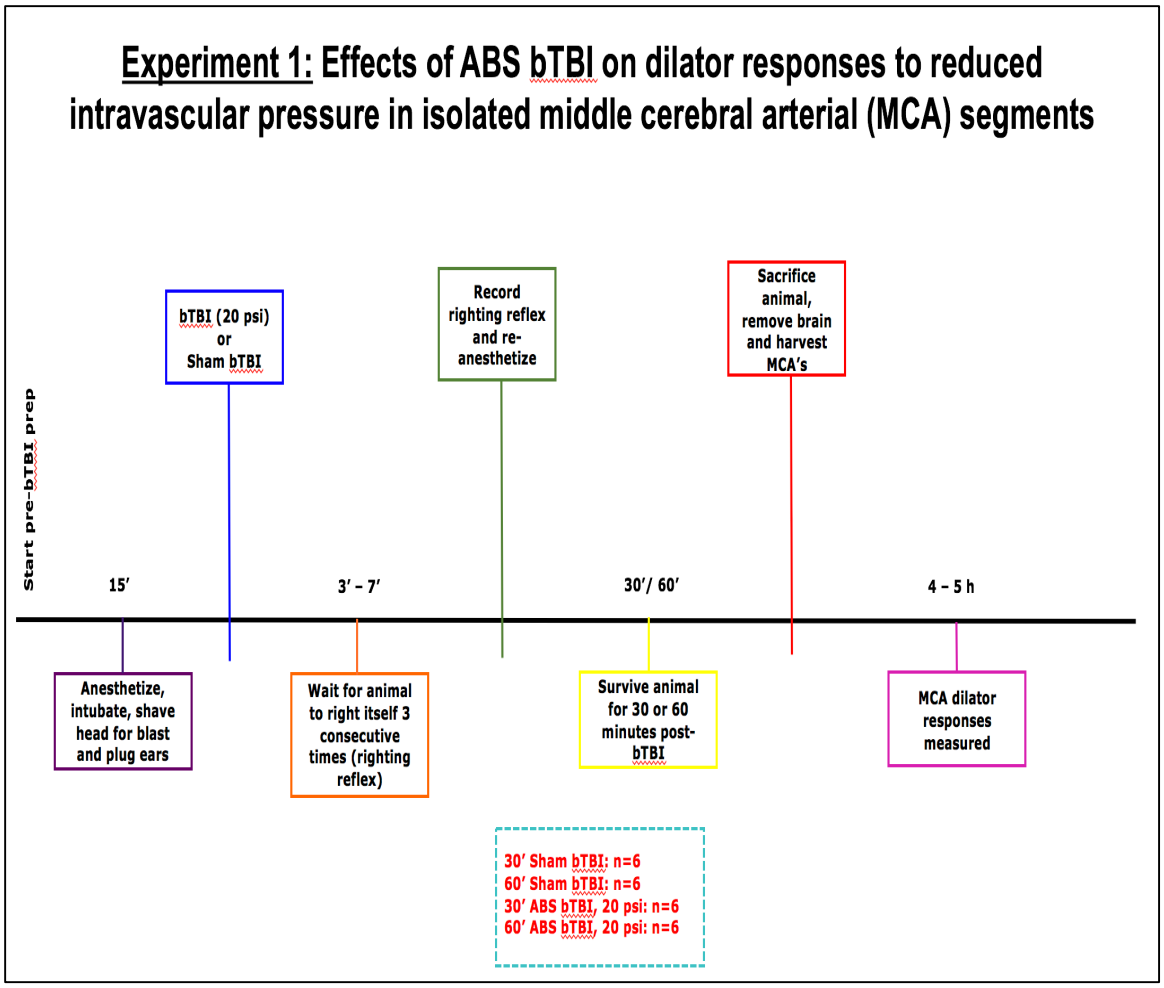

Figure 7: Experiment 1 design

Experimental design and timeline of the effects of ABS bTBI on dilator responses to reduced intravascular pressure in isolated middle cerebral arterial (MCA) segments. Male Sprague Dawley rats were anesthetized, intubated and mechanically ventilated. Animals were blast or sham-injured and 30 or 60 minutes after injury, the animals were sacrificed, the brain removed and the MCA's harvested. MCA's were then mounted on an arteriography system that allows for control of intravascular pressure through the MCA segment and measurement of lumen diameter and the myogenic responses of the arteries were tested by decreasing this intraluminal pressure in increments of 20 mmHg.

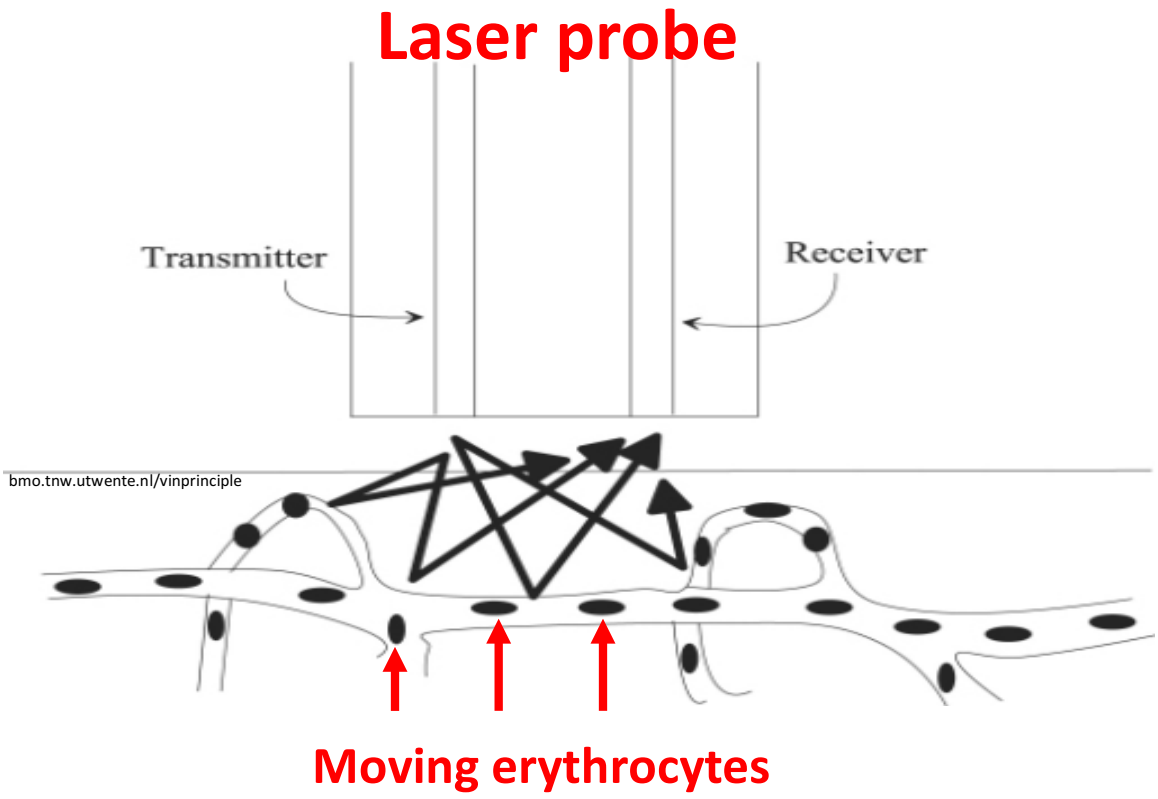

Figure 8: Laser Doppler flowmetry (LDF) mechanism

The technique is based on a laser light hitting moving erythrocytes. Light applied from a laser beam is scattered after entering tissue. The light undergoes a change in wavelength (Doppler shift) directly related to the number and relative velocity of the cells in flux while light hitting static objects remains unchanged. The signals provided by these moving erythrocytes are then bounced back to a detector.

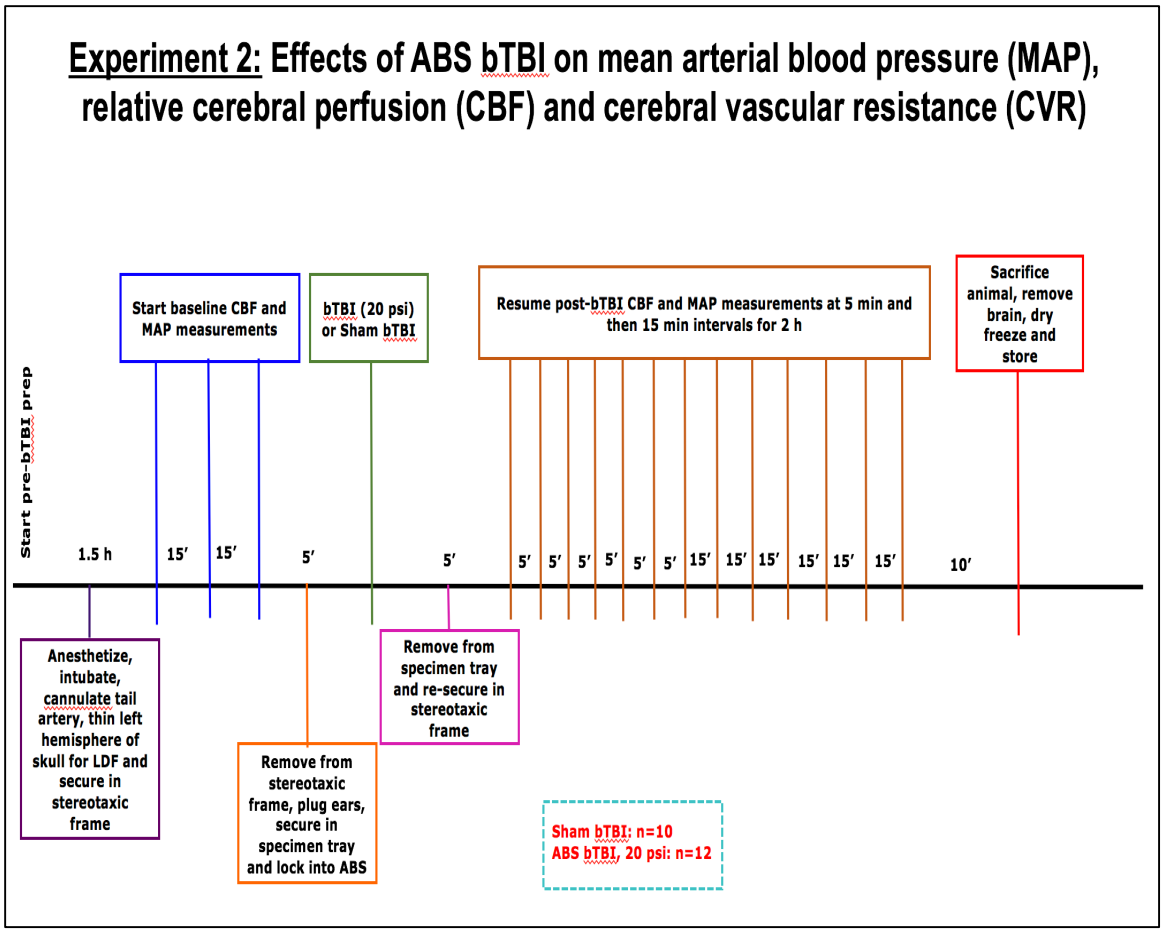

Figure 9: Experiment 2 design

Experimental design and timeline of the effects of ABS bTBI on mean arterial blood pressure (MAP), relative cerebral perfusion (CBF) and cerebral vascular resistance (CVR). Male Sprague Dawley rats were anesthetized, intubated and mechanically ventilated. The tail artery was cannulated for constant observation of MAP and the left hemisphere of the skull was thinned down. The animals were placed on a stereotaxic frame head-holder, the Doppler probe was positioned over the thinned portion of the skull and baseline MAP and CBF measurements were taken for 30 minutes before injury. Animals were removed from the stereotaxic frame, blast or sham-injured, returned to the frame and the Doppler probe was repositioned on the skull. MAP and CBF were then measured for 2 hours after injury: every 5 minutes for the first 30 minutes after injury followed by every 15 minutes for the remaining hour-and-a-half.

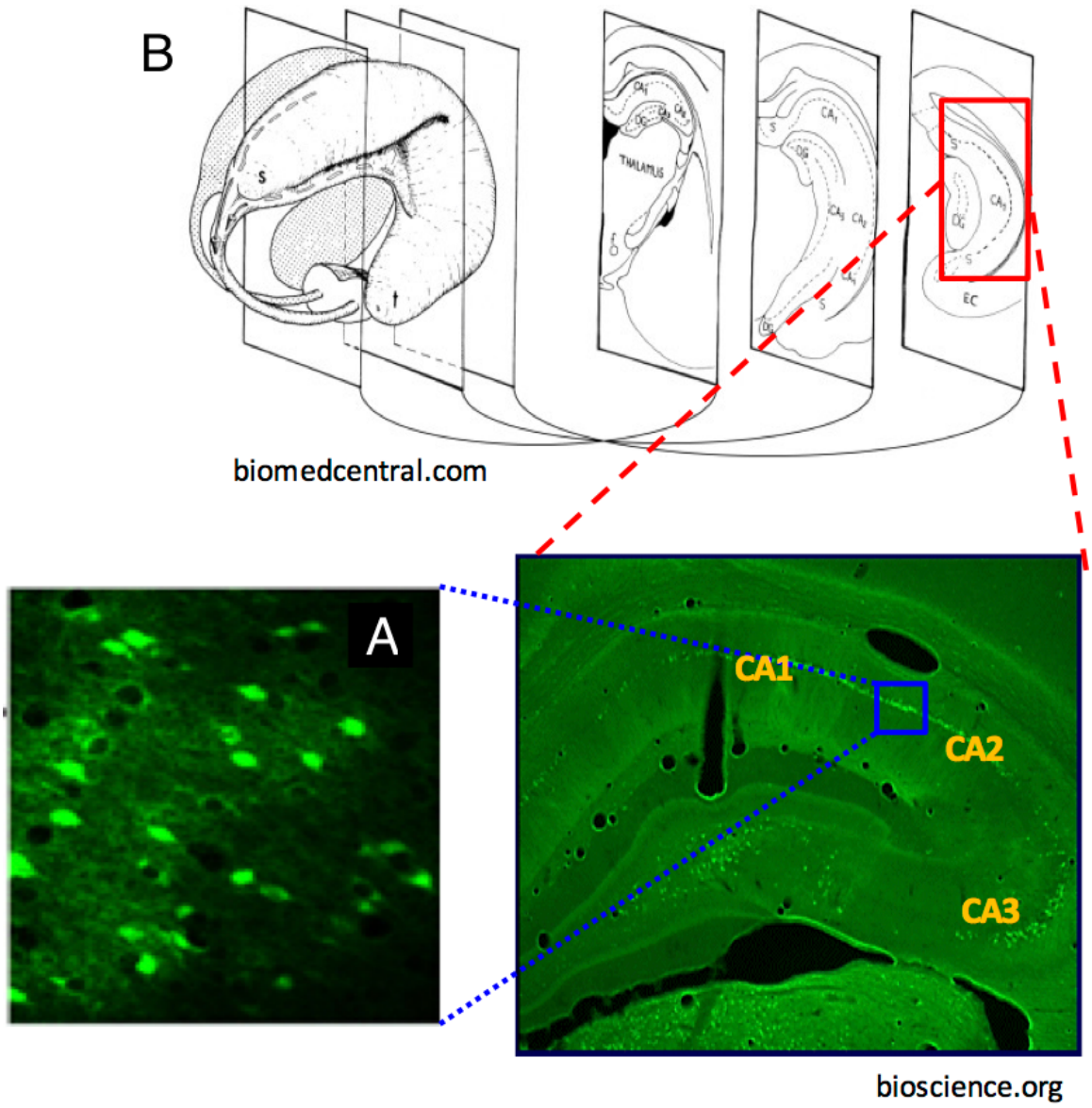

Figure 10: Brain sectioning and FluoroJade (FJ) fluorescence

Schematic of the successive sectioning done front to back on the whole rat brain (**B**). When viewed under a fluorescence microscope, injured or dying neuronal cell bodies (seen here scattered amongst the CA subgroups of the hippocampus) will fluoresce brightly in contrast to the dark-spotted normal, uninjured neighboring neurons (**A**).

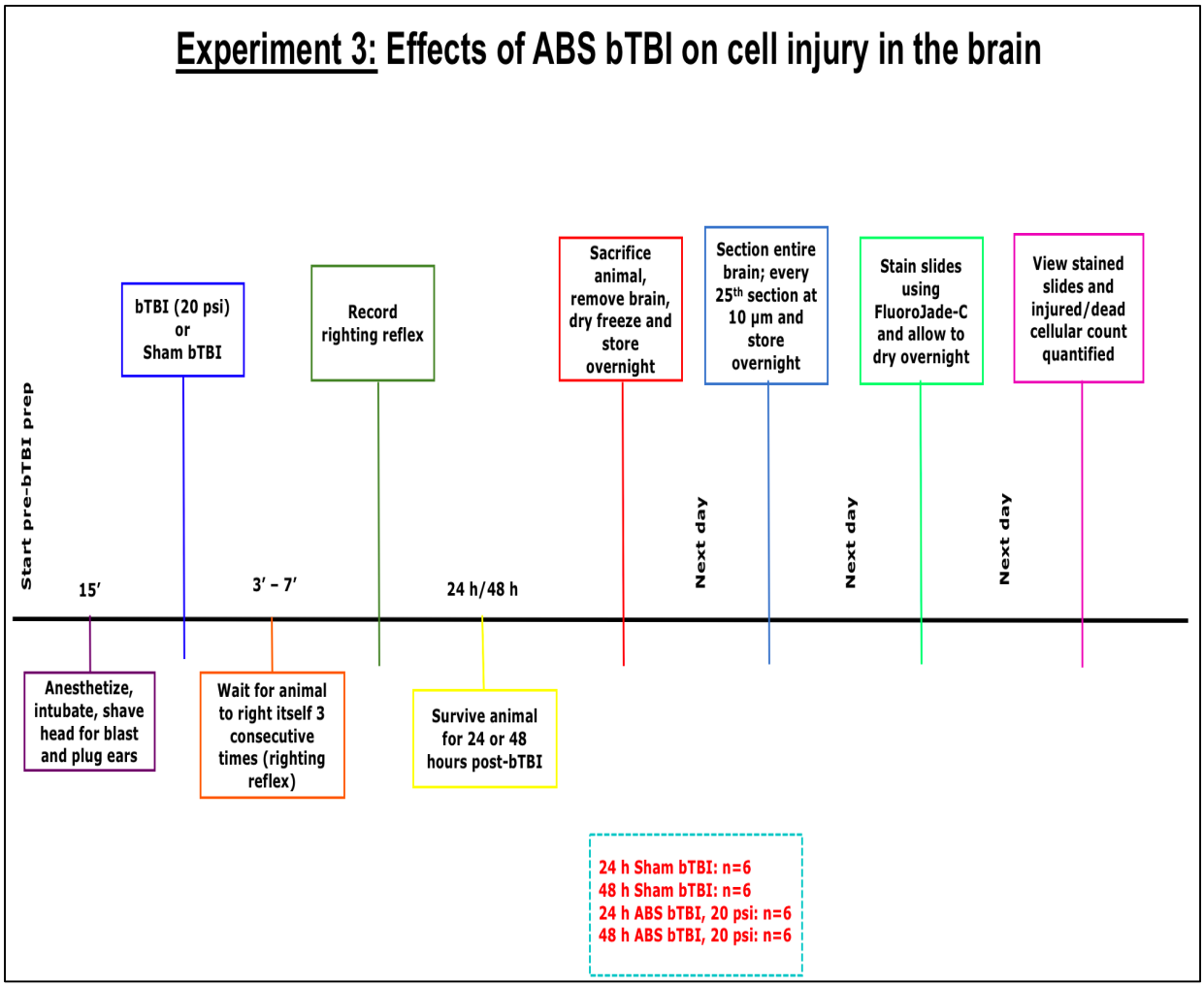

Figure 11: Experiment 3 design

Experimental design and timeline of the effects of ABS bTBI on cell injury in the brain. Male Sprague Dawley rats were anesthetized, intubated and mechanically ventilated. Animals were blast or sham-injured and allowed to recover. 24 or 48 hours after injury, the animals were reanesthetized, sacrificed and the brain harvested and frozen. The entire brain was then sectioned onto slides and stained with FluoroJade-C (FJC). The numbers of FJC-positive cells were then counted by two investigators blinded to experimental group.

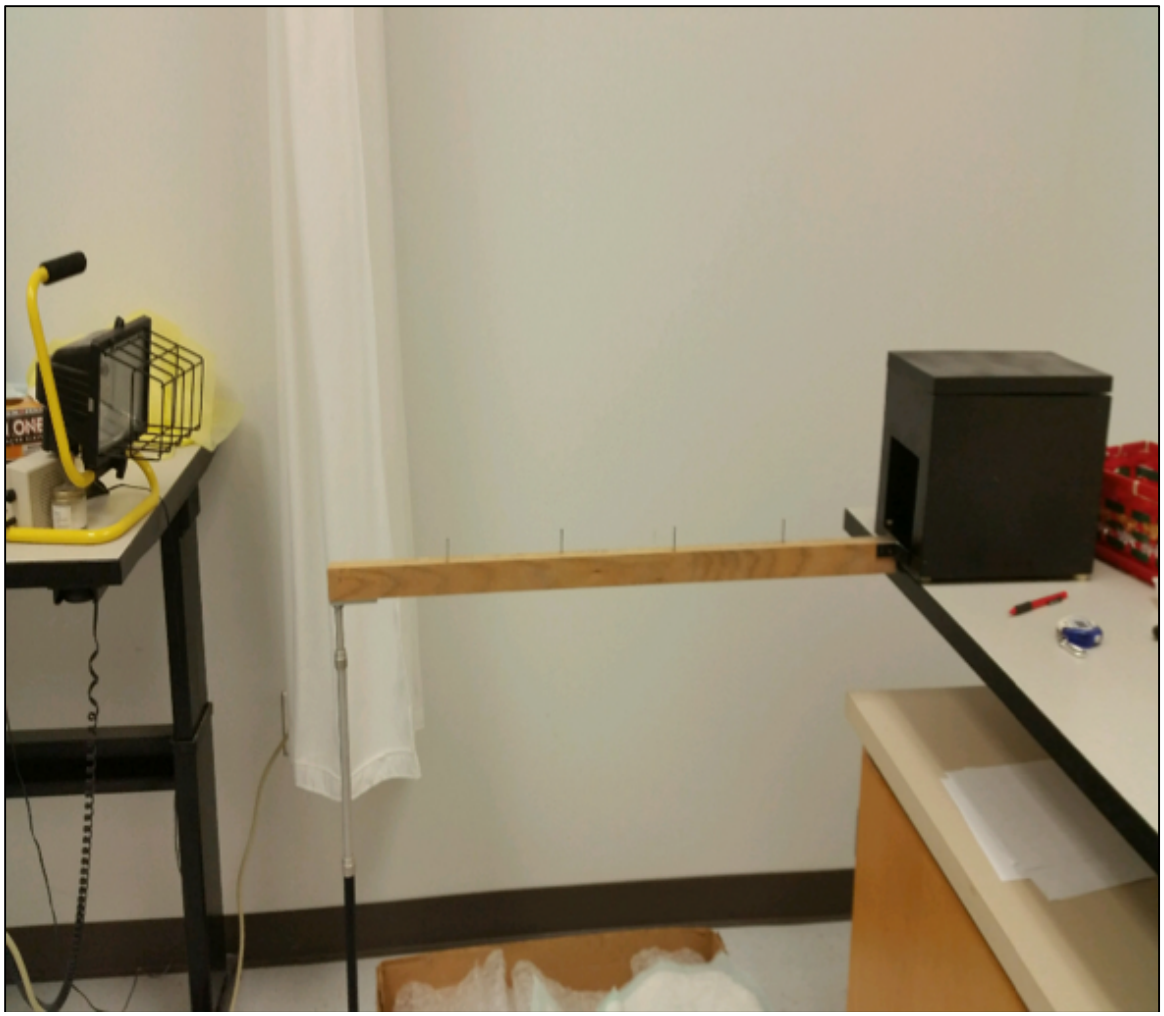

Figure 12: Beam walk apparatus

Set up of the beam walk apparatus. Rats are trained and then timed to traverse a 3 foot long solid pine beam 1 inch in width that contains a darkened goal box attached at the far end with a lamp and white noise generator as an aversive stimulus at the starting end.

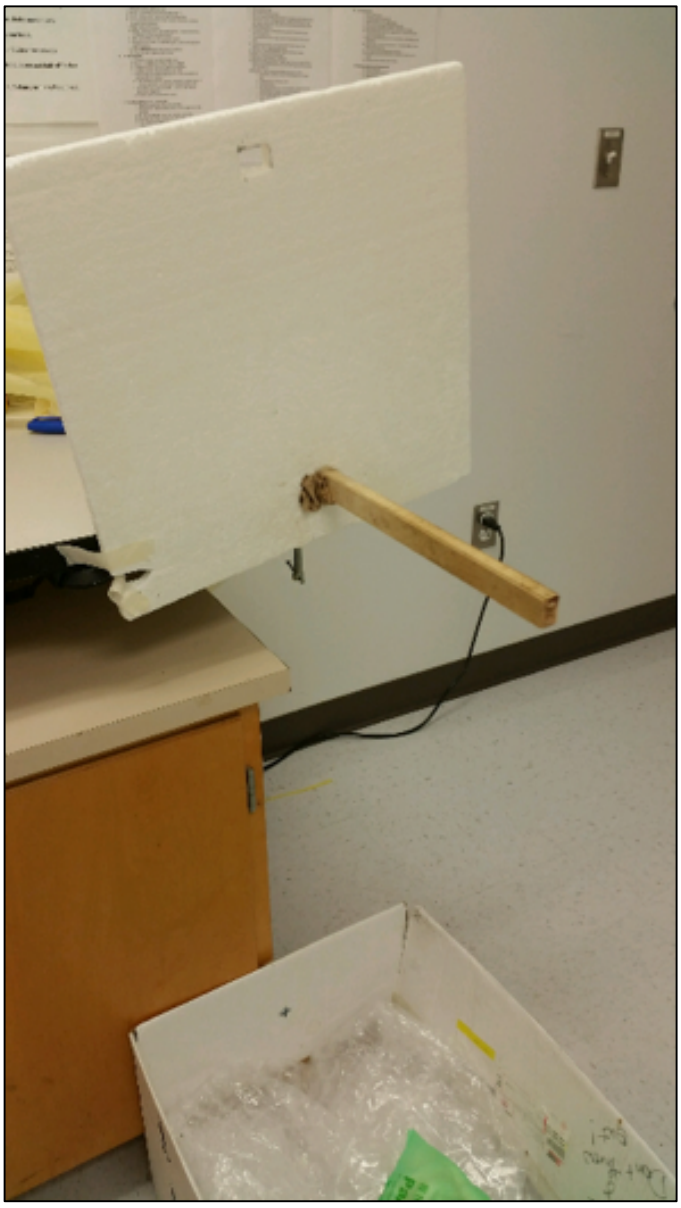

Figure 13: Beam balance apparatus

Set up of the beam balance apparatus. For this task, rats are trained to balance for 60 seconds on a plywood beam approximately 1 foot in length and ½ inch in width that is open-ended on one side with a whiteboard barrier on the other end.

Figure 14: Morris water maze (MWM) pool

(**A**) The water maze consists of a circular pool, 6 feet in diameter filled to a depth of approximately 2.5 feet. The animals escape from the water by locating and climbing onto a transparent Plexiglass goal platform (seen outside and to the right of the pool) 4.5 inches in diameter that stands just beneath the surface of the water. (**B**) Visual cues important for orientation located on the walls of the testing chamber remained constant throughout each testing day.

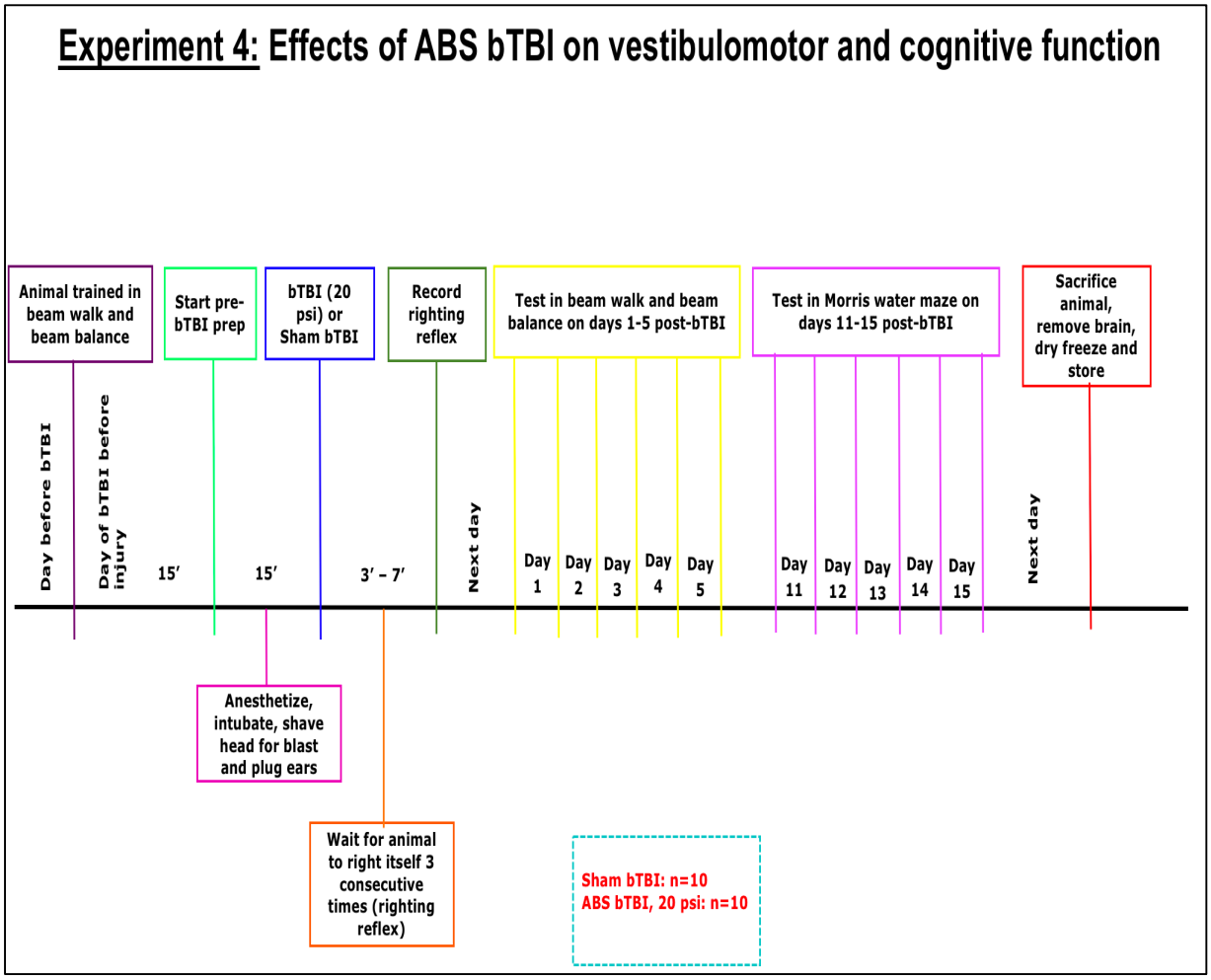

Figure 15: Experiment 4 design

Experimental design and timeline of the effects of ABS bTBI on vestibulomotor and cognitive function. Male Sprague Dawley rats were trained on the beam walk and beam balance tasks on the day before injury and on the day of injury, prior to being injured. Animals were anesthetized, intubated and mechanically ventilated on the day of injury, blast or sham-injured and allowed to recover. On post-injury days 1-5, the animals performances were tested on the beam walk and beam balance tasks. On post-injury days 11-15, animals were tested on the Morris water maze (MWM) task.

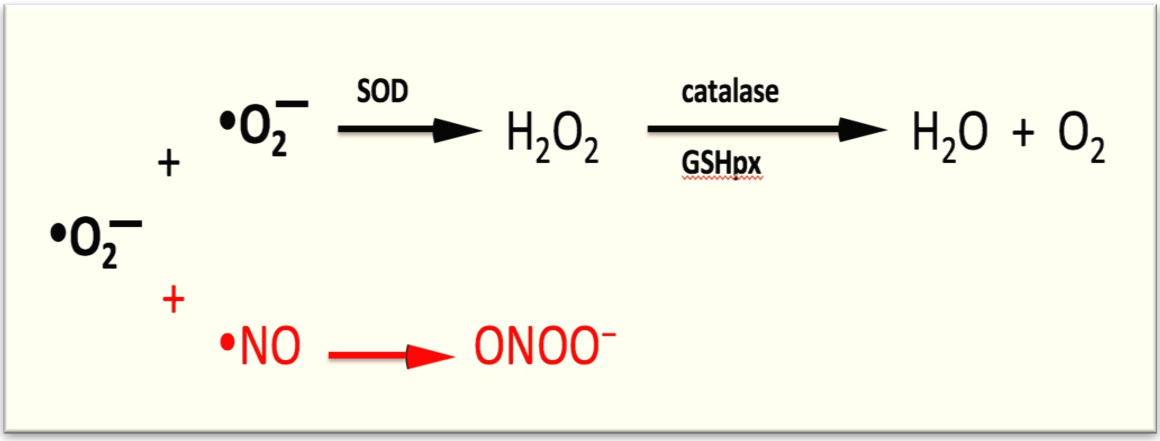

Figure 16: Peroxynitrite formation

Peroxynitrite ($ONOO^-$) is formed when excessive nitric oxide (NO) reacts with superoxide anion ($\bullet O_2^-$) radicals at a rate constant faster than superoxide dismutase (SOD) can scavenge superoxide. Normally, SOD scavenges superoxide anion yielding the formation of hydrogen peroxide (H_2O_2), which is further catalyzed by glutathione peroxidase (GSHpx) into water (H_2O) and oxygen (O_2).

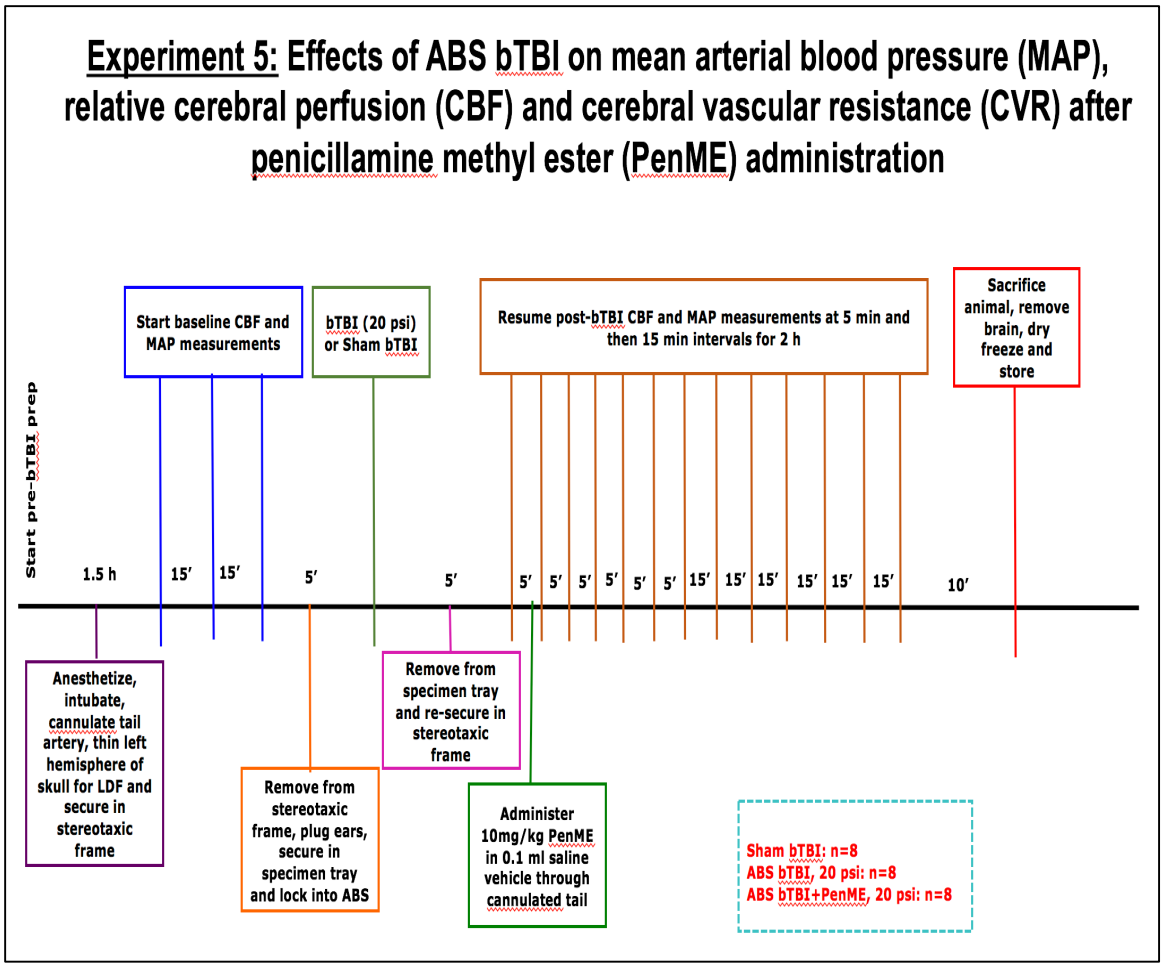

Figure 17: Experiment 5 design

Experimental design and timeline of the effects of ABS bTBI on mean arterial blood pressure (MAP), relative cerebral perfusion (CBF) and cerebral vascular resistance (CVR) after penicillamine methyl ester (PenME) administration. Male Sprague Dawley rats were anesthetized, intubated and mechanically ventilated. The tail artery was cannulated for constant observation of MAP and the left hemisphere of the skull was thinned down. The animals were placed on a stereotaxic frame head-holder, the Doppler probe was positioned over the thinned portion of the skull and baseline MAP and CBF measurements were taken for 30 minutes before injury. Animals were removed from the stereotaxic frame, blast or sham-injured, returned to the frame and the Doppler probe was repositioned on the skull. 5 minutes post-injury, 10 mg/kg of PenME was administered in a 0.1 ml saline vehicle. MAP and CBF were then measured for 2 hours after injury: every 5 minutes for the first 30 minutes after injury followed by every 15 minutes for the remaining hour-and-a-half.

RESULTS

Chapter 3: Study Results

RIGHTING REFLEX

The mean duration of RR suppression for all of the rats subjected to ABS bTBI shockwave exposure (5.19 min ± 2.1) was significantly higher ($P = 0.007$, bTBI vs Sham) than in the Sham group (4.27 min ± 1.6) (**Figure 18**).

EXPERIMENT 1: EFFECTS OF ABS bTBI ON DILATOR RESPONSES TO REDUCED INTRAVASCULAR PRESURE IN ISOLATED MCA SEGMENTS

Mean bTBI overpressure for Experiment 1 was 20.5 psi ± 1.6. In both the 30 and 60-minute Sham groups, MCA diameters increased above baseline as intraluminal pressure was reduced from 100 to 20 mmHg. Dilator responses to progressive reductions in intravascular pressure were significantly reduced in the 30-minute ($P = 0.01$, bTBI vs. Sham) and 60-minute ($P = 0.02$, bTBI vs. Sham) ABS bTBI groups after blast exposure (**Figure 19**).

EXPERIMENT 2: EFFECTS OF ABS bTBI ON MAP, RELATIVE CEREBRAL PERFUSION AND CVR

Mean bTBI overpressure for Experiment 2 was 20.0 psi ± 2.1. To correct for changes in relative perfusion that may have been due to the removal and repositioning of the LDF probe rather than blast-induced changes in perfusion, rats in either group in which perfusion changed more than 20% in either direction of baseline were excluded from the study (see Discussion for details and rationale). Although there was a trend towards elevated MAP in the bTBI group, the differences were not statistically significant ($P =$

0.11, bTBI vs. Sham) (**Figure 20**). In contrast, cerebral perfusion was significantly reduced ($P < 0.0001$, bTBI vs. Sham) (**Figure 21**) and CVR significantly elevated ($P = 0.00042$, bTBI vs. Sham) (**Figure 22**) in the bTBI group for at least two hours post-bTBI.

EXPERIMENT 3: EFFECTS OF ABS bTBI ON CELL INJURY IN THE BRAIN

Mean bTBI overpressure for Experiment 3 was 19.1 psi ± 1.8. The majority of positively stained neuronal cell bodies appeared throughout the cortex in the region overlapping the frontal and parietal/temporal lobes of the sectioned brain. The total numbers of FJC-positive cells were significantly greater 24 hours (566 mean count ± 20.9) ($P = 0.0004$, 24 hour bTBI vs. 24 hour Sham) and 48 hours (456 mean count ± 17.9) ($P = 0.0001$, 48 hour bTBI vs. 48 hour Sham) after ABS bTBI (**Figure 23**) compared to both the 24 hour Sham (147 mean count ± 10.9) and 48 hour Sham (162 mean count ± 11.4) groups, respectively. The mean number of FJC positive cells in the 24 hour bTBI group's frontal lobe region (74 mean count ± 21), parietal/temporal lobe region (462 mean count ± 65.6) and occipital lobe region (38 mean count ± 10.6) were significantly greater than in the 24 hour Sham group's frontal lobe region (20 mean count ± 2.3) ($P = 0.026$, 24 hour bTBI frontal counts vs. 24 hour Sham frontal counts), parietal/temporal region (115 ± 7) ($P < 0.0001$, 24 hour bTBI parietal/temporal counts vs. 24 hour Sham parietal/temporal counts) and occipital region (11 ± 2.4) ($P = 0.035$, 24 hour bTBI occipital counts vs. 24 hour Sham occipital counts) (**Figure 24**). The mean number of FJC positive cells in all three 24 hour bTBI group regions was significantly greater than in all three 24 hour Sham regions ($P < 0.0001$, 24 hour bTBI group vs. 24 hour Sham group). The mean number of FJC positive cells in the 48 hour bTBI group's frontal lobe region (76 mean count ± 19.5) and parietal/temporal lobe region (354 mean count ± 31.3) were significantly greater than

in the 48 hour Sham group's frontal lobe region (23 mean count ± 4.8) ($P = 0.025$, 48 hour bTBI frontal counts vs. 48 hour Sham frontal counts) and parietal/temporal region (130 mean count ± 7.1) ($P < 0.0001$, 48 hour bTBI parietal/temporal counts vs. 48 hour Sham parietal/temporal counts). However, the 48 hour bBTI group's occipital lobe region (28 mean count ± 15.1) was not significantly different from the 48 hour Sham group's occipital region (9 mean count ± 2.4) ($P = 0.239$, 48 hour bTBI occipital counts vs. 48 hour Sham occipital counts) (**Figure 25**). The mean number of FJC positive cells in all three 48 hour bTBI group regions was significantly greater than in all three 48 hour Sham regions ($P < 0.0001$, 48 hour bTBI group vs. 48 hour Sham group). The mean number of FJC positive cells in the frontal lobe region in the 24 hour bTBI group (74 mean count ± 21) and 48 hour bTBI group (76 mean count ± 19.5) and the occipital region in the 24 hour bTBI (38 mean count ± 10.6) and 48 hour bTBI group (28 mean count ± 15.1) were significantly less than those found in the parietal/temporal lobe region in the 24 hour bTBI (462 mean count ± 65.6) and 48 hour bTBI group (354 mean count ± 31.3) ($P = 0.012$, bTBI frontal lobe counts vs. bTBI parietal/temporal lobe counts; $P = 0.010$, bTBI occipital lobe counts vs. bTBI parietal/temporal lobe counts). The mean number of positively stained neuronal cell bodies for the frontal lobe region in both bTBI groups were significantly greater than those in both bTBI groups in the occipital lobe region ($P = 0.007$, bTBI frontal lobe counts vs. bTBI occipital lobe counts) (**Figure 26**).

EXPERIMENT 4: EFFECTS OF ABS bTBI ON VESTIBULOMOTOR AND COGNITIVE FUNCTION

Mean bTBI overpressure for Experiment 4 was 20.9 ± 0.7. Beam walk performance was not significantly different between the bTBI and Sham groups ($P = 0.2$, bTBI vs.

Sham) (**Figure 27**) even though there appeared to be a trend of improved performance between days 1 – 4 in the Sham group. Although there was a trend towards impaired beam balance performance in the bTBI group and improved performance in the Sham group throughout all days tested, the differences were not significantly different ($P = 0.06$, bTBI vs. Sham) (**Figure 28**). Similarly, though there was a trend towards longer MWM latencies to the goal platform in the bTBI group (38.2 seconds ± 3.4) compared to the Sham group (30.2 seconds ± 3.2) across days eleven through fourteen, these latencies were not significantly different between the Sham and bTBI groups ($P = 0.067$, bTBI vs. Sham) (**Figure 29**). However, the inter-trial (trial 1 vs. trial 2 for each pair of trials) differences in latencies were significantly longer for the Sham group compared to the bTBI group in each post-injury day tested ($P = 0.01$, bTBI vs. Sham) (**Figure 30**), indicating that the non-injured animals were able to remember where to find the platform quicker during the second trial, making the difference between the pair of trials larger compared to an animal with impaired working memory who will have shorter differences between the pair of trials.

EXPERIMENT 5: EFFECTS OF ABS bTBI ON MAP, RELATIVE CEREBRAL PERFUSION AND CVR AFTER PenME ADMINISTRATION

Mean bTBI overpressure for Experiment 5 was 20.6 psi ± 2.3. MAP was significantly elevated in the bTBI group compared to the Sham group ($P < 0.01$, bTBI vs. Sham) and the bTBI + PenME group ($P = 0.001$, bTBI vs. bTBI + PenME). However, there were no significant differences between the Sham and bTBI + PenME groups ($P = 0.24$, Sham vs bTBI + PenME) (**Figure 31**), suggesting a possible therapeutic effect of PenME. Relative cerebral perfusion was significantly reduced in the bTBI ($P < 0.0001$, bTBI vs.

Sham) and bTBI + PenME groups ($P < 0.0001$, bTBI + PenME vs. Sham) for at least two hours after mild bTBI. Although there was a trend towards increased perfusion in the bTBI + PenME group beginning 30 minutes post-injury, there were no statistically significant differences in perfusion between the bTBI and bTBI + PenME groups ($P = 0.11$, bTBI vs. bTBI + PenME) (**Figure 32**). Cerebral vascular resistance was significantly elevated in the bTBI group compared to the Sham ($P < 0.01$, bTBI vs. Sham) and bTBI + PenME groups ($P < 0.0001$, bTBI vs. bTBI + PenME). Interestingly, CVR was significantly lower in the bTBI + PenME group compared to the Sham group ($P < 0.0001$, bTBI + PenME vs. Sham) as well as the bTBI group ($P < 0.0001$, bTBI + PenME vs. bTBI) for at least two hrs post-bTBI (**Figure 33**).

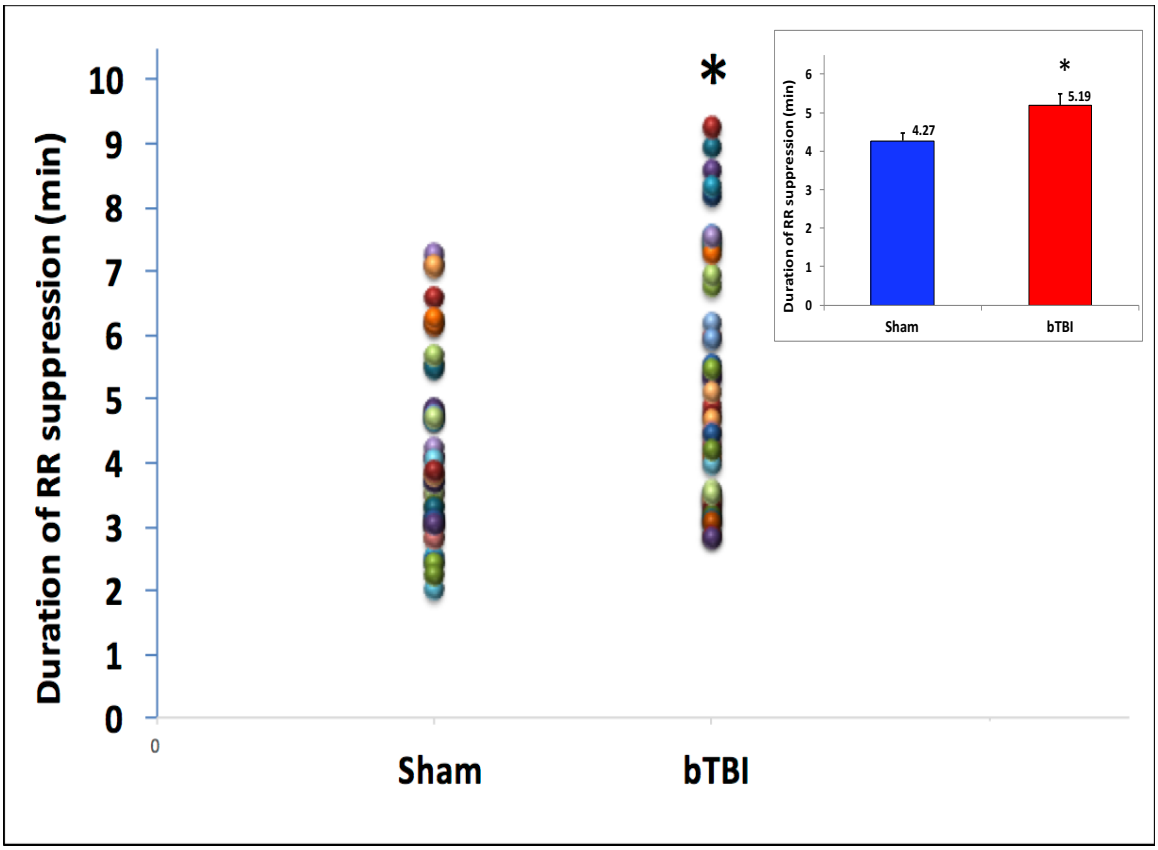

Figure 18: Effects of bTBI on righting reflex (RR) suppression

Duration of RR suppression (n=46/group) was significantly elevated in the bTBI group compared to the Sham group. Mean duration of RR suppression (inset) for the bTBI group (5.19 min ± 2.1) was significantly longer (P = 0.007, bTBI vs. Sham) than in the Sham group (4.27 min ± 1.6). The bTBI group's duration of RR suppression is considered within the range of mild bTBI injury. Values are plotted as means ± SEM. *P = 0.007 vs. Sham.

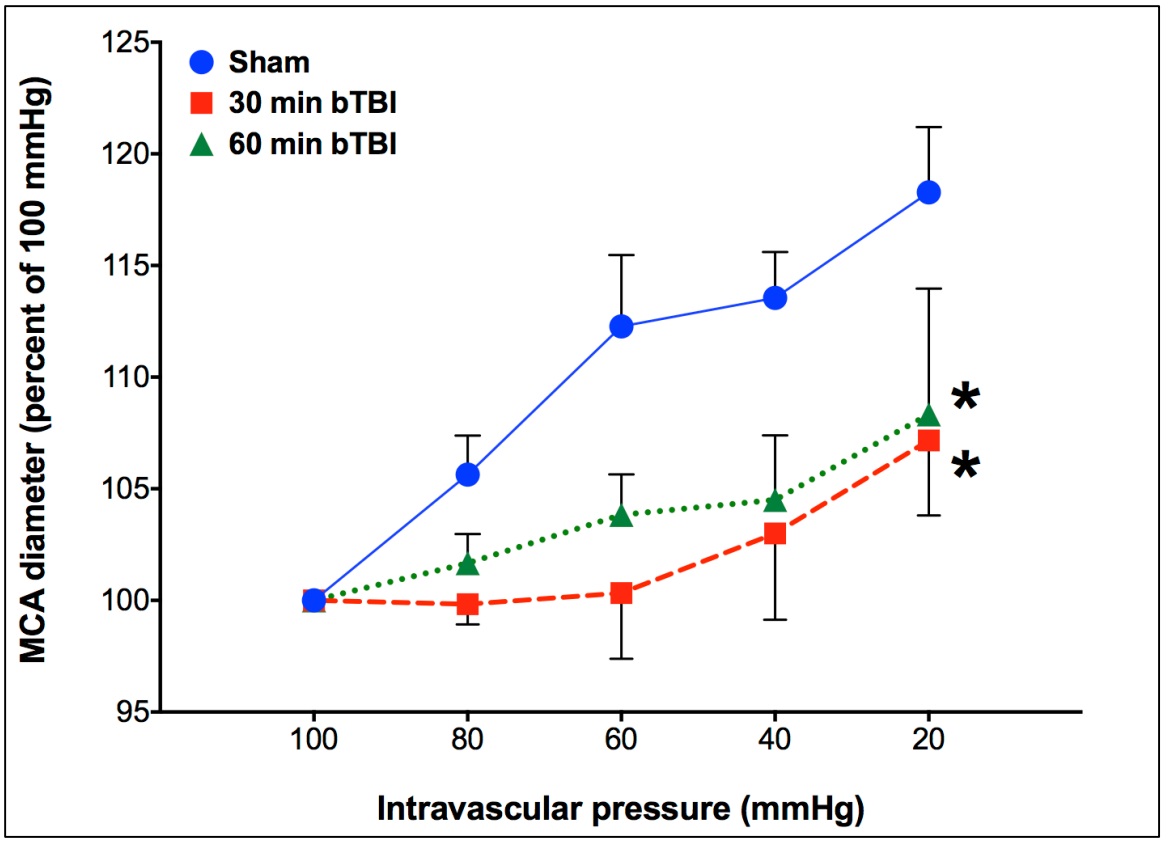

Figure 19: Effects of bTBI on middle cerebral arterial (MCA) responses to reduced intravascular pressure

Dilator responses to progressive reductions in intravascular pressure exhibited impaired vasodilatory responses and were significantly reduced in the 30-minute ($P = 0.01$, bTBI vs. Sham) and 60-minute ($P = 0.02$, bTBI vs. Sham) bTBI groups (n=6/group) after blast exposure compared to the Sham group (n=12). In both the 30 and 60-minute Sham groups, MCA diameters increased above baseline as intraluminal pressure was reduced from 100 to 20 mmHg. Values are plotted as means ± SEM. *$P < 0.05$ vs. Sham.

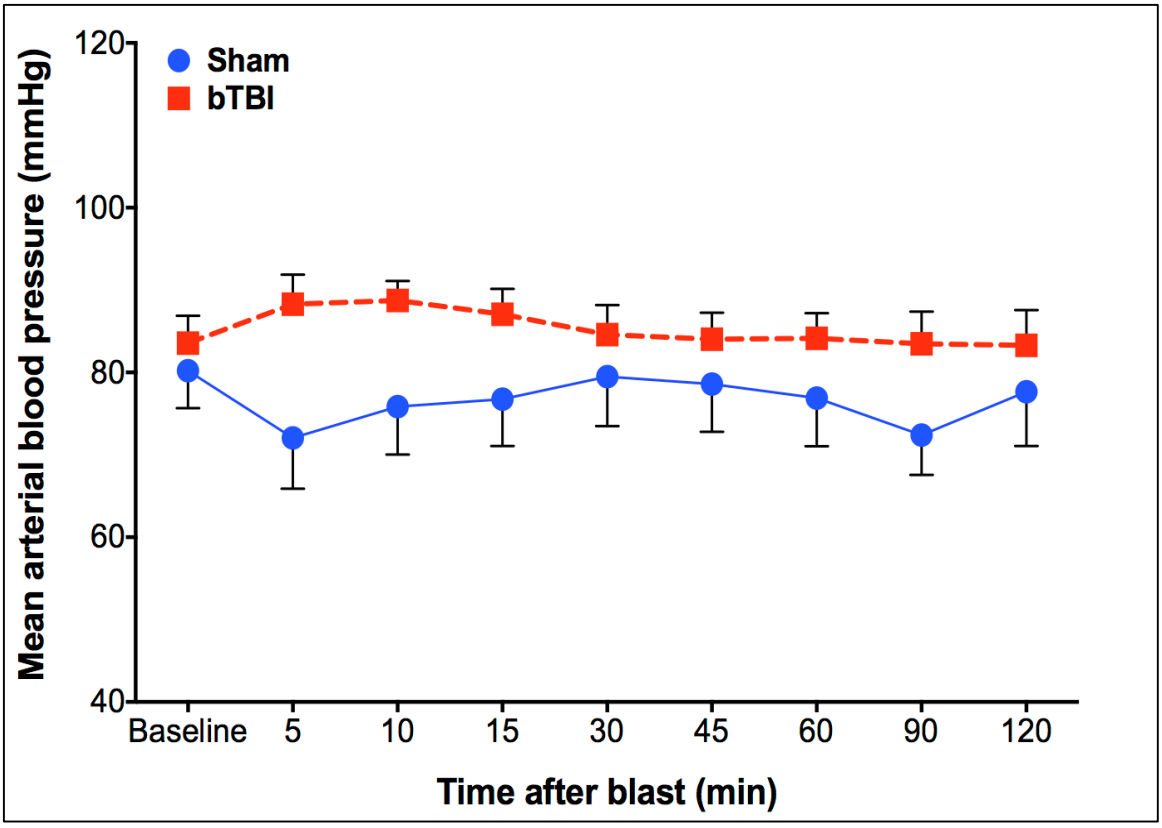

Figure 20: Effects of bTBI on mean arterial blood pressure (MAP)

Although there was a trend towards elevated MAP in the bTBI group (n=12) for at least two hours after injury, the difference in MAP between the bTBI and Sham (n=10) group was not significant ($P = 0.11$, bTBI vs. Sham). Values are plotted as means ± SEM.

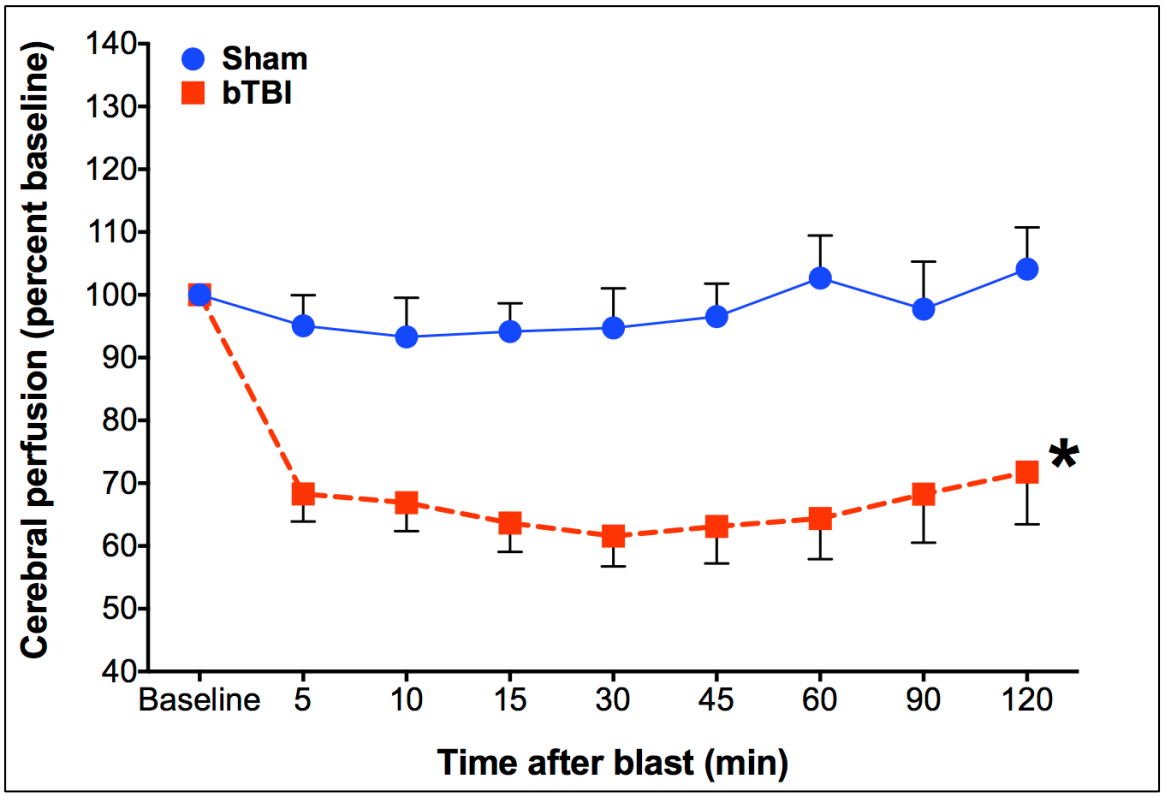

Figure 21: Effects of bTBI on relative cerebral perfusion

Relative cerebral perfusion was significantly reduced ($P < 0.0001$, bTBI vs. Sham) in the bTBI group (n=12) compared to the Sham group (n=10) for at least two hours after injury. To correct for changes in relative perfusion that may have been due to the removal and repositioning of the LDF probe rather than blast-induced changes in perfusion, rats in either group in which perfusion changed more than 20% in either direction of baseline were excluded from the study. Values are plotted as means ± SEM. *$P < 0.0001$ vs. Sham.

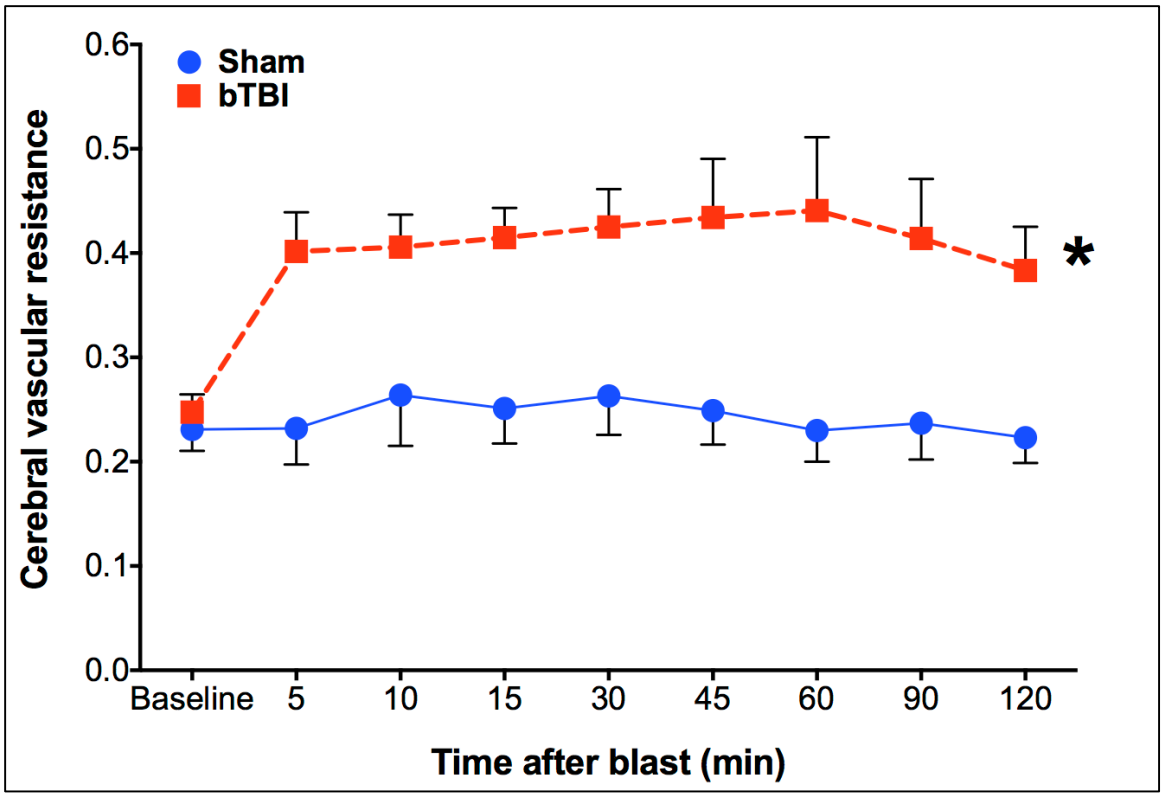

Figure 22: Effects of bTBI on cerebral vascular resistance (CVR)

CVR was significantly elevated ($P = 0.00042$, bTBI vs. Sham) in the bTBI group (n=12) compared to the Sham group (n=10) for at least two hours after injury. Values are plotted as means ± SEM. *$P < 0.001$ vs. Sham.

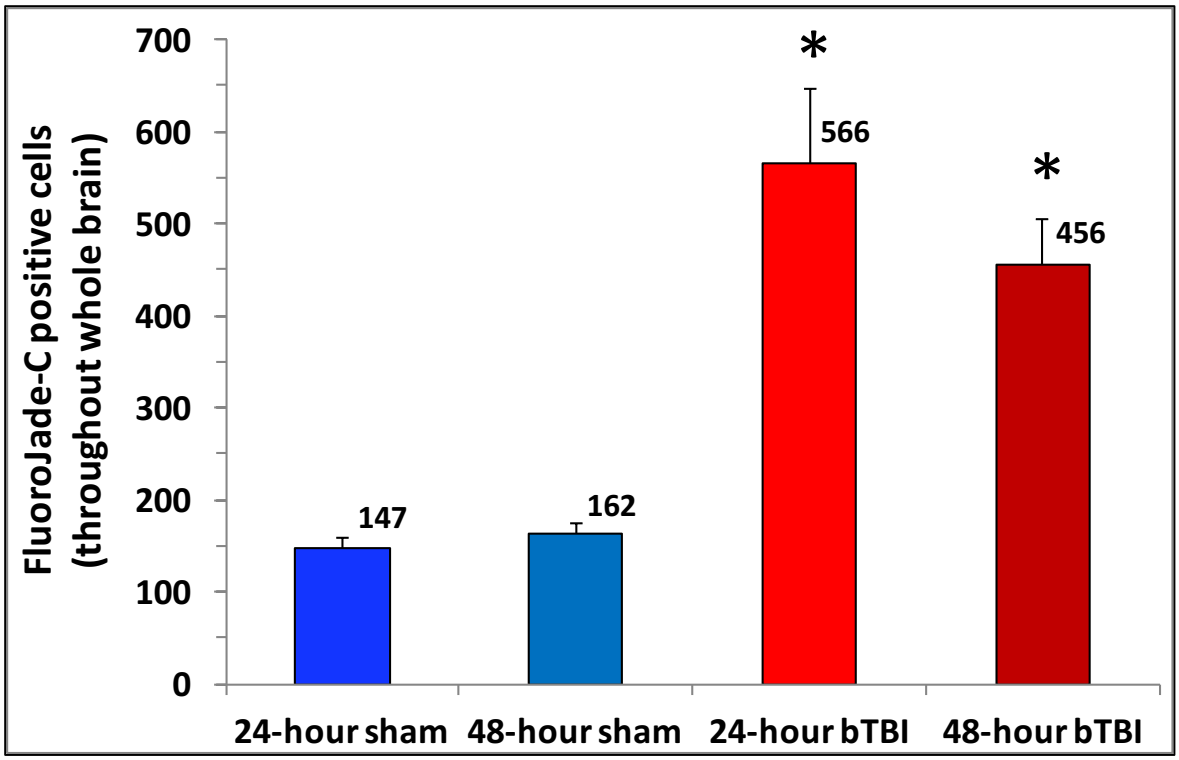

Figure 23: Effects of bTBI on cell injury in the brain

The number of FluoroJade-C (FJC) positive cells counted throughout sixty sections taken from the whole brain were significantly greater 24 hours (n=6) (566 ± 20.9) (P = 0.0004, 24 hour bTBI vs. 24 hour Sham) and 48 hours (n=6) (456 ± 17.9) (P = 0.0001, 48 hour bTBI vs. 48 hour Sham) after bTBI compared to both the 24 hour Sham (n=6) (147 ± 10.9) and 48 hour Sham (n=6) (162 ± 11.4) groups. Values are plotted as means ± SEM. *P < 0.001 vs. Sham.

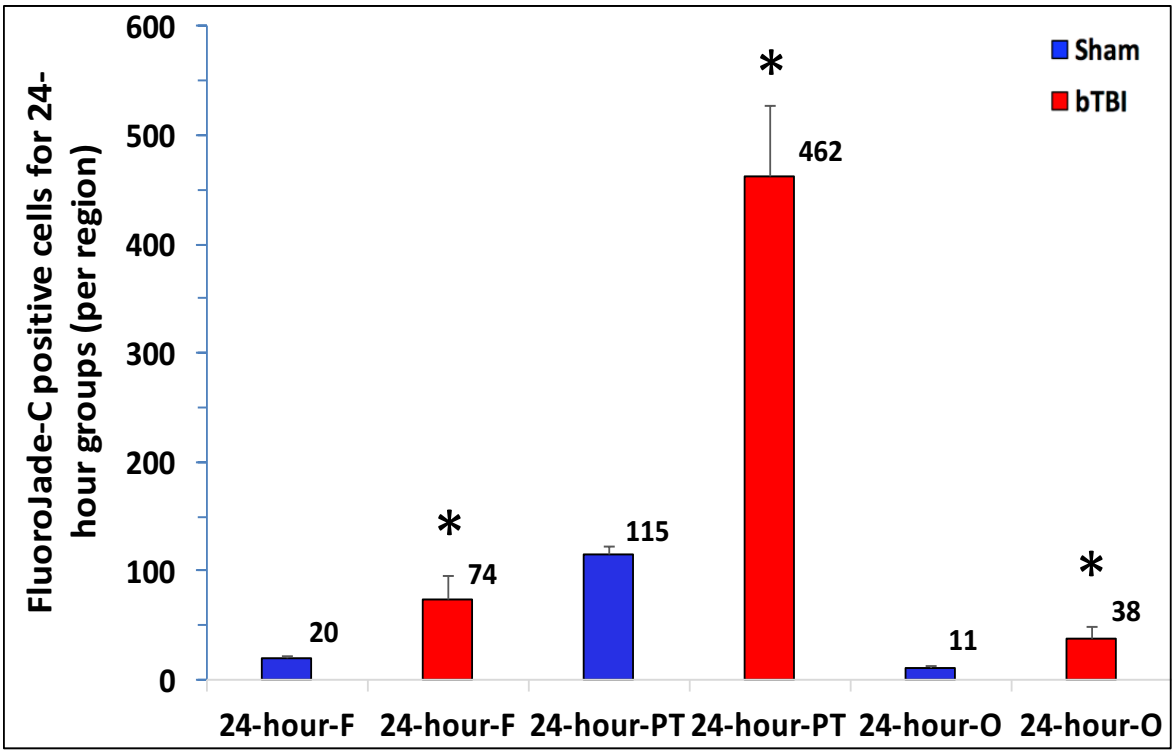

Figure 24: Effects of bTBI on cell injury in the brain – 24-hour group regions

The number of FluoroJade-C (FJC) positive cells in all three 24 hour bTBI group (n=6) regions was significantly greater than in all three Sham (n=6) regions ($P < 0.0001$, bTBI group vs. Sham group). The number of FJC positive cells in the bTBI group's frontal lobe (F) region (n=6) (74 ± 21), parietal/temporal lobe (PT) region (n=6) (462 ± 65.6) and the occipital lobe (O) region (n=6) (38 ± 10.6) were significantly greater than in the Sham group's F region (n=6) (20 ± 2.3) ($P = 0.026$, F bTBI vs. F Sham), PT region (n=6) (115 ± 7) ($P < 0.0001$, PT bTBI vs. PT Sham) and O region (n=6) (11 ± 2.4) ($P = 0.035$, O bTBI vs. O Sham). Values are plotted as means ± SEM. *$P < 0.05$ vs. Sham.

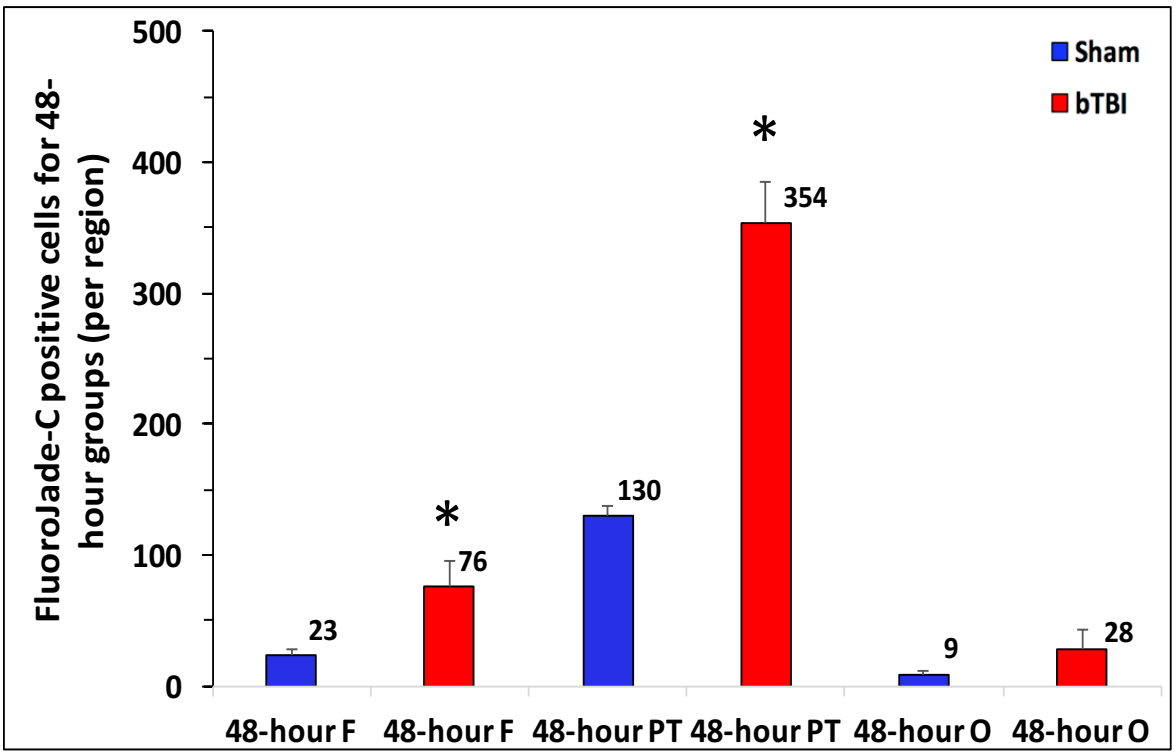

Figure 25: Effects of bTBI on cell injury in the brain – 48-hour group regions

The number of FluoroJade-C (FJC) positive cells in all three 48 hour bTBI group (n=6) regions was significantly greater than in all three Sham (n=6) regions ($P < 0.0001$, 48 hour bTBI group vs. 48 hour Sham group). The number of FJC positive cells in the bTBI group's frontal lobe (F) region (n=6) (76 ± 19.5) and parietal/temporal lobe (PT) region (n=6) (354 ± 31.3) were significantly greater than in the Sham group's F region (n=6) (23 ± 4.8) ($P = 0.025$, F bTBI vs. F Sham) and PT region (n=6) (130 ± 7.1) ($P < 0.0001$, PT bTBI vs. PT Sham). However, the bBTI group's occipital lobe (O) region (n=6) (28 ± 15.1) was not significantly different from the Sham group's O region (n=6) (9 ± 2.4) ($P = 0.239$, O bTBI vs. O Sham). Values are plotted as means ± SEM. *$P < 0.05$ vs. Sham.

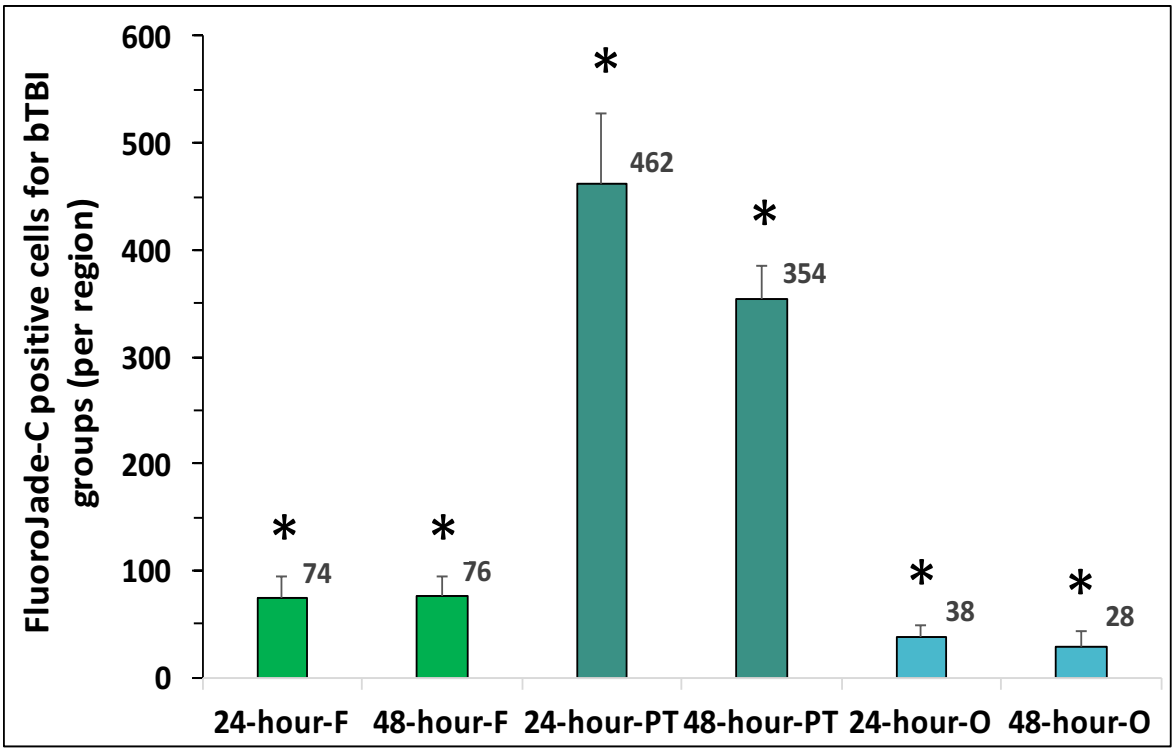

Figure 26: Effects of bTBI on cell injury in the brain – bTBI group regions

The number of FluoroJade-C (FJC) positive cells in the bTBI group's frontal lobe (F) 24 hour group (n=6) (74 ± 21) and 48 hour group (n=6) (76 ± 19.5) and in the occipital lobe's (O) 24 hour group (n=6) (38 ± 10.6) and 48 hour group (n=6) (28 ± 15.1) were significantly less than those found in the group's parietal/temporal lobes (PT) in the 24 hour group (n=6) (462 ± 65.6) and 48 hour group (n=6) (354 ± 31.3) ($P = 0.012$, F vs. PT; $P = 0.010$, O vs. PT). The number of FJC positive cells in both F groups were significantly greater than those in both O groups ($P = 0.007$, F vs. O). Values are plotted as means ± SEM. *$P < 0.05$ vs. each of the other two groups.

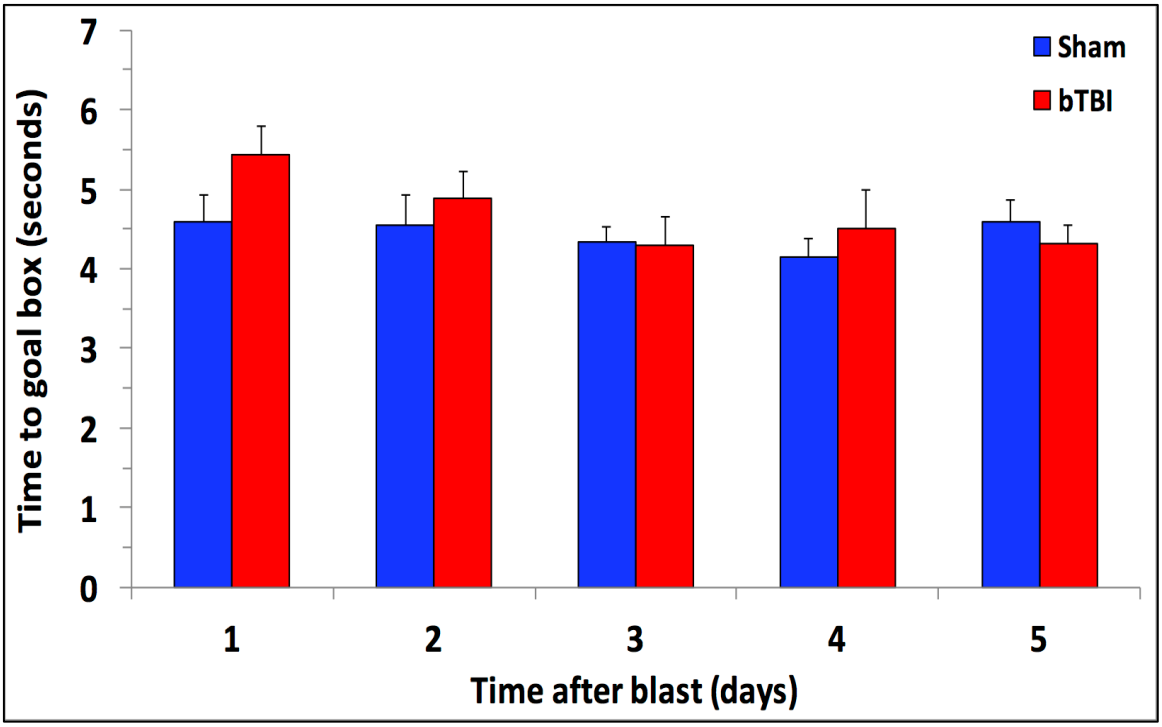

Figure 27: Effects of bTBI on beam walk performance

Beam walk performance was not statistically significantly worse in the bTBI group (n=10) compared to the Sham group (n=10) ($P = 0.2$, bTBI vs. Sham) even though there appeared to be a trend of improved performance between days 1 – 4 in the Sham group. Values are plotted as means ± SEM.

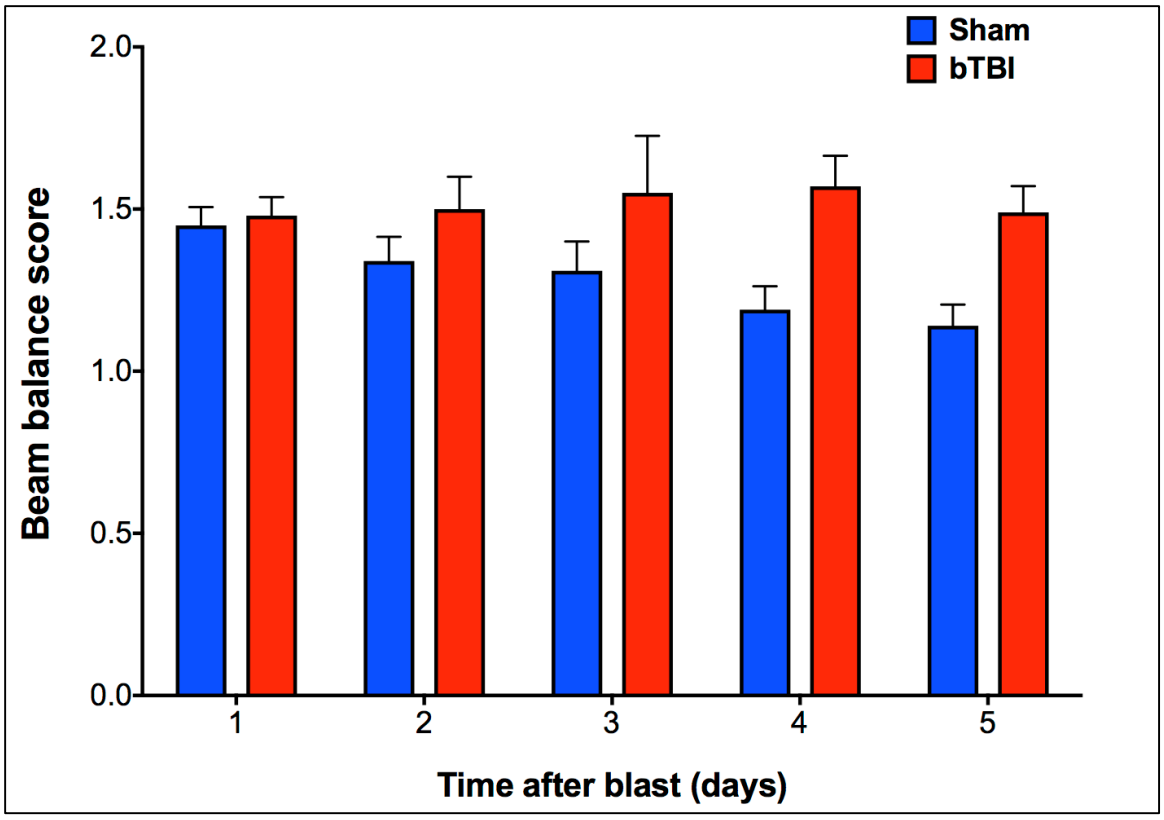

Figure 28: Effects of bTBI beam balance performance

Although there was a trend towards impaired beam balance performance in the bTBI group and improved performance in the Sham group throughout all days tested, performance was not significantly worse in the bTBI group (n=10) compared to the Sham group (n=10) (P = 0.06, bTBI vs. Sham). Values are plotted as means ± SEM.

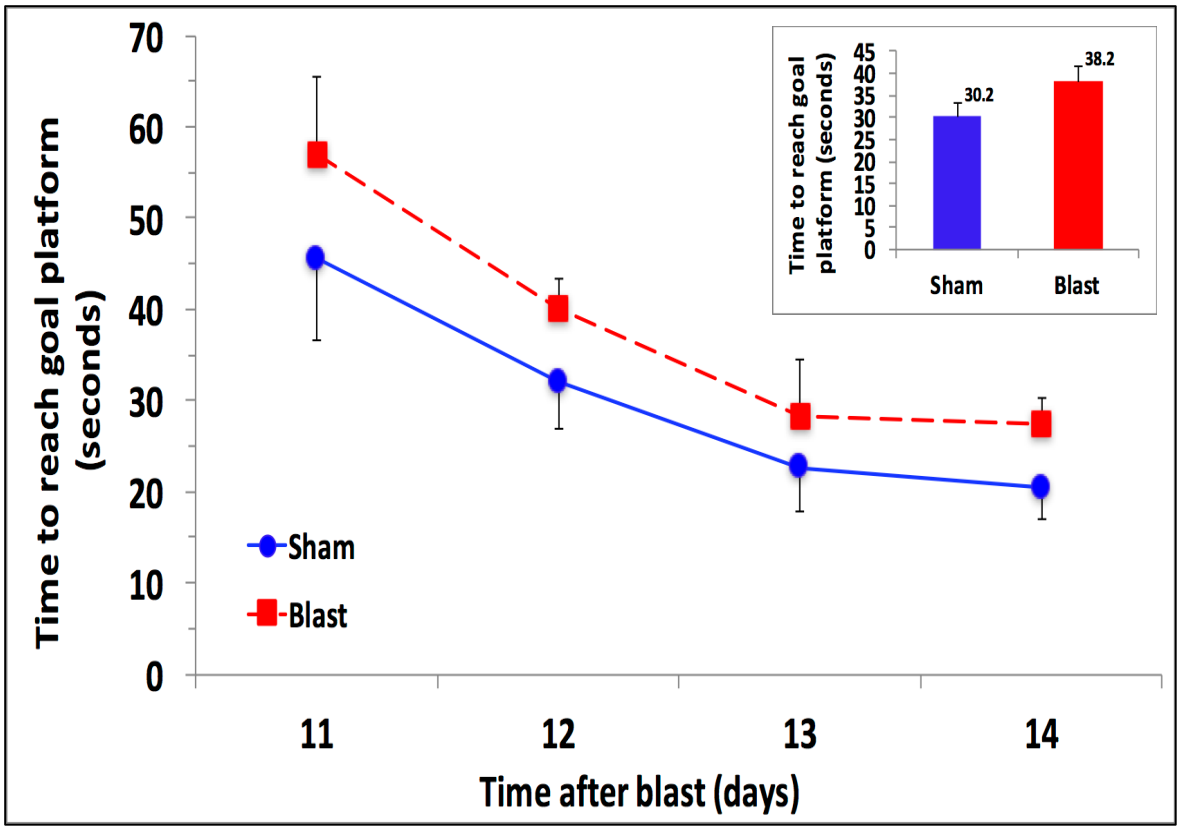

Figure 29: Effects of bTBI on working memory performance – overall latencies

Though there was a trend towards longer overall Morris water maze (MWM) latencies to locate the goal platform (inset) in the bTBI group (n=10) (38.2 sec ± 3.4) across days 11 – 14 post-injury, these latencies were not significantly different ($P = 0.067$, bTBI vs. Sham) from the Sham group (n=10) (30.2 sec ± 3.2). Values are plotted as means ± SEM.

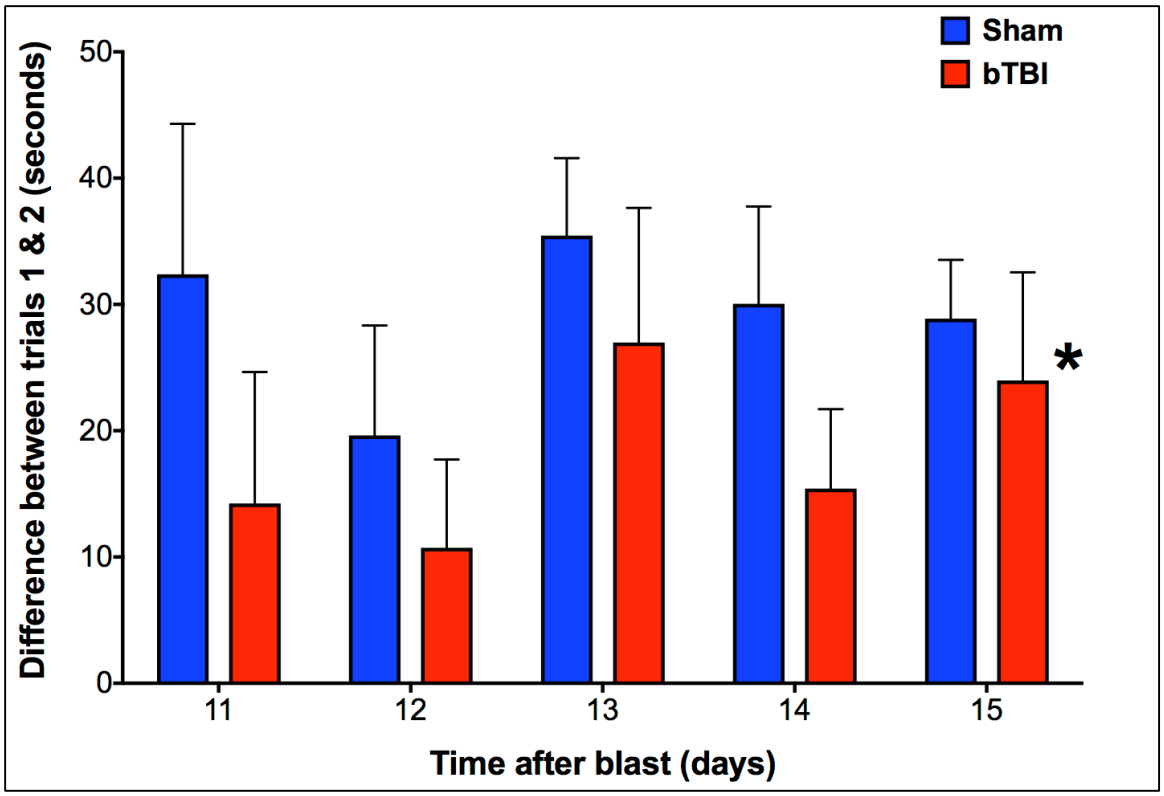

Figure 30: Effects of bTBI on working memory performance – latencies between trial pairs

The differences in latencies to the goal platform between the first and second trials for each pair of trials were significantly longer for the Sham group (n=10) compared to the bTBI group (n=10) in each post-injury day tested ($P = 0.01$, bTBI vs. Sham), indicating impaired working memory in the bTBI group as the injured animals took significantly longer than the Sham animals to find the goal platform on the second trial for each pair of trials on each day tested. Means for each group across all days tested as a whole were compared. Values are plotted as means ± SEM. *$P < 0.01$ vs. Sham.

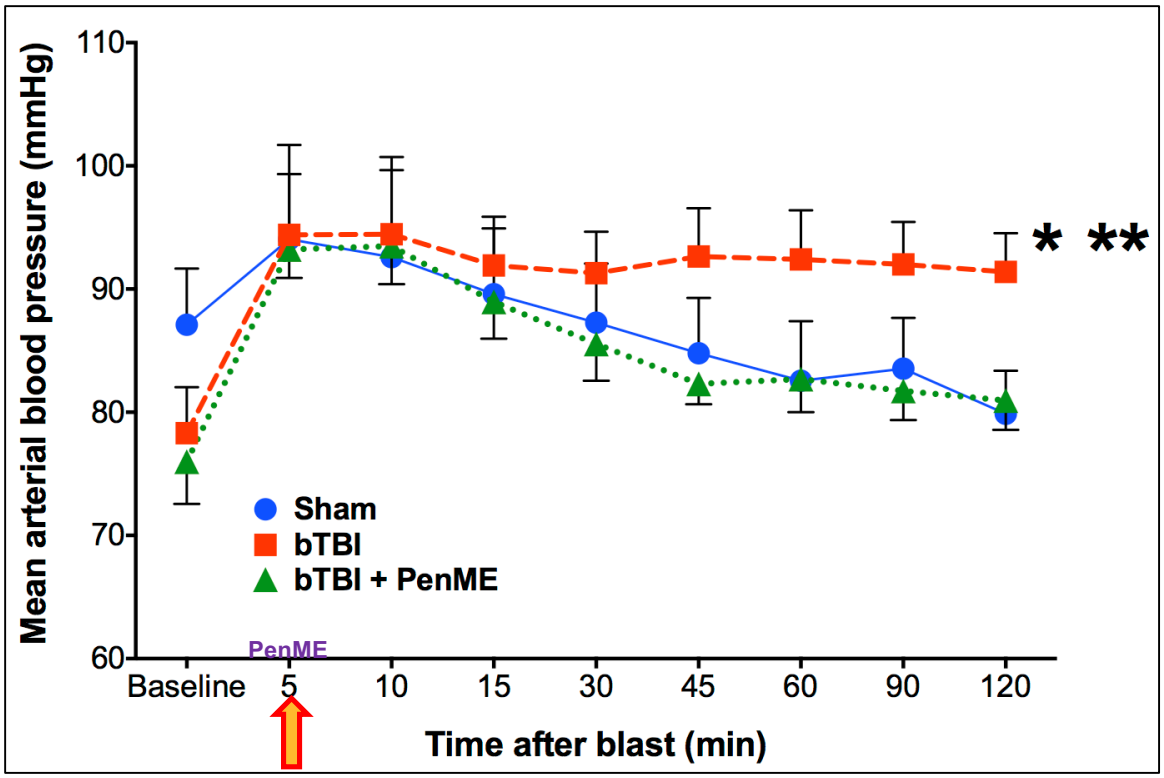

Figure 31: Effects of bTBI on mean arterial blood pressure (MAP) after penicillamine methyl ester (PenME) treatment

MAP was significantly elevated in the bTBI group (n=8) compared to the Sham group (n=8) ($P < 0.01$, bTBI vs. Sham) and bTBI+PenME group (n=8) ($P = 0.001$, bTBI vs. bTBI+PenME) for at least two hours after bTBI. However, there were no statistically significant differences between the Sham and bTBI+PenME groups ($P = 0.24$, Sham vs. bTBI+PenME), suggesting $ONOO^-$ and/or ROS scavenging as well as a possible therapeutic effect of PenME. Values are plotted as means ± SEM. *$P < 0.01$ vs. Sham; **$P = 0.001$ vs. bTBI+PenMe.

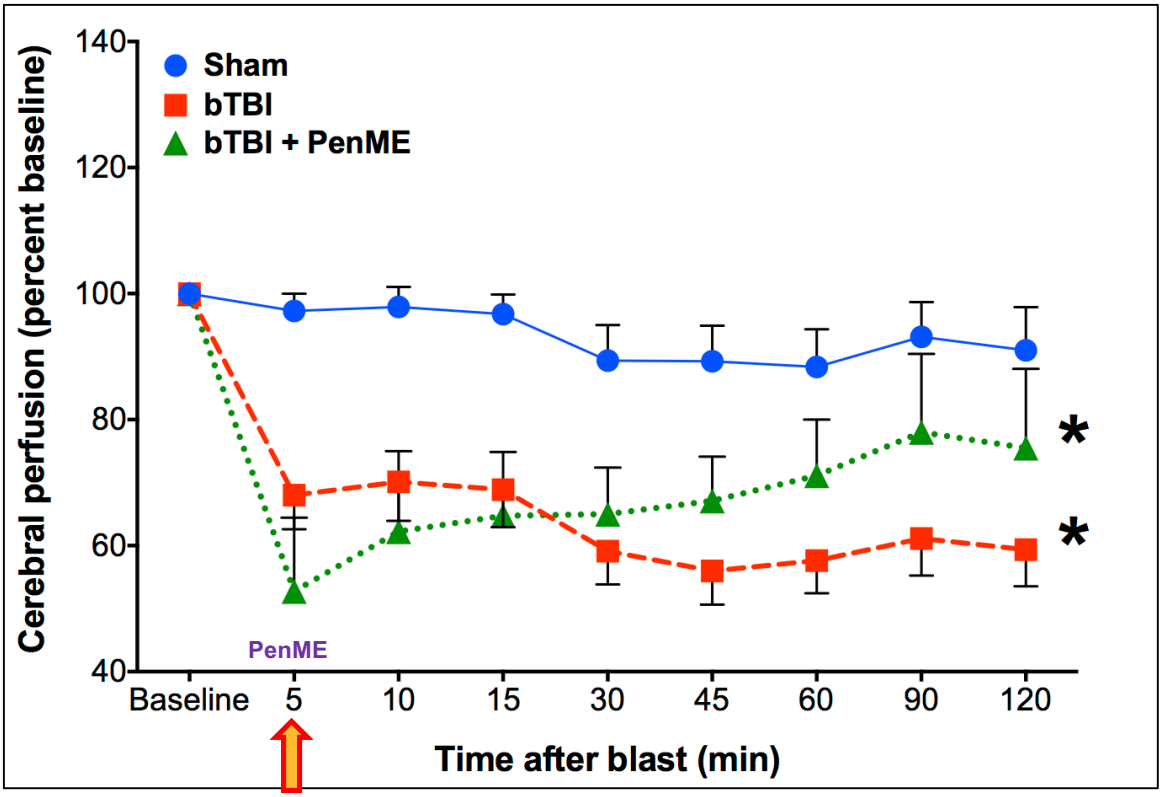

Figure 32: Effects of bTBI on relative cerebral perfusion after penicillamine methyl ester (PenME) treatment

Relative cerebral perfusion was significantly reduced in the bTBI (n=8) ($P < 0.0001$, bTBI vs. Sham) and bTBI+PenME group (n=8) ($P < 0.0001$, bTBI+PenME vs. Sham) compared to the Sham group (n=8) for at least two hours after bTBI. Although there was a trend towards increased perfusion in the bTBI+PenME group beginning 30 minutes post-injury, there were no statistically significant differences in perfusion between the bTBI and bTBI+PenME groups ($P = 0.11$, bTBI vs. bTBI+PenME). Values are plotted as means ± SEM. *$P < 0.0001$ vs. Sham.

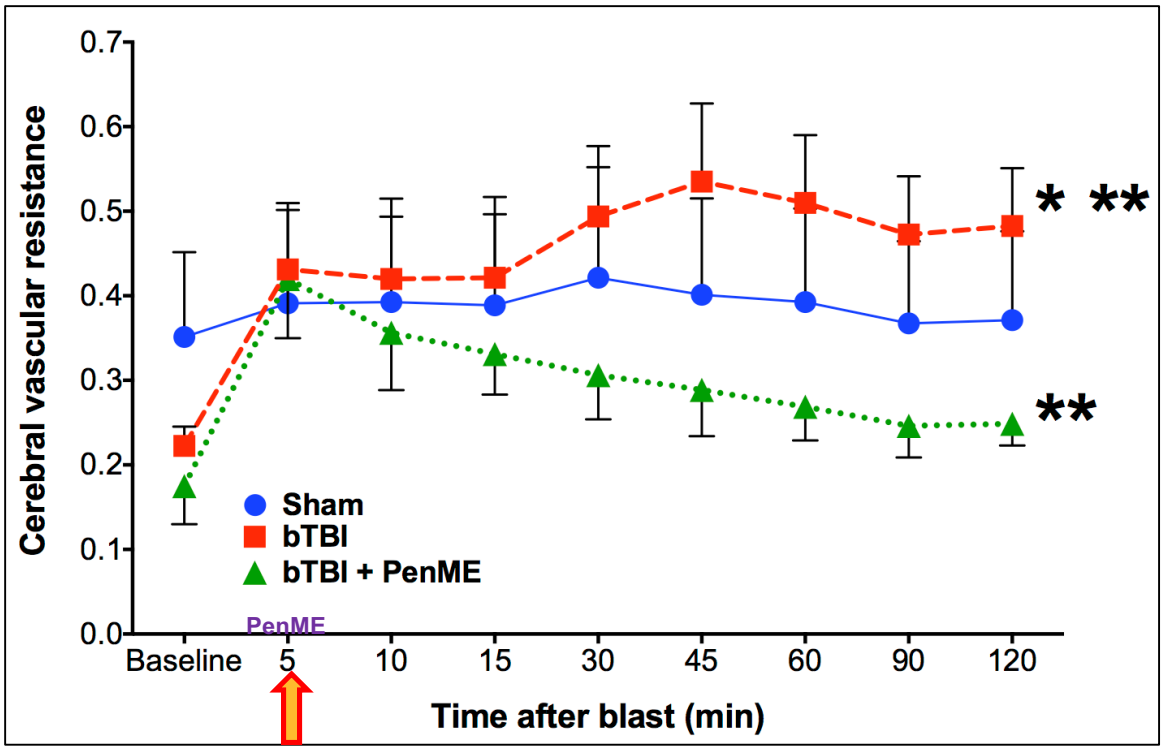

Figure 33: Effects of bTBI on cerebral vascular resistance (CVR) after penicillamine methyl ester (PenME) treatment

CVR was significantly elevated in the bTBI group (n=8) compared to both the Sham group (n=8) ($P < 0.01$, bTBI vs. Sham) and bTBI+PenME group (n=8) ($P < 0.0001$, bTBI vs. bTBI+PenME) but significantly reduced in the bTBI+PenME group compared to the both the Sham group ($P < 0.0001$, bTBI+PenME vs. Sham) and bTBI group ($P < 0.0001$, bTBI+PenME vs. bTBI) for at least two hours after bTBI. Values are plotted as means ± SEM. *$P < 0.01$ vs. Sham; **$P < 0.0001$ vs. Sham and vs. bTBI+PenME.

DISCUSSION

Chapter 4: Discussion and Conclusions

These studies revealed that mild bTBI significantly impaired cerebral compensatory dilator responses to reduced intravascular pressure in MCA segments 30 and 60 minutes after mild bTBI, reduced relative cerebral perfusion without significantly altering MAP for at least two hours after injury and increased CVR, an observation consistent with previous reports of blast-induced vasospasm. Additionally, mild bTBI resulted in significantly impaired working memory up to two-weeks post-bTBI and led to increased numbers of FJC-positive cells throughout the cortex of the rat brain with the greater quantity located amongst the areas overlapping the frontal and parietal/temporal lobes. Significant reductions in blast-induced increases in MAP and significant reductions in CVR after treatment with the $ONOO^-$ scavenger PenME suggest that blast-induced cerebral vascular dysfunction may be due, in part, to the action of oxidants such as $ONOO^-$.

Although shock wave exposure was associated with impaired cerebral perfusion and dilator responses to reduced intravascular pressures, neuronal injury and cognitive dysfunction, the mild shock wave levels used in these studies resulted in durations of suppression of the RR (< 1 minute) only slightly higher than those in Sham-injured rats. Due to the nature of Experiment 2 and 5's protocol of having MAP and relative cerebral perfusion measured at the 5 minute time point post-blast, duration of RR suppression was only recorded for the animals in Experiment 1, 3 and 4.

In experimental studies of bTBI, as in non-blast TBI, there is some overlap between overpressure levels considered to result in "mild" or "moderate" bTBI (DeWitt

et al., 2013). Comparisons across different models of blast injury are difficult due to numerous factors such as variations in the magnitude of blast overpressure ranges used for injury, the assorted details of the experimental setup such as the location of the animal (inside vs. out) relative to the shock tube and the size, length, and type of shock or blast tube used (Cernak, 2005; Cernak and Noble-Haeusslein, 2010; Risling and Davidsson, 2012). Inconsistencies in these blast experiment methodologies confound the proper characterization of injury severity level. A major ambiguity is the duration of suppression of the RR and what range constitutes a mild, moderate or severe bTBI. To that end, my study based our definition of mild bTBI on a duration of suppression of RR less than or equal to 7 minutes; a moderate injury between 8 – 14 minutes; and a severe injury more than 14 minutes (Readnower et al., 2010; DeWitt et al., 2013; Rosenfeld et al., 2013; Wang et al., 2014; Perez-Polo et al., 2015). The RR is a brainstem reflex that is widely used in studies of the effects of anesthetics (Tung et al., 2008; Nguyen et al., 2009) or brain injury (Henninger et al., 2005; Pang et al., 2006; Raghupathi and Huh, 2007). Duration of suppression of the RR is considered to be analogous to duration of loss of consciousness, one of the most consistent criteria for the definition of mild TBI in humans (DeWitt et al., 2013). However, while most definitions of mild TBI in humans assume normal imaging, mild TBI in experimental animals may be associated with significant neuronal injury (for review, see DeWitt et al., 2013).

The pathobiology of primary bTBI consists of a complex set of systemic, cerebral and cerebral vascular events that begin at blast exposure and likely continue for hours to days or weeks afterwards (Long et al., 2009; Cernak and Noble-Haeusslein, 2010; Nakagawa et al., 2011; Risling et al., 2011). Bauman et al. (1997) observed that sublethal

blast overpressure exposure reduced food consumption and exercise performance (rats), possibly due to the development of diffused hemorrhage as well as the progression of pulmonary oxidative and inflammatory responses in the lungs that Chavko et al. (2006) detected, ultimately leading to lung damage. Cheng at al. (2010) noted that 87% of study rats also developed a poor appetite in addition to apnea, limb seizure, and limpness. Long et al. (2009) and Bauman et al. (2009) observed the appearance of widespread white matter fiber degeneration in rats and swine respectively, and astrocytosis (swine) while Ling et al. (2009) reviewed and reported on the clinical finding that exposure to severe bTBI lead to the development of pseudoaneurysms (humans) with both studies observing vasospasm in a significant number of study subjects. Some studies revealed capillary damage, diffuse subarachnoid hemorrhage and edema within the brain parenchyma as well as enlarged intercellular and vascular space in the cortex (Cheng et al., 2010), a dose-dependent rise in ICP (Saljo et al., 2009) and discerned significantly increased BBB permeability at 3 and 24 hours post-blast, increased levels of 4-hydroxynonenal (4-HNE) and 3-nitrotyrosine (3-NT) at 3 hours post-blast and an activated microglia morphology in the hippocampus of animals exposed to blast (Readnower et al., 2010). Tompkins et al. (2013) detected tissue damage, apoptosis initiation and an early inflammatory response within 1 hour post-blast, which remained measureable for up to 3 weeks post-injury. Neuronal swelling (Cernak et al. (2001b), neuronal and glial cells loss (Ahmed et al., 2013), glial and myelin debris in the hippocampus (Cernak et al., 2001b), the development of lasting oxidative stress (Cernak et al., 2001b; Ahmed et al., 2013) and altered antioxidant enzyme defenses (Cernak et al., 2001b) have all been discerned after blast exposure. Predictably, significant impairments

in neurologic and neurobehavioral function in rats on tasks such as rotametric and grip-strength tests (Moochala et al., 2004), beam walking (Long et al., 2009) active avoidance task (Cernak et al., 2001b) and a delayed navigational acquisition of the goal platform in the Morris water maze (Long et al., 2009; Saljo et al., 2009; Tompkins et al., 2013) were also observed. All these results indicate that BBB breakdown, oxidative stress, and microglia activation likely play a role in the neuropathology associated with TBI as a result of blast exposure.

Although the studies above and others focused on the histopathological and behavioral effects of blast exposure (Bauman et al., 1997; Cernak et al., 2001b; Moochhala et al., 2004; Chavko et al., 2006; Park et al., 2007; Bauman et al., 2009; Chen et al., 2009; Elder and Cristian, 2009; Ling et al., 2009; Long et al., 2009; Säljö et al., 2009; Cheng et al., 2010; Elder et al., 2010; Readnower et al., 2010; Chavko et al., 2011; Park et al., 2011; Masel et al., 2012; Ahmed et al., 2013; Tompkins et al., 2013), there have been fewer dedicated to blast's cerebral vascular effects. Previous research suggests that bTBI is associated with cerebral vascular injury (for review, see Elder et al., 2015). Pun et al. (2011) observed histological evidence of narrowing of cerebral vessels in rats one and four days after low level (7.1 or 11.3 psi), whole body explosive blast exposure. Gama Sosa et al. (2014) reported abnormal collagen IV and laminin staining in the primary visual cortex in rats 24 hours after shock wave exposure (10.8 psi) that persisted for up to 10 months post-blast as well as ultrastructural changes in the microvasculature of the frontal cortex. Kwon et al. (2011) and Kovesdi et al. (2011) reported elevated vascular endothelial growth factor levels more than two months after shock wave injury (20.6 psi) in rats. While these results indicate that bTBI results in cerebral vascular injury that may persist for months post-

injury, whether blast-induced vascular injury is associated with acute alterations in cerebral perfusion and cerebral vascular reactivity is unexplored. Due to this lack of information, the importance of identifying what physical and biochemical deficiencies injuries to the cerebral vasculature and the brain exposure to bTBI causes could aid in determining the level of therapeutic and/or rehabilitative success fairly immediately after injury.

My results that relative cerebral perfusion was reduced in the bTBI group indicates that mild bTBI contributed to significant cerebral hypoperfusion that started within five minutes of and persisted for at least two hours after ABS injury. Although my studies indicated that mild bTBI was associated with acute reductions in cerebral perfusion, Bir et al. (2012) using magnetic resonance imaging (MRI) methods to measure relative cerebral perfusion, reported that shock wave exposure (13 – 27 psi) resulted in significantly reduced relative perfusion from 24 to 72 hours post-injury in rats. In my present study, as in that of Bir et al. (2012), the rats were anesthetized with isoflurane, a volatile anesthetic that increases CBF in a concentration-dependent manner (Maekawa et al., 1986; Todd and Weeks, 1996). Considering the vasodilatory properties of isoflurane, it is likely that the blast-induced reductions in cerebral perfusion would have been greater had I and Bir et al. (2012) used a constrictory vasopressor without such dilator effects such as epinephrine (adrenaline) (Naftalin and Yagiela, 2002; Becker and Reed, 2012).

The myogenic vascular response, first described by Bayliss (1902), a major mechanism contributing to autoregulation of CBF, is characterized by vasoconstriction if perfusion pressure increases and vasodilatation if perfusion pressure decreases (Kontos, 1981; Paulson, 1990). Impairment of endothelium-dependent dilator responses to acetylcholine in isolated basilar arteries harvested from rats subjected to single or repeated

shock wave exposure (30 psi) were reported by Toklu et al. (2015). These investigators also reported that constrictor responses to endothelin-1 in basilar arterial segments were enhanced by shock wave exposure. Increased endothelin-dependent vasoconstriction after blast would be consistent with my observations that CVR was significantly increased by bTBI. Alford et al. (2011) reported that blast exposure *in vitro* resulted in increased sensitivity to the constrictor effects of endothelin-1 within one hour of simulated blast exposure (high velocity uniaxial stretch) in a highly aligned monolayer of VSMC's on an elastic substrate. Furthermore, 24 hours after high velocity stretch injury, the VSMC's exhibited prolonged hyperconstriction dependent on the force of the stretch. Baumann et al. (2009) reported cerebral arterial vasospasm after blast exposure in swine *in vivo*. Armonda et al. (2006) reported cerebral vasospasm in 48% of patients with severe blast-related trauma. While cerebral vasospasm often was observed after bTBI in combat casualties and is common after subarachnoid hemorrhage (SAH), Armonda et al. (2006) observed vasospasm in bTBI patients even in the absence of SAH. Together, my results and those of Toklu et al. (2015), Alford et al. (2011) and Armonda et al. (2006) suggest that blast exposure may contribute to cerebral arterial vasospasm observed in patients after bTBI (Ling et al., 2009; Armonda et al., 2006).

Impact (i.e. non-blast) TBI was followed by cerebral hypoperfusion in some patients (Bouma et al., 1991; Bouma et al., 1992a; Bouma et al., 1992b) and experimental animals (Yamakami and McIntosh, 1989; DeWitt et al., 1992; DeWitt et al., 1997b). Clinical TBI (Bouma and Muizelaar, 1990) and FPI TBI (Lewelt et al., 1980; DeWitt et al., 1986; Engelborghs et al., 2000) impaired cerebral vascular responses to changes in arterial blood pressure (i.e. autoregulation). Impact TBI also was associated with impaired

cerebral vascular compensatory responses to changes in $PaCO_2$ (Overgaard and Tweed, 1974; Wei et al., 1980a; Wei et al., 1980b), PaO_2 (Lewelt et al., 1982) and hematocrit (DeWitt et al., 1997a). Though many of these similarities exist between impact TBI and bTBI, there are key differences between the two. While impact TBI's present some evidence of external and/or penetrating injury, primary bTBI's may occur in the absence of external injures, thus internal injuries can be unrecognized and their severity underestimated. Exposure to a blast wave can cause hidden brain damage and the potential for neurological consequences days to month after the blast event. Additionally, in contrast to concussive and/or penetrating wounds where the nature of the injury is often focal, blast injuries are often multifocal.

Impact TBI markedly increased the mortality and morbidity due to posttraumatic insults such as hemorrhagic hypotension (HH) (Luerssen et al., 1988; Chesnut et al., 1993a; Chesnut et al., 1993b; Navarro et al., 2012), in part, because traumatic cerebral vascular injury impairs or abolishes compensatory cerebral vasodilation to reduced intravascular pressure (Lewelt et al., 1980; DeWitt and Prough, 2003; DeWitt and Prough, 2009). Although my study did not include any groups exposed to HH, my results and those of Toklu et al. (2015) indicate that bTBI-impaired cerebral vasodilatory responses from blast exposure, like impact TBI, may increase vulnerability to post-blast HH. This hypothesis is supported by the work of Long et al. (2009) who observed that survival rates were significantly lower in rats subjected to HH after blast injury than in Sham-injured rats. Unfortunately, the likelihood that combatants exposed to bTBI also sustain posttraumatic HH is high.

Hemorrhage is the leading cause of death in combat including those killed instantly

(killed in action) and those who died after transport to a medical facility (died of wounds) (Alam et al., 2005). Chambers et al. (2005) reported that 90% of patients treated by the U.S. Marine Forward Resuscitation Surgical System (FRSS) during OIF sustained penetrating injuries and, therefore, some degree of hemorrhage. More than 80% of the most critically ill patients treated by the FRSS presented in class three or four hemorrhagic shock and 40% of patients who required treatment during transport to higher echelons of care were treated for systemic arterial hypotension (Chambers et al., 2005). Nelson et al. (2006) observed that 100% of blast-injured patients with persistent arterial hypotension (systolic blood pressure < 90) died while all of those without hypotension survived their injuries. Although the results of Nelson et al. (2006) suggest that HH after blast injury is associated with higher mortality, it was a small study (n=14) and it is possible that mortality was high in the patients with hypotension because they were more seriously injured. While these results are inconclusive, they are consistent with a hypothesis that blast injury, like non-blast injury, renders patients more vulnerable to post-blast HH.

The majority of positively stained neuronal cell bodies appeared localized throughout the cortex in the areas overlapping the frontal and parietal/temporal lobes and slightly superior to the CA1 region while the particularly vulnerable hippocampal CA3 region did not present with a consistently localized pattern throughout each sectioned brain tissue examined. Though the significance of this observed parietal/temporal lobe positively stained localization during the time points examined warrants further studies, my observed results that the numbers of FJC-positive cells increased in the brains of rats subjected to mild bTBI (as compared to Sham) (**Figure 34**) are consistent with previous reports of blast-induced neuronal injury. Sajja et al. (2012) observed significant increases in the numbers

of FJ-positive neurons in the rat hippocampus 24 and 48 hours after 117 kPa (17 psi) shock wave exposure. In a separate study, Sajja et al. (2014) reported significant increases in the numbers of FJ-positive neurons in the pre-frontal cortices of rats three to 168 hours after 117 kPa shock wave exposure. Although most studies of the histopathological effects of primary blast used shock tubes, Kuehn et al. (2010) observed increases in the numbers of FJ-positive neurons in the cerebellum and hippocampus of rats subjected to severe blast exposure (427-517 kPa, 62-75 psi) in a blast tube powered by nail gun blank cartridges. In addition to neuronal injury assessed using FJ staining, Cho et al. (2013) observed reduced neuronal nuclear antigen (NeuN) immunoreactivity in the hippocampus from 24 hours to two weeks post-blast using an ABS shock tube similar to ours and comparable in shock wave amplitudes (19 psi, 129 kPa). As NeuN is a protein expressed by mature neurons, these results indicate that blast injury is associated with the loss of hippocampal neurons that continues for at least two weeks post-blast. Although these studies were limited to assessments of acute neuronal injury (< two weeks), Sajja, et al. (2015) reported significant increases in the numbers of FJ-positive neurons in the amygdala and prefrontal cortex that persisted for at least six months after 117 kPa ABS shock wave exposure. Together, these reports and my results indicate that mild primary blast/shock wave exposure results in significant neuronal injury that lasts at least six months post-blast.

FJC, an anionic tribasic derivative dye of fluorescein, is extremely acidic (Shmued et al., 1997). FJC has an improved signal to noise ratio, therefore creating a better compound for visualizing finer neuronal morphology on almost any tissue section type or thickness (Shmued et al., 1997; Schmued and Hopkins, 2000; Schmuck et al., 2009). Comparisons of silver staining methods and FJ techniques using a time course of cellular

disintegration, revealed that silver staining is apparent sooner after neurotoxicity compared to FJ, implying that FJ contains specificity for the delayed assured phase of the neurodegenerative process (Shmued et al., 1997; Schmuck et al., 2009). In comparison with other indicators of neural injury, FJC exhibited the greatest signal to background ratio, highest resolution, maximum contrast and affinity for neurons, making it ideal for localizing degenerating nerve cell bodies (Shmued et al., 1997; Schmued and Hopkins, 2000; Schmuck et al., 2009).

In addition to cerebral vascular and cellular injury, I observed significant impairment in working memory. The differences between the time to the goal platform in trials 1 and 2 of the MWM were significantly greater in the Sham than in the bTBI group on days 11 - 15 after blast or Sham blast. Since rats with normal working memory were able to remember where to find the goal platform more rapidly during the second trial of each pair of trials, their differences between the trial pairs would be larger. An animal with impaired working memory will not remember as quickly on the second trial where to locate the goal platform, thus having a smaller, much shorter difference between the trial pairs. My results indicate significant deficits in working memory in the bTBI group. These results are consistent with previous reports of blast-induced memory deficits. Sajja et al. (2014) and Cho et al. (2013) reported working memory deficits (novel object recognition) one to two weeks after 117 kPa shock wave exposure while Sajja et al. (2015) observed deficits in working memory that persisted for at least three months after. Spatial memory deficits (Barnes maze) were observed in rats two weeks (Kamnaksh et al., 2012) or two months (Kovesdi et al., 2011) after 138 kPa (20 psi) or 142 kPa (21 psi) shock wave exposure, respectively. These results indicate that mild bTBI results in working and spatial memory

deficits that may persist as long as three months after blast exposure.

Several experimental studies have implicated a significant role of the toxic ROS/RNS $ONOO^-$ in the pathophysiology of impact (i.e. non-blast) TBI (Hall, 1995b; Wei et al., 1996; Hall et al., 1999; Hall et al., 2004) and bTBI (DeWitt and Prough, 2009; Masel et al., 2012). NO, a potent vasodilator, has been identified as the endothelium-derived relaxing factor (EDRF) (Angus and Cocks, 1987; Gryglewski and Moncada, 1987; Guo et al., 2016) that relaxes VSMC's and dilates all types of blood vessels. NO is synthesized by enzymes (nitric oxide synthases, NOS) that convert L-arginine to NO and citrulline (Alderton et al., 2001). There are three distinctive NOS isoforms: neuronal NOS (nNOS) predominantly but not exclusively localized in neuronal tissue and constitutively expressed; inducible NOS (iNOS) which is normally present in miniscule quantities but can be induced in a wide range and variety of tissues; and endothelial NOS (eNOS) which was first identified in vascular endothelial cells and also constitutively expressed (Alderton et al., 2001; Guo et al, 2016). In addition to its powerful vasodilatory properties, NO transmits cellular signals for various physiological functions (Liu et al., 2002). Immediately after impact TBI, the activity of nNOS and eNOS was increased, resulting in elevated concentrations of NO as early as five minutes post-injury (Zunic et al., 2005) and returning to baseline 30 minutes after FPI (Wada et al., 1998) to several hours after controlled cortical impact injury (CCI) (Wada et al., 1998; Cherian et al., 2000). Cernak et al. (2001a.) reported that bTBI resulted in increases in the expression of iNOS messenger RNA (mRNA) and brain nitrite/nitrate levels while Abdul-Muneer et al. (2013) reported that iNOS immunoreactivity increased within one hour of mild (123 kPa) shock wave exposure. Cho et al. (2013) reported evidence of superoxide production (dihydroethidium

fluorescence) starting four hours and persisting for at least two weeks after 129.2 kPA ± 3.0 (18.7 psi ± 0.4) shock wave exposure in an ABS.

Though NO may affect myriad cellular functions either directly or indirectly (Förstermann and Sessa, 2012; Roszer, 2012), the balance between detrimental neurotoxicity and beneficial importance as a cellular signaling molecule for various physiological functions is contingent upon many aspects such as the NO synthase isoform producing NO, the particular microenvironment in which it is being produced as well as the cell type in charge of production (Liu et al., 2015). It has been considered that iNOS-derived NO is accountable for some of the more neurotoxic activity of NO post-injury (Garry et al., 2015). Although originally isolated and expressed primarily in activated macrophages (Stuehr et al., 1991; Robinson et al., 2011), iNOS expression ensues after exposure to pro-inflammatory cytokines such as interferon-gamma (IFN-ɣ) and/or microbial products such as bacterial lipopolysaccharides (LPS).

Increased NO levels due to TBI may result in the production of elevated concentrations of $ONOO^-$. The $ONOO^-$ reaction is calculated to transpire *in vivo* (Beckman et al., 1990; Squadrito and Pryor, 1995) and is formed when excessive iNOS-produced NO (Garry et al., 2015) reacts with NADPH (nicotinamide adenine dinucleotide phosphate) oxidase-generated superoxide anion ($\bullet O_2^-$) radicals (Beckman et al., 1990; Angeloni et al., 2015). With one of the largest rate constants known for reactions of NO (Huie and Padmaja, 1993; Kobayashi et al., 1995), the reaction is near diffusion controlled and the rate of formation of $ONOO^-$ is first order in both concentrations of NO and $\bullet O_2^-$ (Huie and Padmaja, 1993; Goldstein and Czapski, 1995;

Kobayashi et al., 1995); thus, formation of $ONOO^-$ depends upon the actions of both NOS and SOD.

$ONOO^-$ may oxidatively attack a wide range of cellular targets such as cell membrane lipids and ion channels (lipid peroxidation) (Hall et al., 2016), the thiol groups and tyrosine residues of proteins and enzymes (hydroxylation and nitration) (Singh et al., 2007; Sun et al, 2012), nucleic acids (fragmentation) (Zhang et al., 2002) and the mitochondria through calcium efflux and inhibition of the electron transport chain (mitochondrial dysfunction) (Hall et al., 1999; Zhang et al., 2002; Singh et al., 2007). Studies utilizing a mouse impact acceleration (IA) head injury model provided evidence implicating $ONOO^-$ in the pathophysiology of TBI (Hall, 1995b; Hall et al., 1999). Previous studies have observed $ONOO^-$-related impairment in cerebral arterial reactivity (DeWitt et al., 2001; Maneen et al., 2006; Maneen and Cipolla, 2007). In pial arteries in cats (Wei et al., 1996) and isolated pressurized MCA's in rats (Elliot et al., 1998; DeWitt et al., 2001), $ONOO^-$ exposure produced dose-dependent dilation (cats) and constriction (rats) and resulted in decreased or eliminated vasodilatory responses to progressive reductions in intravascular pressure (rats). Observations during the development of hemorrhagic and endotoxic shock indicated endogenous $ONOO^-$-production in vascular hyporeactivity (Zingarelli et al., 1997; Zhao et al., 2000) while systemic $ONOO^-$ administration caused a decreased vascular reactivity which resembled this shock-induced occurring hyporeactivity (Szabo et al., 1996; Benkusky et al., 1999), further associating $ONOO^-$ with decreased vasoreactivity. Blast-induced $ONOO^-$ formation is further supported by evidence that bTBI is associated with increases in 3-NT immunoreactivity in the hippocampus and cortex (Masel et al., 2012). Abdul-Muneer et

al. (2013) reported that mild bTBI was followed by increases in 3-NT immunoreactivity in rat brain microvessels. The evidence of blast-induced increases in NO (Cernak et al., 2001a; Abdul-Muneer et al., 2013), $\bullet O_2^-$ (Cho et al., 2013) and 3-NT immunoreactivity in the brain and cerebral vasculature (Masel et al., 2012; Abdul-Muneer et al., 2013) provide support for the hypothesis that bTBI results in increases in brain tissue and vascular $ONOO^-$ levels.

$ONOO^-$-mediated damage after impact TBI can be reduced through the administration of scavengers. Penicillamine (Pen) and PenME are non-specific $ONOO^-$ scavengers that improve outcome after TBI (Hall et al., 1999; Hall et al., 2004; Singh et al., 2007). A strong derivative of the amino acid cysteine, Pen or ß,ß-dimethyl cysteine, a sulfur-containing monothiol (Walshe, 1956; Khayyal et al., 1967), has an extensive record of clinical use in both copper- and non-copper based disease and disorders due to its regard as a prevailing copper chelator (Tran-Ho et al., 1997; Weiss and Stremmel, 2012; Fieten et al., 2013b; Riha et al., 2016) that promotes the detoxification of numerous metal ions located in the body (Khayyal et al., 1967). Stable in its reduced form, Pen is a highly soluble degradation product of penicillin that binds copper at its SH-group, is swiftly excreted by the kidneys (Walshe, 1956) and forms fairly stable chelates with all biologically effective trace metals as well as iron and zinc (Lenz and Martell, 1964). Previous studies revealed efficacy in diminishing hepatic copper concentrations (dogs) (Twedt et al., 1979; Mandigers et al., 2005; Hoffmann et al., 2006), enhancing urinary copper excretion and reducing toxicity due to Wilson's disease (humans) (Walshe, 1956; Weiss and Stremmel, 2012), promoting iron excretion and effective in cases of lead poisoning (Boulding and Baker, 1957) and in subsiding the degree of inflammation (Fieten et al., 2013a) brought

about by such heavy-metal poisoning. While Pen has limited BBB permeability and acts intravascularly, PenME or methyl (2R)-2-amino-3-methyl-3-sulfanylbutanoate is a lipophilic, blood brain barrier-penetrable free radical scavenger (Hall et al., 1999) that can act extravascularly in cerebral vessels and tissues. Hall et al. (1999) reported that both Pen and PenME improved early neurological outcome (grip test) after TBI in mice, suggesting that the intravascular scavenging of $ONOO^-$ contributed to the therapeutic effects of Pen. Moreover, bTBI can result in BBB breakdown leading to increased BBB permeability (Readnower et al., 2010; Garman et al., 2011), thereby increasing the access of substances that normally wouldn't traverse the BBB. I tested the PenME compound as our experimental free radical scavenger in order to determine the cerebral vascular effects of $ONOO^-$ scavenging after bTBI as measured by relative cerebral perfusion and mean arterial pressure in rats treated with PenME after blast injury. Together, these studies indicating that bTBI results in increases in NO and superoxide and 3-NT immunoreactivity (DeWitt and Prough, 2009; Masel et al., 2012), suggest $ONOO^-$ formation is likely after bTBI and that $ONOO^-$ contributes to blast-induced cerebral vascular dysfunction.

Our results of a trend towards increases in cerebral perfusion and significant reductions in cerebral vascular resistance after treatment with PenMe suggests that blast-induced cerebral vascular dysfunction may be due, in part, to the actions of $ONOO^-$.

The manner in which blast energy is transmitted into the brain remains controversial (Rossle, 1950; Cernak et al., 2001b; Courtney and Courtney, 2009; Long et al., 2009; Taylor and Ford, 2009; Chafi et al., 2010; Bolander et al., 2011; Courtney and Courtney, 2011; Koliatsos et al., 2011; Nakagawa et al., 2011). One of several theorized mechanisms is the transfer of kinetic blast energy to the cerebral vasculature and brain via

the great vessels of the thorax (Rossle, 1950; Cernak et al., 2001b; Courtney and Courtney, 2009; Courtney and Courtney, 2011; Nakagawa et al., 2011). In studies of intracranial pressure oscillations in a non-protected, whole-body shock wave-exposed Rhesus monkey (Romba and Martin, 1961) and in a swine outfitted with a lead-and-foam-lined vest that covered the chest and upper abdomen (Bauman et al., 2009), similar occurrences of significant increases in intraparenchymal and intravascular pressure pulses were observed. Experimental blast studies in rodents demonstrated that protecting the torso virtually eliminated axonopathy and fiber degeneration (Long et al., 2009) while the use of a plexiglass covering around the torso of blast-injured mice abolished axonal nerve cell damage compared to non-shielded mice who suffered up to 80% axonal damage (Koliatsos et al., 2011). In contrast, in ferrets (Rafaels et al., 2012) and rabbits (Rafaels et al., 2011) with thoracic and abdominal protection, apnea, meningeal bleeding (ferrets) and multifocal subdural and SAH's (rabbits) as well as fatalities were observed. In a rat model in which blast overpressures were delivered exclusively to the head through direct cranial blast injury (dcBI) (Kuehn et al., 2010), sub-lethal injury resulted in apnea, SAH's in the path of the blast wave, abnormal immunoglobulin (IgG) immunolabeling, cleaved caspase-3 and beta-amyloid precursor protein (β-APP), FJC staining in brain regions not over-lapping the SAH's and abnormalities on the behavioral Rotarod task. These results of blast-induced brain injury in the presence of thoracic protection indicate that primary blast exposure to the head in the absence of thoracic injury is sufficient to produce significant brain injury. We used head-only blast exposure to determine blast effects to the head alone, excluding the possibility of indirect brain injury through thoracic transmission of the blast wave.

Due to recent combat operations in almost the last two decades, the incidence of bTBI has risen significantly (Cernak et al., 2001b; Warden, 2006; Martin et al., 2008). 52% - 63% of U.S. combat TBI's from OIF are blast-related injuries (Galarneau, et al., 2008; Wojcik, et al., 2010) with the Department of Defense reporting (January 2015) that over 73% of all U.S. military casualties were caused by explosive weaponry. The increase in explosive injuries and the fact that alterations in brain functionality could modify the posttraumatic course of neurological performance, emphasize the importance of clarifying the structural and biochemical impairments to the brain and the cerebral vasculature that exposure to bTBI causes.

My findings of increased RR suppression, impaired cerebral dilator responses to reduced intravascular pressure, reduced cerebral perfusion, a sustained increased MAP, increased CVR, acute cellular injury/degeneration and impaired working memory and/or cognitive dysfunction are fairly consistent with both prior non-blast (i.e. impact) and blast TBI studies. Though the blast pressure levels employed in my study produced brief unconsciousness in the injured animals, this small increase in RR suppression indicated a mild injury. Impaired cerebral dilatory properties to reduced intravascular pressures could potentially be a function of the vasospasm and VSMC hyperconstriction previously reported after blast exposure, ultimately leading to the reduced cerebral perfusion and increase in MAP observed. Prolonged reduction in cerebral perfusion may have also contributed to the acute cellular injury/degeneration detected throughout the whole brain which in turn may have been a factor in the impaired cognitive dysfunction seen with the behavioral tasks.

STUDY CONSIDERATIONS

These studies had aspects worth noting. They were conducted using an ABS, a new shock tube that circumvents some of the problems associated with experimental blast research. Overpressure waves generated by older blast/shock tubes may be altered by reflected pressure waves produced when the primary blast wave impacts the closed end of the tube (Kovacs et al., 2014). The use of a reflected wave suppressor in the ABS prevents or minimizes the effects of shock wave reflection. A potential limitation of shock tubes is the impact of fragments produced by the rupture of membranes (Alphonse et al., 2014). Acetate membranes, which are utilized in other ABS devices, produce true Friedlander-type overpressure waves but the fragments produced may act as projectiles, resulting in a combined insult of primary blast wave exposure plus secondary impacts from acetate fragments. In contrast, Mylar ruptures without producing fragments, making it better suited for studies of the effects of primary blast/shock wave exposure. Although the incomplete rupture of Mylar membranes may alter the shape of the overpressure wave in some studies (Alphonse et al., 2014), the overpressure wave generated by the ABS using Mylar membranes in the present study closely resembled an idealized Friedlander wave.

I used isoflurane as the primary anesthetic for all of our studies because it is the most widely used general anesthetic for TBI studies in rats (Kochanek et al., 1995; Adelson et al., 1996; Singleton et al., 2001; Hallam et al., 2004; Statler et al., 2006; Hawkins et al., 2013; Rowe et al., 2013; Sell et al., 2016). However, previous human (Pauca and Dripps, 1973; Lam et al., 1994; Matta et al., 1999) and animal (Drummond et al., 1986; Flynn et al., 1992; Koenig et al., 1994; Farber et al., 1997; Masamoto et al., 2007) studies indicated that isoflurane is a cerebral vasodilator (Eger, 1984; Schwinn et al., 1990; Iida et al., 1998)

because of direct effects on vascular smooth muscle (Altura et al., 1980; Longnecker and Harris, 1980; Akata, 2007). Among the observations detected due to isoflurane administration in the previously mentioned studies are significant effects (increases) on CBF (Cucchiara et al., 1974; Van Aken et al., 1986; Olsen et al., 1994; Matta et al., 1999; Sloan, 2002; Oshima et al., 2003; Schlunzen et al., 2006; Kimme et al., 2007; Li et al., 2013), cerebral blood volume (CBV) (Matta et al., 1999; Sloan, 2002; Kimme et al., 2007), a reduced rate of cerebral oxygen consumption ($CMRO_2$) (Cucchiara et al., 1974), permeability and neurovascular coupling (Sloan, 2002; Oshima et al., 2003; Masamoto et al., 2009) and a disturbance in the CBF autoregulation mechanism (McPherson and Traystman, 1988; Olsen et al., 1994). Though administration of volatile anesthetics can also affect net decreases in CBF (Mielck et al., 1998; Mielck et al., 1999), the reduced CBF observed after blast and impact TBI could possibly be even lower if not for the increased CBF effect of isoflurane. If an anesthetic possesses a substantial vasodilatory effect on the cerebral vasculature that could be countered by a conflicting vasoconstrictory influence (such as a non-impact TBI blast injury or exposure), the vasodilatory effect of the volatile anesthetic may be compromised (Sakabe et al., 1983; Mielck et al., 1998; Mielck et al., 1999; Patel and Drummond, 2010). Of the commonly used volatile anesthetics, isoflurane ranks second to last in the order of vasodilating effectiveness (Reinstrup et al., 1995; Kuroda et al., 1997). While isoflurane-mediated vasodilation may have contributed to some of the blast effects I observed, the minimum alveolar concentration (MAC) that was utilized as an anesthetic for my studies (1 MAC = ~1.5% for rats) is likely negligible in terms of causing such cerebral vasodilation as to be solely responsible for the reductions in CBF I observed in our experimental animals after blast overpressure exposure.

A third limitation was the use of LDF to measure relative cerebral perfusion. Laser Doppler perfusion measurements are expressed as a percentage of baseline values. Ideally, the LDF probe remains in exactly the same location for the duration of the measurements. However, in my studies, baseline LDF measurements were recorded, the rats were removed from the stereotaxic frame, subjected to ABS bTBI or Sham injury then returned to the stereotaxic frame. Although I attempted to replace the probe in exactly the same location from which the baseline measurements were made, in some cases I may have been unable to do so. In order to compensate for misplaced probes in both the Sham and bTBI groups, I excluded all animals in which the first measurement after the probe was replaced yielded values 20% higher or lower than baseline in the absence of comparable changes in MAP (five excluded Sham rats and three bTBI rats). However, it is important to note that relative cerebral perfusion was significantly ($P < 0.02$, bTBI vs Sham) reduced by mild bTBI even if all animals were included in the calculations of relative cerebral perfusion. Additionally, it is possible that thinning of the skull for LDF measurements may have compromised skull integrity, thus contributing to injury induction/severity. However, the scalp covering the thinned area was tightly sutured before administration of blast injury. I observed no evidence of skull fracture in the rats subjected to bTBI. While minor surface hemorrhage under the thinned area was observed in four out of the twelve rats subjected to bTBI, no hemorrhage was observed in eight of the twelve.

A fourth potential limitation is that FJC-positive cells were counted throughout the entire brain, while relative cerebral perfusion was measured only at one location in the cerebral cortex. Working memory performance is particularly sensitive to hippocampal injury but hippocampal perfusion was not measured. Therefore, I cannot definitively

establish that blast-induced reductions in cerebral perfusion contributed to either the neuronal injury seen and quantified throughout the brain or the observed impaired working memory performance.

Finally, it is important to note that, although FJ is widely used to stain injured and/or dying neuronal cells (Schmued et al., 1997; Larsson et al., 2001; Sato el al., 2001; Hellmich et al., 2005a; Hellmich et al., 2005b), there is evidence that FJ-positive cells may be injured but not necessarily dying (Wang et al., 2015) and FJ may stain non-neural cells (e.g. activated microglia, astrocytes) under circumstances when combined with specific markers for detection of GFAP or activated CD68 microglia (Damjanac et al., 2007).

SUMMARY

My results indicate that mild bTBI levels associated with statistically significant but small increases in the duration of suppression of RR's produced significant reductions in relative cerebral perfusion and significant increases in CVR. Additionally, these studies indicated that mild bTBI resulted in significant increases in the numbers of FJC-positive cells throughout the brain and significant impairments in working memory performance. My results that cerebral vasodilator responses to reduced intravascular pressure in MCA segments were impaired by mild bTBI suggest that blast exposure may result in increased vulnerability to hemorrhagic hypotension. Finally, my results that PenME significantly reduced blast-induced increases in CVR suggests that $ONOO^-$ contributes to the impairment of cerebral vascular function after bTBI.

FUTURE DIRECTIONS

Hemorrhage is a common complication of closed head (blunt trauma) injuries, penetrating injuries and explosive blast injuries and frequently accompanies TBI.

Uncontrolled hemorrhage can ultimately lead to decreases in blood pressure (arterial hypotension) that, in conjunction with impaired cerebral vasodilatory responses, may further contribute to cerebral hypoperfusion and, perhaps, cerebral ischemia (DeWitt and Prough, 2003; DeWitt and Prough, 2009). In the laboratory setting, hypotension after TBI was linked with reduced CBF (Giri et al., 2000; Matsushita et al., 2001) and brain tissue oxygen levels (Giri et al., 2000), impaired ion homeostasis (Stiefel et al., 2005) and impaired energy metabolism (Ishige et al., 1988). Hemorrhage associated with TBI results in poor neurologic outcomes and can nearly double the morbidity and mortality compared with TBI alone (Miller et al., 1978; Wald et al., 1993). Rapid infusion of large volumes of plasma expanders is the standard treatment for patients with combined TBI and hemorrhage (Bullock et al., 1996). In uninjured patients, the infusion of fluid results in hemodilution (Messmer, 1975; Mirhashemi et al., 1987; Chatpun and Cabrales, 2010), which reduces arterial O_2 content, blood viscosity and vascular resistance and therefore increases CBF. However, TBI impairs normal dilator responses to blood viscosity (DeWitt et al., 1996), potentially reducing the efficacy of volume expanders.

As stated above, our results that blast reduced dilator responses to reduced intravascular pressure in isolated MCA's suggest that blast exposure increases vulnerability to post-blast HH. This hypothesis could be tested by measuring cerebral perfusion, MAP and CVR for two hours post-bTBI in rats subjected to (n = 8/group):

a. Sham bTBI

b. Sham bTBI + HH (MAP reduced to 50 mmHg for 45 minutes)

c. bTBI

d. bTBI + HH (starting 15 minutes post-bTBI)

Hemorrhagic hypotension would be followed by reinfusion of shed blood (see Ishige et al., 1988; Cherian et al., 1994; Hellmich et al., 2005b; Navarro et al., 2012).

If reductions in cerebral perfusion were greater in the bTBI + HH than in the HH group alone, we plan to conduct a second set of experiments to determine whether the administration of PenME would improve cerebral perfusion after bTBI + HH in rats subjected to (n = 8/group):

a. bTBI + HH

b. Sham bTBI + HH + 10 mg/kg PenME 5 minutes post-Sham bTBI

c. bTBI + HH + 10 mg/kg PenME 5 minutes post-bTBI

These studies will test the hypothesis that relative cerebral perfusion will be significantly lower after bTBI followed by HH than in HH alone and that this increased vulnerability to post-blast hemorrhagic hypotension is due, in part, to the effects of $ONOO^{-}$.

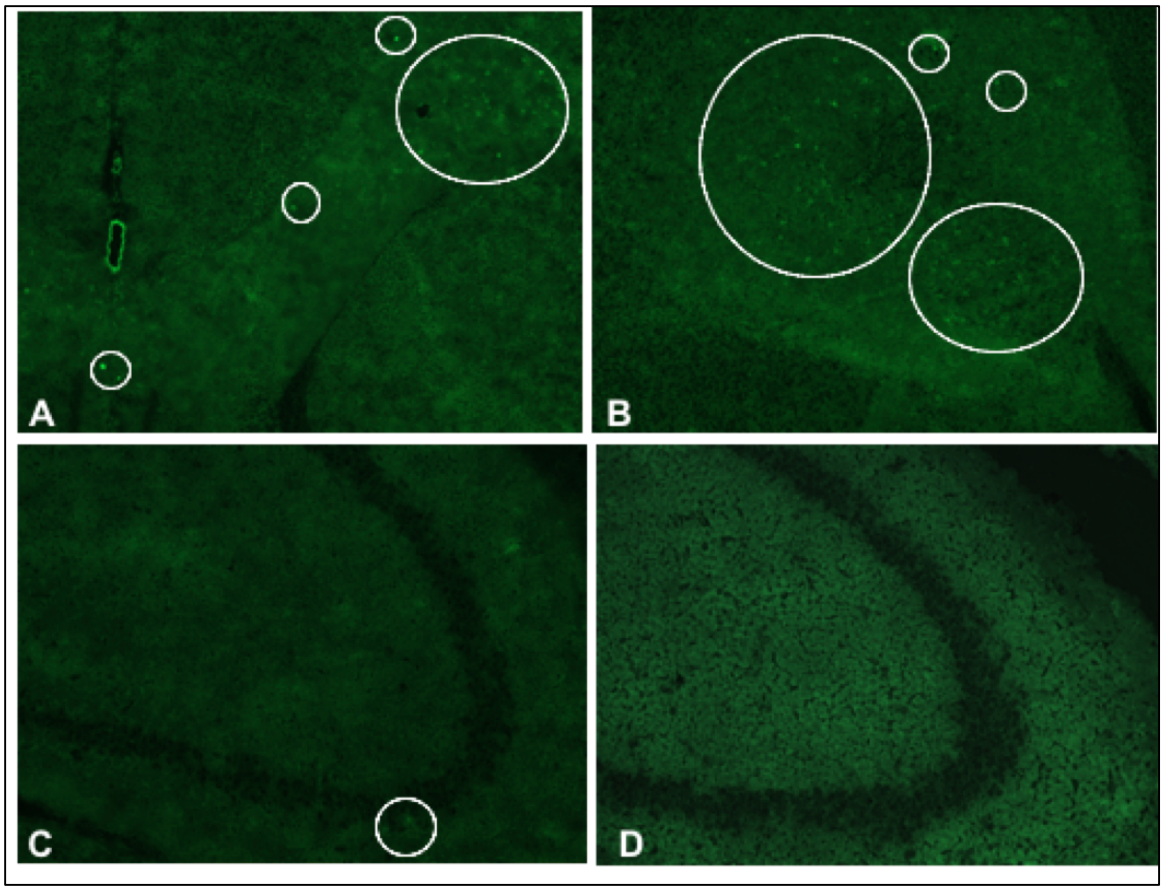

Figure 34: FluoroJade-C (FJC) stained brain sections

FluoroJade-C stained brain sections in a bTBI rat. Circled FJC-positive cell bodies in the (**A**) frontal cortex edge and the corpus callosum (**B**) cortex (**C**) cortex and hippocampus or (**D**) Sham rats 24 hours after injury.

Appendix A: Preclinical Common Data Elements for ABS bTBI

Animal Characteristics	
Species	Rat
Age Range	3-5 mo. prior to TBI
Sex	Male
Animal Vendor	Charles River
Strain	Sprague-Dawley
Weight Range	350-480g pre-TBI
Animal History	
Pre-TBI Housing	Group housed; 12hr light/dark cycle; food and water ad libitum; AAALAC accredited animal care facility maintained to USDA standards
Anesthetic Type	Isoflurane (4% for induction, 2% for maintenance), intubated and mechanically ventilated
Anesthetic Route	Inhaled
Analgesia Type	Acetaminophen suppository
Injury Severity	Mild (blast)
Number of Injury Exposures	Single
Post-TBI Housing	Group housed unless separated for fighting, etc.; 12hr light/dark cycle; food and water ad libitum; AAALAC accredited animal care facility maintained to USDA standards
Euthanasia Type	4% isoflurane followed by decapitation
Assessments and Outcomes	
Acute Neurological Assessment	Neuroscore
Righting Reflex Response Time	mild = ≤ 7 minutes; moderate = 8-14 minutes; severe = > 14 minutes
Learning and Memory Tests	Morris water maze
Sensory/Motor Tests	Beam walk; beam balance
Anxiety and Depression Tests	N/A
Histopathology	Cellular/neuronal injury
Injury Model Characteristics	
Injury Model	Advanced Blast Simulator (ABS) TBI
Device Mfg	ORA, Inc., Fredericksburg, VA
Animal Stabilization Method	Rat's dorsal scalp perpendicular to the ABS blast wave with only the cranium located inside ABS device and supported by a sling to prevent head movement. The rest of the body lies in a left lateral position outside the ABS device on a specimen tray that locks into the blast chamber.
Impact Location Side	Central dorsal

ABS Blast TBI

Blast Induced Delivery Device	Advanced Blast Simulator device is a shock tube designed by David Ritzel (Dyn-FX Consulting, Ltd. Ontario, Canada) and produced by Steve Parks (ORA, Inc., Fredericksburg, VA)
Pressure Wave Type	Single pulse blast-waves (Friedlander-style over/under pressure waves)
Detonation Type	Shock waves produced using compressed gas driver and Mylar® membranes
Detonation Material Quantity	Mylar® sheets (4)
Driver Gas	Compressed air
Pressure Wave Medium	Air
Distance From Detonation	6 feet, 7 inches (2 m)
Blast Tube or Column Area	90 square inches (581 cm^2)
Blast Tube Length	14 feet (4.27 m)
Shock Tube Driven Section Length	10 inches (254 mm)
Membrane Thickness	0.016 inches (0.4 mm/sheet)
Membrane Burst Method	Non-debris complete rupture
Membrane Burst Pressure	179.33 psi ± 3.0 (1236.4 kPa ± 20.7)
Tube End Configuration	Cylindrical reflected wave suppressor
Distance Between Animal and Tube	Rat's head is inside of tube
Animal Orientation to Blast Wave	Perpendicular
Overpressure Peak	20.88 psi (138 kPa)
Overpressure Rise Time	0.37 millisec ± 0.006
Overpressure Wave Duration	3.50 millisec ± 0.063
Impulse	N/A
Reflective Wave Overpressure	N/A
Blast Wind Pressure	N/A
Pressure Sensor Type	Piezoelectric pressure probe transducers
Pressure Sensor Sampling Frequency	20 microsec time sample ≥ 50 kHz sample rate
Incident Pressure Time History	< 2 microsec
Body Exposure	Head only
Protective Shielding Location	N/A
Protective Shielding Type	N/A
Reflective Surfaces	N/A
Primary Blast Effects	Absence of external injury: occluded blood vessels, cerebral vasospasm, SAH hemorrhage, tympanic membrane rupture, neuronal death/degeneration
Secondary Blast Effects	N/A
Tertiary Blast Effects	N/A

Quaternary Blast Effects	N/A
Systemic Injuries	N/A
Extracranial Injuries	N/A
Pre-BINT Surgical Procedures	Isoflurane (4% initial, 2% to maintain until blast injury), intubated, mechanically ventilated and the top of the scalp is shaved
Post-BINT Surgical Procedures	Measurement of duration of righting reflex suppression and removal of intubation tube

References

Abdul-Muneer, P.M., Schuetz, H., Wang, F., Skotak, M., Jones, J., Gorantla, S., Zimmerman, M.C., Chandra, N., and Haorah, J. (2013). Induction of oxidative and nitrosative damage leads to cerebrovascular inflammation in an animal model of mild traumatic brain injury induced by primary blast. Free Radic Biol Med 60, 282 – 291.

Adelson, P.D., Robichaud, P., Hamilton, R.L., and Kochanek, P.M. (1996). A model of diffuse traumatic brain injury in the immature rat. J Neurosurg 85(5), 877 – 884.

Ahmed, F.A., Kamnaksh, A., Kovesdi, E., Long, J.B. and Agoston, D.V. (2013). Long-term consequences of single and multiple mild blast exposure on select physiological parameters and blood-based biomarkers. Electrophoresis 34(15), 2229 – 2233.

Ahn, D.S., Choi, S.K., Kim, Y.H., Cho, Y.E., Shin, H.M., Morgan, K.G., and Lee, Y.H. (2007). Enhanced stretch-induced myogenic tone in the basilar artery of spontaneously hypertensive rats. J Vasc Res 44(3), 182 – 191.

Akata, T. (2007). General anesthetics and vascular smooth muscle: direct actions of general anesthetics on cellular mechanisms regulating vascular tone. Anesthesiology 106(2), 365 – 391.

Alam, H.B., Burris, D., DaCorta, J.A., and Rhee, P. (2005). Hemorrhage control in the battlefield: role of new hemostatic agents. Mil Med 170(1), 63 – 69.

Alderton, W.K., Cooper, C.E., and Knowles, R.G. (2001). Nitric oxide synthases: structure, function and inhibition. Biochem J 357(Pt 3), 593 – 615.

Alford, P.W., Dabiri, B.E., Goss, J.A., Hemphill, M.A., Brigham, M.D., and Parker, K.K. (2011). Blast-induced phenotypic switching in cerebral vasospasm. Proc Natl Acad Sci 108(31), 12705 – 12710.

Allen, S.P., Wade, S.S., and Prewitt, R.L. (1997). Myogenic tone attenuates pressure-induced gene expression in isolated small arteries. Hypertension 30(2 Pt 1), 203 – 208.

Alley, M.D., Schimizze, B.R., and Son, S.F. (2011). Experimental modeling of explosive blast-related traumatic brain injuries. Neuroimage 54(Suppl 1), S45 – S54.

Alphonse, V.D., Siva Sai Suijith Sajja, V., Kemper, A.R., Ritzel, D.V., Duma, S.M., and VandeVord, P.J. (2014). Membrane characteristics for biological blast overpressure testing using blast simulators. Biomed Sci Instrum 50, 248 – 25.

Althaus, J.S., Oien, T.T., Fici, G.J., Scherch, H.M., Sethy, V.H., and VonVoigtlander, P.F. (1994). Structure activity relationships of peroxynitrite scavengers: an approach to nitric oxide neurotoxicity. Res Commun Chem Pathol Pharmacol 83 (3), 243 – 254.

Altura, B.M., Altura, B.T., Carella, A., Turlapaty, P.D., and Weinberg, J. (1980). Vascular smooth muscle and general anesthetics. Fed Proc 39(5), 1584 – 1591.

Anderson, R.J. (2008). Shell shock: an old injury with new weapons. Mol Interv 8(5), 204 – 218.

Angeloni, C., Prata, C., Dalla Sega, F.V., Piperno, R. and Hrelia, S. (2015). Traumatic brain injury and NADPH oxidase: a deep relationship. Oxid Med Cell Longev 2015(370312), 1 – 10.

Angus, J.A. and Cocks, T.M. (1987). Vasodilation and the discovery of endothelium-derived relaxing factor. Med J Aust 146(5), 250 – 253.

Arciniegas, D.B., Held, K., and Wagner P. (2002). Cognitive impairment following traumatic brain injury. Curr Treat Options Neurol 4(1), 43 – 57.

Armonda, R.A., Bell, R.S., Vo, A.H., Ling, G., DeGraba, T.J., Crandall, B., Ecklund, J., and Campbell, W.W. (2006). Wartime traumatic cererbral vasospasm: recent review of combat casualties. Neurosurgery 59(6), 1215 – 1225.

Arun, P., Abu-Taleb, R., Oguntayo, S., Tanaka, M., Wang, Y., Valiyaveettil, M., Long, J.B., Zhang, Y., and Nambiar, M.P. (2013). Distinct patterns of expression of traumatic brain injury biomarkers after blast exposure: role of compromised cell membrane integrity. Neurosci Lett 552, 87 – 91.

Atkinson, C.L., Carter, H.H., Naylor, L.H., Dawson, E.A., Marusic, P., Hering, D., Schlaich, M.P., Thijssen, D.H., and Green, D.J. (1985). Opposing effects of shear-mediated dilation and myogenic constriction on artery diameter in response to handgrip exercise in humans. J Appl Physiol 119(8), 858 – 864.

Attwell, D., Buchan, A.M., Charpak, S., Lauritzen, M., Macvicar, B.A., and Newman, E.A. (2010). Glial and neuronal control of brain blood flow. Nature 468(7321), 232 – 243.

Avidan, V., Hersch, M., Armon, Y., Spira, R., Aharoni, D., Reissman, P., and Schecter, W.P. (2005). Blast lung injury: clinical manifestations, treatment, and outcome. Am J Surg 190(6), 927 – 931.

Balaban, C., Jackson, R.L., Liu, J., Gao, W. and Hoffer, M.E. (2016). Intracranial venous injury, thrombosis and repair hallmarks of mild blast traumatic brain injury in

rats: lessons from histological and immunohistochemical studies of decalcified sectioned heads and correlative microarray analysis. J Neurosci Methods 272, 56 – 68.

Banchoff, T.F. and Lovett, S.T. (2015). Differential Geometry of Curves and Surfaces. 2nd ed. Chapman and Hall/CRC Press: Boca Raton, FL.

Bass, C.R., Panzer, M.B., Rafaels, K.A., Wood, G., Shridharani, J., and Capehart, B. (2012). Brain injuries from blast. Ann Biomed Eng 40(1), 185 – 202.

Bass, C.R., Rafaels, K.A., and Salazar, R.S. (2008). Pulmonary injury risk assessment for short-duration blasts. J. Trauma 65, 604 – 615.

Bauman, R.A., Elsayed, N., Petras, J.M. and Widholm, J. (1997). Exposure to sublethal blast overpressure reduces the food intake and exercise performance of rats. Toxicology 121(1), 65 – 79.

Bauman, R.A., Ling, G., Tong, L., Januszkiewicz, A., Agoston, D., Delanerolle, N., Kim, Y., Ritzel, D., Bell, R., Ecklund, J., Armonda, R., Bandak, F., and Parks, S. (2009). An introductory characterization of a combat-casualty-care relevant swine model of closed head injury resulting from exposure to explosive blast. J Neurotrauma 26(6), 841 – 860.

Bayliss, W.M. (1902). On the local reactions of the arterial wall to changes of internal pressure. J Physiol 28(3), 220 – 231.

Becker, D.E. and Reed, K.L. (2012). Local anesthetics: review of pharmacological considerations. Anesth Prog 59(2), 90 – 101.

Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A., and Freeman, B.A. (1990). Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 87(4), 1620 – 1624.

Benkusky, N.A., Lewis, S.J. and Kooy, N.W. (1999). Peroxynitrite-mediated attenuation of alpha- and beta-adrenoceptor agonist-induced vascular responses in vivo. Eur J Pharmacol 364(2-3), 151 – 158.

Binder, L.M. (1986). Persisting symptoms after mild head injury: a review of the postconcussive syndrome. J Clin Exp Neuropsychol 8(4), 323 – 346.

Bir, C., VandeVord, P., Shen, Y., Raza, W., and Haacke, E.M. (2012). Effects of variable blast pressures on blood flow and oxygen saturation in rat brain as evidenced using MRI. Magn Reson Imaging 30(4), 527 – 534.

Black, A.H., Nadel, L., and O'Keefe, J. (1977). Hippocampal function in avoidance learning and punishment. Psychol Bull 84(6), 1107 – 1129.

Bolander, R., Mathie, B., Bir, C., Ritzel, D., and VandeVord, P. (2011). Skull flexure as a contributing factor in the mechanism of injury in the rat when exposed to a shock wave. Ann Biomed Eng 39(10), 2550 – 2559.

Born, C.T. (2005). Blast trauma: the fourth weapon of mass destruction. Scand J Surg 94(4), 279 – 285.

Boulding, J.E. and Baker, R.A. (1957). The treatment of metal poisoning with penicillamine. Lancet 270(7003), 985.

Bouma, G.J. and Muizelaar, J.P. (1990). Relationship between cardiac output and cerebral blood flow in patients with intact and with impaired autoregulation. J Neurosug 73(3), 368 – 374.

Bouma, G.J., Muizelaar, J.P., Bandoh, K., and Marmarou, A. (1992a). Blood pressure and intracranial pressure-volume dynamics in severe head injury: relationship with cerebral blood flow. J Neurosurg 77(1), 15 – 19.

Bouma, G.J., Muizelaar, J.P., Choi, S.C., Newlon, P.G., and Young, H.F. (1991). Cerebral circulation and metabolism after severe traumatic brain injury: the elusive role of ischemia. J Neurosurg 75(5), 685 – 693.

Bouma, G.J., Muizelaar, J.P., Stringer, W.A., Choi, S.C., Fatouros, P., and Young, H.F. (1992b). Ultra-early evaluation of regional cerebral blood flow in severely head-injured patients using xenon-enhanced computerized tomography. J Neurosurg 77(3), 360 – 368.

Brooks, D.N. (1972). Memory and head injury. J Nerv Ment Dis 155(5), 350 – 355.

Bryan, R.M., Eichler, M.Y., Swafford, M.W.G., Johnson, T.D., Suresh, M.S., and Childres, W.F. (1996). Stimulation of α_2 adrenoceptors dilates the rat middle cerebral artery. Anesthesiology 85(1), 82 – 90.

Bullock, R., Chesnut, R.M., Clifton, G., Ghajar, J., Marion, D.W., Narayan, R.K., Newell, D.W., Pitts, L.H., Rosner, M.J. and Wilberger, J.W. (1996). Guidelines for the management of severe head injury. Brain Trauma Foundation. Eur J Emerg Med 3(2), 109 – 127.

Butler, T.I., Kassed, C.A., and Pennypacker, K.R. (2003). Signal transduction and neurosurvival in experimental models of brain injury. Brain Res Bull 59(5), 339 – 351.

Capruso, D.X. and Levin, H.S. (1992). Cognitive impairment following closed head injury. Neurol Clin 10(4), 879 – 893.

Cereijido, M., Valdes, J., Shoshani, L., and Contreras, R.G. (1998). Role of tight junctions in establishing and maintaining cell polarity. Annu Rev Physiol 60, 161 – 177.

Cernka, I. (2005). Animal models of head trauma. NeuroRx 2(3), 410 – 422.

Cernak, I. (2015). Blast injuries and blast-induced neurotrauma: overview of pathophysiology and experimental knowledge models and findings, in: *Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects*. F.H. Kobeissy (ed). CRC Press/Taylor & Francis: Boca Raton, Chapter 45.

Cernak, I., and Noble-Haeusslein, L.J. (2010). Traumatic brain injury: an overview of pathobiology with emphasis on military populations. J Cereb Blood Flow Metab 30(2), 255 – 266.

Cernak, I., Wang, Z., Jiang, J., Bian, X., and Savic, J. (2001a). Cognitive deficits following blast injury-induced neurotrauma: possible involvement of nitric oxide. Brain Inj 15(7), 593 – 612.

Cernak, I., Wang, Z., Jiang, J., Bian, X., and Savic, J. (2001b). Ultrastructural and functional characteristics of blast injury-induced neurotrauma. J Trauma 50(4), 695 – 706.

Chafi, M.S., Karami, G., and Ziejewski, M. (2010). Biomechanical assessment of brain dynamic responses due to blast pressure waves. Ann Biomed Eng 38(2), 490 – 504.

Chambers, L.W., Rhee, P., Baker, B.C., Perciballi, J., Cubano, M., Compeggie, M., Nace, M., and Bohman, H.R. (2005). Initial experience of US Marine Corps forward resuscitative surgical system during Operation Iraqi Freedom. Arch Surg 140(1), 26 – 32.

Champion, H.R., Holcomb, J.B., and Young, L.A. (2009). Injuries from explosions: physics, biophysics, pathology, and required research focus. J Trauma 66(5), 1468 – 1477.

Chatpun, S. and Cabrales, P. (2010). Effects of plasma velocity modulation on cardiac function during moderate hemodilution. Asian J Transfus Sci 4(2), 102 – 108.

Chavko, M., Prusaczyk, W.K., and McCarron, R.M. (2006). Lung injury and recovery after exposure to blast overpressure. J Trauma 61(4), 933 – 942.

Chavko, M., Watanabe, T., Adeeb, S., Lankasky, J., Ahlers, S.T., and McCarron, R.M. (2011). Relationship between orientation to a blast and pressure wave propagation inside the rat brain. J Neurosci Methods 195(1), 61 – 66.

Chen, Y.C., Smith, D.H., and Meaney, D.F. (2009). *In-vitro* approaches for studying blast-induced traumatic brain injury. J Neurotrauma 26(6), 861 – 876.

Cheng, J., Gu, J., Ma, Y., Yang, T., Kuang, Y., Li, B., and Kang, J. (2010). Development of a rat model for studying blast-induced traumatic brain injury. J Neurol Sci 294(1-2), 23 – 28.

Cherian, L., Goodman, J.C., and Robertson, C.S. (2000). Brain nitric oxide changes after controlled cortical impact injury in rats. J Neurophysiol 83(4), 2171 – 2178.

Cherian, L., Robertson, C.S., Contant, C.F. and Bryan, R.M. (1994). Lateral cortical impact injury in rats: cerebrovascular effects of varying depth of cortical deformation and impact velocity. J Neurotrauma 11(5), 573 – 585.

Cherian, L., Robertson, C.S., and Goodman, J.C. (1996). Secondary insults increase injury after controlled cortical impact in rats. J Neurotrauma 13(7), 371 – 383.

Chesnut, R.M., Marshall, L.F., Klauber, M.R., Blunt, B.A., Baldwin, N., Eisenberg, H.M., Jane, J.A., Marmarou, A., and Foulkes, M.A. (1993a). The role of secondary brain injury in determining outcome from severe head injury. J Trauma 34(2), 216 – 222.

Chesnut, R.M., Marshall, S.B., Piek, J., Blunt, B.A., Klauber, M.R., and Marshall, L.F. (1993b). Early and late systemic hypotension as a frequent and fundamental source of cerebral ischemia following severe brain injury in the Traumatic Coma Data Bank. Acta Neurochir Suppl 59, 121 – 125.

Chien, S. and Lipowsky, H.H. (1982). Correlation of hemodynamics in macrocirculation and microcirculation. Int J Microcirc Clin Exp 1(4), 351 – 365.

Cho, H.J., Sajja, V.S.S.S., VandeVord, P.J., and Leea, Y.W. (2013). Blast induces oxidative stress, inflammation, neuronal loss and subsequent short-term memory impairment in rats. Neuroscience 253, 9 – 20.

Clark, R.E., Broadbent, N.J., and Squire, L.R. (2005). Hippocampus and remote spatial memory in rats. Hippocampus 15(2), 260 – 272.

Clemedson, C.J. (1956). Blast injury. Physiol Rev 36(3), 336 – 354.

Courant, R. and Friedrichs, K.O. (1948). Supersonic flow and shock waves. Interscience Publishers: New York, NY.

Courtney, A.C. and Courtney, A.W. (2009). A thoracic mechanism of mild traumatic brain injury due to blast pressure waves. Med Hypotheses 72(1), 76 – 83.

Courtney, M.W. and Courtney, A.C. (2011). Working toward exposure thresholds for blast-induced traumatic brain injury: thoracic and acceleration mechanisms. Neuroimage 54(Suppl 1), S55 – S61.

Cucchiara, R.F., Theye, R.A., and Michenfelder, J.D. (1974). The effects of isoflurane on canine cerebral metabolism and blood flow. Anesthesiology 40(6), 571 – 574.

Cullis, I.G. (2001). Blast waves and how they interact with structures. J R Army Med Corps 147(1), 16 – 26.

Damjanac, M., Rioux Bilan, A., Barrier, L., Pontcharraud, R., Anne, C., Hugon, J., and Page, G. (2007). Fluoro-Jade® B staining as useful tool to identify activated microglia and astrocytes in a mouse transgenic model of Alzheimer's disease. Brain Res 1128(1), 40 – 49.

DePalma, R.G., Burris, D.G., Champion, H.R., and Hodgson, M.J. (2005). Blast injuries. N Engl J Med 352(13), 1335 – 1342.

DeWitt, D., Jenkins, L.W., Wei, E.P., Lutz, H., Becker, D.P., and Kontos, H.A. (1986). Effects of fluid-percussion brain injury on regional cerebral blood flow and pial arteriolar diameter. J Neurosurg 64(5), 787 – 794.

DeWitt, D.S., Mathew, B.P., Chaisson, J.M., and Prough, D.S. (2001). Peroxynitrite reduces vasodilatory responses to reduced intravascular pressure, calcitonin gene-related peptide and cromakalim in isolated middle cerebral arteries. J Cereb Blood Flow Metab 21(3), 253 – 261.

DeWitt, D.S., Perez-Polo, R., Hulsebosch, C.E., Dash, P.K., and Robertson, C.S. (2013). Challenges in the development of rodent models of mild traumatic brain injury. J Neurotrauma 30(9), 688 – 701.

DeWitt, D.S. and Prough, D.S. (2003). Traumatic cerebral vascular injury: the effects of concussive brain injury on the cerebral vasculature. J Neurotrauma 20(9), 795 – 825.

DeWitt, D.S. and Prough, D.S. (2009). Blast-induced brain injury and posttraumatic hypotension and hypoxemia. J Neurotrauma 26(6), 877 – 887.

DeWitt, D.S. and Prough, D.S. (2012). Assessment of cerebral vascular dysfunction after traumatic brain injury, in: *Animal Models of Acute Neurological Injuries II: Injury*

and Mechanistic Assessments. J. Chen, X. M. Xu and J. H. Zhang (eds.). Humana Press: New York.

DeWitt, D.S., Prough, D.S., Deal, D.D., Vines, S.M. and Hoen, H. (1996). Hypertonic saline does not improve cerebral oxygen delivery after head injury and mild hemorrhage in cats. Crit Care Med 24(1), 109 – 117.

DeWitt, D.S., Prough, D.S., Taylor, C.L., Whitley, J.M., Deal, D.D., and Vines, S.M. (1992). Regional cerebrovascular responses to progressive hypotension after traumatic brain injury in cats. Am J Physiol 263(4 Pt 2), H1276 – H1284.

DeWitt, D.S., Prough, D.S., Uchida, T., Deal, D.D., and Vines, S.M. (1997a). Effects of nalmefene, CG3703, tirilazad or dopamine on cerebral blood flow, oxygen delivery, and electroencephalographic activity after traumatic brain injury and hemorrhage. J Neurotrauma 14(12), 931 – 941.

DeWitt, D.S., Smith, T.G., Deyo, D.S., Miller, K.R., Uchida, T., and Prough, D.S. (1997b). L-arginine or superoxide dismutase prevents or reverses cerebral hypoperfusion after fluid-percussion traumatic brain injury. J Neurotrauma 14(4), 223 – 233.

D'Hooge, R. and De Deyn, P.P. (2001). Applications of the Morris water maze in the study of learning and memory. Brain Res Brain Res Rev 36(1), 60 – 90.

Dixon, C.E., Kochanek, P.M., Yan, H.Q., Schiding, J.K., Griffith, R.G., Baum, E., Marion, D.W., and DeKosky, S.T. (1999). One-year study of spatial memory performance, brain morphology, and cholinergic markers after moderate controlled cortical impact in rats. J Neurotrauma 16(2), 109 – 122.

Dixon, C.E., Lyeth, B.G., Povlishock, J.T., Findling, R.L., Hamm, R.J., Marmarou, A., Young, H.F., and Hayes, R.L. (1987). A fluid percussion model of experimental brain injury in the rat. J Neurosurg 67(1), 110 – 119.

Dowling, L.W. (1917). Projective Geometry. McGraw-Hill: New York, NY.

Dressler, R.F. (1954). Turbulent flow in shock tubes of varying cross section. J Research Nat Bureau Standards 53(4), 253 – 260.

Drummond, J.C., Todd, M.M., Scheller, M.S., and Shapiro, H.M. (1986). A comparison of the direct cerebral vasodilating potencies of halothane and isoflurane in the New Zealand white rabbit. Anesthesiology 65(5), 462 – 467.

Duff, R.E. and Blackwell, A.N. (1966). Explosive driven shock tubes. Rev Sci Instrum 37(5), 579 – 586.

Edvinson, L., Mackenzie, E.T., and McCullogh, J. (1993). Cerebral Blood Flow and Metabolism. Raven Press: New York.

Eger, E.I. 2nd (1984). The pharmacology of isoflurane. Br J Anaesth 56 Suppl 1, 71S – 99S.

Elder, G.A. and Cristian, A. (2009). Blast-related mild traumatic brain injury: mechanisms of injury and impact on clinical care. Mt Sinai J Med 76(2), 111 – 118.

Elder, G.A., Gama Sosa, M.A., De Gasperi, R., Stone, J.R., Dickstein, D.L., Haghighi, F., Hof, P.R., and Ahlers, S.T. (2015). Vascular and inflammatory factors in the pathophysiology of blast-induced brain injury. Front Neurol 6, 1 – 22.

Elder, G.A., Mitsis, E.M., Ahlers, S.T., and Cristian, A. (2010). Blast-induced mild traumatic brain injury. Psychiatr Clin North Am 33(4), 757 – 781.

Elliot, S.J., Lacey, D.J., Chilian, W.M., and Brzezinska, A.K. (1998). Peroxynitrite is a contractile agonist of cerebral artery smooth muscle cells. Am J Physiol 275(5 Pt 2), H1585 – H1591.

Elsayed, N.M. (1997). Toxicology of blast overpressure. Toxicology 121(1), 1 – 15.

Elsayed, N.M., Fitzpatrick, T.M., and Dodd, K.T. (1997). Free-radical associated response in blood of sheep, rabbits, and rats after a single exposure to high energy impulse noise (blast). Environ Nutr Interact 1, 11 – 22.

Engelborghs, K., Haseldonckx, M., Van Reempts, J., Van Rossem, K., Wouters, L., Borgers, M., and Verlooy, J. (2000). Impaired autoregulation of cerebral blood flow in an experimental model of traumatic brain injury. J Neurotrauma 17(8), 667 – 677.

Eskridge, S.L., Macera, C.A., Galarneau, M.R., Holbrook, T.L., Woodruff, S.I., MacGregor, A.J., Morton, D.J., and Shaffer, R.A. (2012). Injuries from combat explosions in Iraq: injury type, location and severity. Injury 43(10), 1678 – 1682.

Eyre, J.A., Essex, T.J., Flecknell, P.A., Bartholomew, P.H., and Sinclair, J.I. (1988). A comparison of measurements of cerebral blood flow in the rabbit using laser Doppler spectroscopy and radionuclide labeled microspheres. Clin Phys Physiol Meas 9(1), 65 – 74.

Farber, N.E., Harkin, C.P., Niedfeldt, J., Hudetz, A.G., Kampine, J.P., and Schmeling, W.T. (1997). Region-specific and agent-specific dilation of intracerebral microvessels by volatile anesthetics in rat brain slices. Anesthesiology 87(5), 1191 – 1198.

Feeney, D.M., Gonzalez, A., and Law, W.A. (1982). Amphetamine, haloperidol, and experience interact to affect rate of recovery after motor cortex injury. Science 217(4562), 855 – 857.

Fieten, H., Dirksen, K., Van den Ingh, T.S.G., Winter, E., Watson, A.L., Leegwater, P.A.J. and Rothuizen, J. (2013a). D-penicillamine treatment of copper associated hepatitis in Labrador retrievers. Vet J 196(3), 522 – 527.

Fieten, H., Hugen, S., Van den Ingh, T.S.G., Hendriks, W.H., Vernooij, J.C.M., Bode, P., Watson, A.L., Leegwater, P.A.J. and Rothuizen, J. (2013b). Urinary excretion of copper, zinc and iron with and without D-penicillamine administration in relation to hepatic copper concentration in dogs. Veterinary J 197(2), 468 – 473.

Förstermann, U. and Sessa, W.C. (2012). Nitric oxide synthases: regulation and function. Eur Heart J 33(7), 829 – 837.

Flynn, N.M., Buljubasic, N., Bosnjak, Z.J., and Kampine, J.P. (1992). Isoflurane produces endothelium-independent relaxation in canine middle cerebral arteries. Anesthesiology 76(3), 461 – 467.

Friedlander, F.G. (1946). The diffraction of sound pulses; diffraction by a semi-infinite plane. Proc R Soc Lond A Math Phys Sci 186(1006), 322 – 344.

Galarneau, M.R., Woodruff, S.I., Dye, J.L., Mohrle, C.R. and Wade, A.L. (2008). Traumatic brain injury during Operation Iraqi Freedom: findings from the United States Navy-Marine Corps Combat Trauma Registry. J Neurosurg 108(5), 950 – 957.

Gama Sosa, M.A., De Gasperi, R., Janssen, P.L., Yuk, F.J. Anazodo, P.C., Pricop, P.E., Paulino, A.J., Wicinski, B., Shaughness, M.C., Maudlin-Jeronimo, E., Hall, A.A., Dickstein, D.L., McCarron, R.M., Chavko, M., Hof, P.R., Ahlers, S.T., and Elder, G.A. (2014). Selective vulnerability of the cerebral vasculature to blast injury in a rat model of mild traumatic brain injury. Acta Neuropathol Commun 2(67), 1 – 18.

Garman, R.H., Jenkins, L.W., Switzer, R.C., Bauman, R.A., Tong, L.C., Swauger, P.V., Parks, S.A., Ritzel, D.V., Dixon, C.E., Clark, R.S.B., Bayir, H., Kagan, V., Jackson, E.K., and Kochanek, P.M. (2011). Blast exposure in rats with body shielding is characterized primarily by diffuse axonal injury. J Neurotrauma 28(6), 947 – 959.

Garry, P.S., Ezra, M., Rowland, M.J., Westbrook, J. and Pattinson, K.T.S. (2015). The role of the nitric oxide pathway in brain injury and its treatment – from bench to bedside. Exp Neurol 263, 235 – 243.

Giri, B.K., Krishnappa, I.K., Bryan, R.M. Jr., Robertson, C., and Watson, J. (2000). Regional cerebral blood flow after cortical impact injury complicated by a secondary insult in rats. Stroke 31(4), 961 – 967.

Giunta, B., Obregon, D., Velisetty, R., Sanberg, P.R., Borlongan, C.V., and Tan, J. (2012). The immunology of traumatic brain injury: a prime target for Alzheimer's disease prevention. J Neuroinflammation 9, 185.

Globus, M.Y., Alonso, O., Dietrich, W.D., Busto, R., and Ginsberg, M.D. (1995). Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. J Neurochem 65(4), 1704 – 1711.

Golding, E.M., Contant, C.F., Robertson, C.S., and Bryan, R.M. (1998). Temporal effect of severe controlled cortical impact injury in the rat on the myogenic response of the middle cerebral artery. J Neurotrauma 15(11), 973 – 984.

Golding, E.M., Robertson, C.S., and Bryan, R.M.Jr. (1999a). The consequences of traumatic brain injury on cerebral blood flow and autoregulation: a review. Clin Exp Hypertens 21(4), 299 – 332.

Golding, E.M., Robertson, C.S., and Bryan, R.M. (1999b). Loss of myogenic responses in the middle cerebral artery following severe head injury in the rat. J Neurosci 36, 253 – 262.

Golding, E.M., Steenberg, M.L., Contant, C.F.Jr., Krishnappa, I., Robertson, C.S., and Bryan, R.M.Jr. (1999c). Cerebrovascular reactivity to CO(2) and hypotension after mild cortical impact injury. Am J Physiol 277(4 Pt 2), H1457 – H1466.

Goldstein, L.B. and Davis, J.N. (1990). Beam-walking in rats: studies towards developing an animal model of functional recovery after brain injury. J Neurosci Methods 31(2), 101 – 107.

Goldstein, S. and Czapski, G. (1995). The reaction of $NO^{\bullet}$ with $O_2^{\bullet-}$ and $HO_2^{\bullet}$. Free Radic Biol Med 19(4), 505 – 510.

Gronwall, D. and Wrightson, P. (1974). Delayed recovery of intellectual function after minor head injury. Lancet 2(7881), 605 – 609.

Gryglewski, R.J. and Moncada, S. (1987). Secretory function of vascular endothelium. Adv Prostaglandin Thromboxane Leukot Res 17A, 397 – 404.

Guo, Z.N., Shao, A., Tong, L.S., Sun, W., Liu, J., and Yang, Y. (2016). The role of nitric oxide and sympathetic control in cerebral autoregulation in the setting of subarachnoid hemorrhage and traumatic brain injury. Mol Neurobiol 53(6), 3606 – 3615.

Haberl, R.L., Heizer, M.L., Marmarou, A., and Ellis, E.F. (1989). Laser-Doppler assessment of brain microcirculation: effect of systemic alterations. Am J Physiol 256(4 Pt 2), H1247 – H1254.

Hall, E.D. (1995a). Mechanisms of secondary CNS injury, in: *Neurosurgery 96: Manual of Neurosurgery*. J.D. Palmer (ed). Churchill-Livingstone: New York, pps. 505 – 510.

Hall, E.D. (1995b). The mouse head injury model: utility in the discovery of acute cerebroprotective agents, in: *Central Nervous System Trauma Research Techniques*. S.T. Ohnishi and T. Ohnishi (eds). CRC Press: Boca Raton, pps. 213 – 233.

Hall, E.D. and Braughler, J.M. (1993). Free radicals in CNS injury. Res Publ Assoc Res Nerv Ment Dis 71, 81–105.

Hall, E.D., Detloff, M.R., Johnson, K., and Kupina, N.C. (2004). Peroxynitrite-mediated protein nitration and lipid peroxidation in a mouse model of traumatic brain injury. J Neurotrauma 21(1), 9 – 20.

Hall, E.D., Kupina, N.C., and Althaus, J.S. (1999). Peroxynitrite scavengers for the acute treatment of traumatic brain injury. Ann N Y Acad Sci 890: 462 – 468.

Hall, E.D., Wang, J.A., Bosken, J.M., and Singh, I.N. (2016). Lipid peroxidation in brain or spinal cord mitochondria after injury. J Bioenerg Biomembr 48(2), 169 – 174.

Hallam, T.M., Floyd, C.L., Folkerts, M.M., Lee, L.L., Gong, Q.Z., Lyeth, B.G., Muizelaar, J.P., and 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(5), 521 – 539.

Hamit, H.F. (1973). Primary blast injuries. IMS Ind Med Surg 42(3), 14 – 21.

Hamm, R.J. (2001). Neurobehavioral assessment of outcome following traumatic brain injury in rats: an evaluation of selected measures. J Neurotrauma 18(11), 1207 – 1216.

Hamm, R.J., Dixon, C.E., Gbadebo, D.M., Singha, A.K., Jenkins, L.W., Lyeth, B.G., and Hayes, R.L. (1992). Cognitive deficits following traumatic brain injury produced by controlled cortical impact. J. Neurotrauma 9(1), 11 – 20.

Hamm, R.J., Lyeth, B.G., Jenkins, L.W., O'Dell, D.M., and Pike, B.R. (1993). Selective cognitive impairment following traumatic brain injury in rats. Behav Brain Res 59(1-2), 169 – 173.

Hamm, R.J., Temple, M.D., Pike, B.R., O'Dell, D.M., Buck, D.L., and Lyeth, B.G. (1996). Working memory deficits following traumatic brain injury in the rat. J Neurotrauma 13(6), 317 – 323.

Hanley, C.J. (2007). U.S. Military struggles to defeat IED's. *The Associated Press Online*, August 20.

Hawkins, B.T. and Davis, T.P. (2005). The blood-brain barrier/neurovascular unit in heath and disease. Pharmacol Rev 57(2), 173 – 185.

Hawkins, B.E., Cowart, J.C., Parsley, M.A., Capra, B.A., Eidson, K.A., Hellmich, H.L., Dewitt, D.S., and Prough, D.S. (2013). Effects of trauma, hemorrhage and resuscitation in aged rats. Brain Res 1496, 28 – 35.

Hellmich, H.L., Capra, B., Eidson, K., Garcia, J., Kennedy, D., Uchida, T., Parsley, M., Cowart, J., DeWitt, D., and Prough, D. (2005a). Dose-dependent neuronal injury after traumatic brain injury. Brain Res 1044(2), 144 – 154.

Hellmich, H.L., Garcia, J.M., Shimamura, M., Shah, S.A., Avila, M.A., Uchida, T., Parsely, M.A., Capra, B.A., Eidson, K.A., Kennedy, D.R., Winston, J.H., DeWitt, D.S., and Prough, D.S. (2005b). Traumatic brain injury and hemorrhagic hypotension suppress neuroprotective gene expression in injured hippocampal neurons. Anesthesiology 102(4), 806 – 814.

Henninger, N., Dutzmann, S., Sicard, K.M., Kollmar, R., Bardutzky, J., and Schwab, S. (2005). Impaired spatial learning in a novel rat model of mild cerebral concussion injury. Exp Neurol 195(2), 447 – 457.

Henshall, B.D. (1957). On some aspects of the use of shock tubes in aerodynamic research. HM Stationary Office: London.

Hicks, S.P. and D'Amato, C.J. (1975). Motor-sensory cortex-corticospinal system and developing locomotion and placing in rats. Am J Anat 143(1), 1 – 42.

Hiebert, J.B., Shen, Q., Thimmesch, A.R., and Pierce, J.D. (2015). Traumatic brain injury and mitochondrial dysfunction. Am J Med Sci 350(2), 132 – 138.

Hoffman, G., Van den Ingh, T.S.G., Bode, P. and Rothuizen, J. (2006). Copper-associated chronic hepatitis in Labrador retrievers. J Vet Intern Med 20, 856 – 861.

Huie, R.E. and Padmaja, S. (1993). The reaction of NO with superoxide. Free Radic Res Commun 18(4), 195 – 199.

Iida, H., Ohata, H., Iida, M., Watanabe, Y., and Dohi, S. (1998). Isoflurane and sevoflurane induce vasodilation of cerebral vessels via ATP-sensitive K^+ channel activation. Anesthesiology 89(4), 954 – 960.

Intaglietta, M. and Zweifach, B.W. (1974). Microcirculatory basis of fluid exchange. Adv Biol Med Phys 15(0), 111 – 159.

Ishige, N., Pitts, L.H., Berry, I., Nishimura, M.C., and James, T.L. (1988). The effects of hypovolemic hypotension on high energy phosphate metabolism of traumatized brain in rats. J Neurosurg 68(1), 129 – 136.

Itoh, Y. and Suzuki, N. (2012). Control of brain capillary blood flow. J Cereb Blood Flow Metab 32(7), 1167 – 1176.

Jenkins, L.W., Moszynski, K., Lyeth, B.G., Lewelt, W., DeWitt, D.S., Allen, A., Dixon, C.E., Povlishock, J.T., Majewski, T.J., Clifton, G.L., Young, H.F., Becker, D.P., and Hayes, R.L. (1989). Increased vulnerability of the mildly traumatized rat brain to cerebral ischemia: the use of controlled secondary ischemia as a research tool to identify common or different mechanisms contributing to mechanical and ischemic brain injury. Brain Res 477(1-2), 211 – 224.

Johnson, P.C. (1977). The myogenic response and the microcirculation. Microvasc Res 13(1), 1 – 18.

Johnson, P.C. (1989). The myogenic response in the microcirculation and its interaction with other control systems. J Hypertens Suppl 7(4), S33 – S39.

Johnson, P.C. and Henrich, H.A. (1975). Metabolic and myogenic factors in local regulation of the microcirculation. Fed Proc 34(11), 2020 – 2024.

Kabu, S., Jaffer, H., Petro, M., Dudzinski, D., Stewart, D., Courtney, A., Courtney, M., and Labhasetwar, V. (2015). Blast-associated shock waves result in increased brain vascular leakage and elevated ROS levels in a rat model of traumatic brain injury. PLoS One 10(5), 1 – 19.

Kamnaksh, A., Kwon, S.K., Kovesdi, E., Ahmed, F., Barry, E.S., Grunberg, N.E., Long, J. and Agoston, D. (2012). Neurobehavioral, cellular, and molecular consequences of single and multiple mild blast exposure. Electrophoresis 33(24), 3680 – 3692.

Khayyal, M.T., Girgis, N.I. and McConnell, E. (1967). The use of penicillamine as an adjuvant to tartar emetic in the treatment of experimental schistosomiasis. Bull World Health Organ 37(3), 387 – 392.

Kimme, P., Ledin, T., and Sjöberg, F. (2007). Dose effect of sevoflurane and isoflurane anesthetics on cortical blood flow during controlled hypotension in the pig. Acta Anaesthesiol Scand 51(5), 607 – 613.

Kobayashi, K., Miki, M. and Tagawa, S. (1995). Pulse-radiolysis study of the reaction of nitric oxide with superoxide. J Chem Soc Dalton Trans 17, 2885 – 2889.

Kocsis, J.D. and Tessler, A. (2009). Pathology of blast-related brain injury. J Rehabil Res Dev 46(6), 667 – 672.

Kochanek, P.M., Marion, D.W., Zhang, W., Schiding, J.K., White, M., Palmer, A.M., Clark, R.S., O'Malley, M.E., Styren, S.D., and Ho, C. (1995). Severe controlled cortical impact in rats: assessment of cerebral edema, blood flow, and contusion volume. J Neurotrauma 12(6), 1015 – 1025.

Koehler, R.C., Roman, R.J., and Harder, D.R. (2009). Astrocytes and the regulation of cerebral blood flow. Trends Neurosci 32(3), 160 – 169.

Koenig, H.M., Pelligrino, D.A., Wang, Q., and Albrecht, R.F. (1994). Role of nitric oxide and endothelium in rat pial vessel dilation responses to isoflurane. Anesth Analg 79(5), 886 – 891.

Koliatsos, V.E., Cernak, I., Xu, L., Song, Y., Savonenko, A., Crain, B.J., Eberhart, C.G., Frangakis, C.E., Melnikova, T., Kim, H., and Lee, D. (2011). A mouse model of blast injury to the brain: initial, pathological, neuropathological, and behavioral characterization. J Neuropathol Exp Neurol 70(5), 399 – 416.

Kontos, H.A. (1981). Regulation of the cerebral circulation. Annu Rev Physiol 43, 397 – 407.

Kontos, H. A. and Povlishock, J. T. (1986). Oxygen radicals in brain injury. Cent Nerv Syst Trauma 3(4), 257 – 263.

Kontos, H. A. and Wei, E. P. (1986). Superoxide production in experimental brain injury. J. Neurosurg 64(5), 803 – 807.

Kovacs, S.K., Leonessa, F., and Ling, G.S.F. (2014). Blast TBI models, neuropathology, and implications for seizure risk. Front Neurol 5(47), 1 – 11.

Kovesdi, E., Czeiter, E., Tamas, A., Reglodi, D., Szellar, D., Pal, J., Bukovis, P., Doczi, T., and Buki, A. (2007). Rescuing neurons and glia: is inhibition of apoptosis useful? Prog Brain Res 161, 81 – 95.

Kovesdi, E., Gyorgy, A.B., Kwon, S.K., Wingo, D.L., Kamnaksh, A., Long, J.B., Kasper, C.E., and Agoston, D.V. (2011). The effect of enriched environment on the

outcome of traumatic brain injury; a behavioral, proteomics, and histological study. Front Neurosci 5, 42.

Kovesdi, E., Kamnaksh, A., Wingo, D., Ahmed, F., Grunberg, N.E., Long, J.B., Kasper, C.E., and Agoston, D.V. (2012). Acute minocycline treatment mitigates the symptoms of mild blast-induced traumatic brain injury. Front Neurol 3, 1 – 18.

Kroppenstedt, S.N., Kern, M., Thomale, U.W., Schneider, G.H., Lanksch, W.R., and Unterberg, A.W. (1999). Effect of cerebral perfusion pressure on contusion volume following impact injury. J Neurosurg 90(3), 520 – 526.

Kuehn, R., Simard, P.F., Driscoll, I., Keledjian, K., Ivanova, S., Tosun, C., Williams, A., Bochicchio, G., Gerzanich, V., and Simard, J.M. (2010). Rodent model of direct cranial blast injury. J Neurotrauma 28(10), 2155 – 2169.

Kuroda, Y., Murakami, M., Tsuruta, J., Murakawa, T., and Sakabe, T. (1997). Blood flow velocity of middle cerebral artery during prolonged anesthesia with halothane, isoflurane, and sevoflurane in humans. Anesthesiology 87(3), 527 – 32.

Kwon, S.K.C., Kovesdi, E., Gyorgy, A.B., Wingo, D., Kamnaksh, A., Walker, J., Long, J.B., and Agoston, D.V. (2011). Stress and traumatic brain injury: a behavioral, proteomics, and histological study. Front Neurol 2, 12.

Lam, A.M., Mayberg, T.S., Eng, C.C., Cooper, J.O., Bachenberg, K.L., and Mathisen, T.L. (1994). Nitrous oxide-isoflurane anesthesia causes more cerebral vasodilation than an equipotent dose of isoflurane in humans. Anesth Analg 78(3), 462 – 468.

Lammie, G.A., Piper, I.R., Thomson, D., and Brannan, F. (1999). Neuropathologic characterization of a rodent model of closed head injury–addition of clinically relevant secondary insults does not significantly potentiate brain damage. J Neurotrauma 16(7), 603 – 615.

Lang, E.W., Diehl, R.R., and Mehdorn, M. (2001). Cerebral autoregulation testing after aneurysmal subarachnoid hemorrhage: the phase relationship between arterial blood pressure and cerebral blood flow velocity. Crit Care Med 29(1), 158 – 163.

Larsson, E., Lindvall, O., and Kokaia, Z. (2001). Stereological assessment of vulnerability of immunocytochemically identified striatal and hippocampal neurons after global cerebral ischemia. Brain Res 913(2), 117 – 132.

Lenz, G.R. and Martell, A.E. (1964). Metal chelates of some sulfur-containing amino acids. Biochemistry 3, 745 – 750.

Lenzi, P., Zoccoli, G., Walker, A.M., and Franzini, C. (1999). Cerebral blood flow regulation in REM sleep: a model for flow-metabolism coupling. Arch Ital Biol 137(2-3), 165 – 179.

Leonardi, A.D., Bir, C.A., Ritzel, D.V., and VandeVord, P.J. (2011). Intracranial pressure increases during exposure to a shock wave. J Neurotrauma 28(1), 85 – 94.

Leonardi, A.D., Keane, N.J., Bir, C.A., Ryan, A.G., Xu, L., and Vandevord, P.J. (2012). Head orientation affects the intracranial pressure response resulting from shock wave loading in the rat. J Biomech 45(15), 2595 – 2602.

Leung, L.Y., VandeVord, P.J., Dalcengio, A.L., Bir, C., Yang, K.H., and King, A.I. (2008). Blast related neurotrauma: a review of cellular injury. Mol Cell Biomech 5(3), 155 – 168.

Lewelt, W., Jenkins, L.W., and Miller, J.D. (1980). Autoregulation of cerebral blood flow after experimental fluid percussion injury of the brain. J Neurosurg 53(4), 500 – 511.

Lewelt, W., Jenkins, L.W., and Miller, J.D. (1982). Effects of experimental fluid percussion injury of the brain on cerebrovascular reactivity to hypoxia and to hypercapnia. J Neurosurg 56(3), 332 – 338.

Levin, H.S., Benton, A.L., and Grossman, R.G. (1982). Neurobehavioral consequences of closed head injury. Oxford University Press: New York.

Levin, H.S., Goldstein, F.C., High, W.M., and Eisenberg, H.M. (1988). Disproportionately severe memory deficit in relation to normal intellectual functioning after closed head injury. J Neurol Neurosurg Psychiatry 51(10), 1294 – 1301.

Li, C.X., Patel, S., Auerbach, E.J., and Zhang, X. (2013). Dose-dependent effect of isoflurane on regional cerebral blood flow in anesthetized macaque monkeys. Neurosci Lett 541, 58 – 62.

Ling, G., Bandak, F., Armonda, R., Grant, G., and Ecklund, J. (2009). Explosive blast neurotrauma. J Neurotrauma 26(6), 815 – 825.

Liu, H., Li, J., Zhao, F., Wang, H., Qu, Y. and Mu, D. (2015). Nitric oxide synthase in hypoxic or ischemic brain injury. Rev Neurosci 26(1), 105 – 117.

Liu, P.K., Robertson, C.S., and Valadka, A. (2002). The association between neuronal nitric oxide synthase and neuronal sensitivity in the brain after brain injury. Ann N Y Acad Sci 962, 226 – 241.

Loane, D.J. and Faden, A.I. (2010). Neuroprotection for traumatic brain injury: translational challenges and emerging therapeutic strategies. Trends Pharmacol Sci 31(12), 596 – 604.

Long, J.B., Bentley, T.L., Wessner, K.A., Cerone, C., Sweeney, S., and Bauman, R.A. (2009). Blast overpressure in rats: recreating a battlefield injury in the laboratory. J Neurotrauma 26(6), 827 – 840.

Longnecker, D.E. and Harris, P.D. (1980). Microcirculatory actions of general anesthetics. Fed Poc 39(5), 1580 – 1583.

Lozano, D., Gonzales-Portillo, G.S., Acosta, S., de la Pena, I., Tajiri, N, Kaneko, Y., and Borlongan, C.V. (2015). Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatr Dis Treat 11, 97 – 106.

Luerssen, T.G., Klauber, M.R., and Marshall, L.F. (1988). Outcome from head injury related to patient's age. A longitudinal prospective study of adult and pediatric head injury. J Neurosurg 68(3), 409 – 416.

MacGregor, A.J., Dougherty, A.L., and Galarneau, M.R. (2011). Injury-specific correlates of combat-related traumatic brain injury in Operation Iraqi Freedom. J Head Trauma Rehabil 26(4), 312 – 318.

Maekawa, T., Tommasino, C., Shapiro, H.M., Keifer-Goodman, J., and Kohlenberger, R.W. (1986). Local cerebral blood flow and glucose utilization during isoflurane anesthesia in the rat. Anesthesiology 65(2), 144 – 151.

Mandigers, P.J., Van den Ingh, T.S.G., Bode, P. and Rothuizen, J. (2005). Improvement in liver pathology after 4 months of D-penicillamine in 5 Doberman pinschers with subclinical hepatitis. J Vet Intern Med 19(1), 40 – 43.

Maneen, M.J. and Cipolla, M.J. (2007). Peroxynitrite diminishes myogenic tone in cerebral arteries: role of nitrotyrosine and F-actin. Am J Physiol Heart Circ Physiol 292(2), H1042 – H1050.

Maneen, M.J., Hannah, R., Vitullo, L., DeLance, N., and Cipolla, M.J. (2006). Peroxynitrite diminishes myogenic activity and is associated with decreased vascular smooth muscle F-actin in rat posterior cerebral arteries. Stroke 37(3), 894 – 899.

Martin, E.M., Lu, W.C., Helmick, K., French, L., and Warden, D.L. (2008). Traumatic brain injuries sustained in the Afghanistan and Iraq wars. J Trauma Nurs 15(3), 94 – 99.

Martin, W.A. (1958). A review of shock tubes and shock tunnels. General Dynamics Convair-Astronautics Division: San Diego, CA.

Masamoto, K., Kim, T., Fukuda, M., Wang, P., and Kim, S.G. (2007). Relationship between neural, vascular, and BOLD signals in isoflurane-anesthetized rat somatosensory cortex. Cereb Cortex 17(4), 942 – 950.

Masamoto, K., Fukuda, M., Vazquez, A., and Kim, S.G. (2009). Dose-dependent effect of isoflurane on neurovascular coupling in rat cerebral cortex. Eur J Neurosci 30(2), 242 – 250.

Masel, B.E., Bell, R.S., Brossart, S., Grill, R.J., Hayes, R.L., Levin, H.S., Rasband, M.N., Ritzel, D.V., Wade, C.E., and DeWitt, D.S. (2012). Galveston brain injury conference 2010: clinical and experimental aspects of blast Injury. J Neurotrauma 29(12), 2143 – 2171.

Mathew, B.P., DeWitt, D.S., Bryan, R.M., Bukoski, R.D., and Prough, D.S. (1999). Traumatic brain injury reduces myogenic responses in pressurized rodent middle cerebral arteries. J Neurotrauma 16(12), 177 – 186.

Matsushita, Y., Bramlett, H.M., Kuluz, J.W., Alonso, O., and Dietrich, W.D. (2001). Delayed hemorrhagic hypotension exacerbates the hemodynamic and histopathologic consequences of traumatic brain injury in rats. J Cereb Blood Flow Metab 21(7), 847 – 856.

Matta, B.F., Heath, K.J., Tipping, K., and Summors, A.C. (1999). Direct cerebral vasodilatory effects of sevoflurane and isoflurane. Anesthesiology 91(3), 677 – 680.

Mchedlishvili, G. (1980). Physiological mechanisms controlling cerebral blood flow. Stroke 11(3), 240 – 248.

McLean, A., Temkin, N.R., Dikem, S., and Wyler, A.R. (1983). The behavioral sequelae of head injury. J Clin Neuropsychol 5(4), 361 – 376.

McPherson, R.W. and Traystman, R.J. (1988). Effects of isoflurane on cerebral autoregulation in dogs. Anesthesiology 69(4), 493 – 499.

Mellor, S.G. (1988). The pathogenesis of blast injury and its management. Br J Hosp Med 36(6), 536 – 539.

Meserve, B.E. (1983). Fundamental Concepts of Geometry. Reprint. Dover Publications: New York, NY. Originally published: Addison-Wesley Publications: Reading, MA.

Messmer, K. (1975). Hemodilution. Surg Clin North Am 55(3), 659 – 678.

Mielck, F., Stephan, H., Buhre, W., Weyland, A., and Sonntag, H. (1998). Effects of 1 MAC desflurane on cerebral metabolism, blood flow and carbon dioxide reactivity in humans. Br J Anaesth 81(2), 155 – 160.

Mielck, F., Stephan, H., Weyland, A., and Sonntag, H. (1999). Effects of one minimum alveolar anesthetic concentration sevoflurane on cerebral metabolism, blood flow, and CO2 reactivity in cardiac patients. Anesth Analg 89(2), 364 – 369.

Miller, J.D. and Becker, D.P. (1982). Secondary insults to the injured brain. J R Coll Surg Edinb 27(5), 292 – 298.

Miller, J.D., Sweet, R.C., Narayan, R. and Becker, D.P. (1978). Early insults to the injured brain. JAMA 240(5), 439 – 442.

Mirhashemi, S., Messmer, K. and Intaglietta, M. (1987). Tissue perfusion during normovolemic hemodilution investigated by a hydraulic model of the cardiovascular system. Int J Microcirc Clin Exp 6(2), 123 – 136.

Moochhala, S.M., Md, S., Lu, J., Teng, C.H., and Greengrass, C. (2004). Neuroprotective role of aminoguanidine in behavioral changes after blast injury. J Trauma 56(2), 393 – 403.

Morris, R.G.M. (1981). Spatial localization does not require the presence of local cues. Learning and Motivation 12, 239 – 260.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11(1), 47 – 60.

Morris, R.G., Garrud, P., Rawlins, J.N., and O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. Nature 297(5868), 681 – 683.

Moser, E.I. and Kropf, E., and Moser, M.B. (2008). Place cells, grid cells, and the brain's spatial representation system. Annu Rev Neurosci 31, 69 – 89.

Murkin, J.M. (2007). Cerebral autoregulation: the role of CO_2 in metabolic homeostasis. Semin Cardiothrac Vasc Anesth 11(4), 269 – 273.

Naftalin, L.W. and Yagiela, J.A. (2002). Vasoconstrictors: indications and precautions. Dent Clin North Am 46(4), 733 – 746.

Nakagawa, A., Manley, G.T, Gean, A.D., Ohtani, K., Armonda, R., Tsukamoto, A., Yamamoto, H., Takayama, K., and Tominaga, T. (2011). Mechanisms of primary blast-induced traumatic brain injury: insights from shock-wave research. J Neurotrauma 28(6), 1101 – 1119.

Navarro, J.C., Pillai, S., Cherian, L., Garcia, R., Grill, R.J., and Robertson, C.S. (2012). Histopathological and behavioral effects of immediate and delayed hemorrhagic shock after mild traumatic brain injury in rats. J Neurotrauma 29(2), 322 – 334.

Nelson, T.J., Wall, D.B., Stedje-Larson, E.T., Clark, R.T., Chambers, L.W., and Bohman, H.R. (2006). Predictors of mortality in close proximity blast injuries during Operation Iraqi Freedom. J Am Coll Surg 202(3), 418 – 422.

Ngo, T., Mendis, P., Gupta, A., and Ramsay, J. (2007). Blast loading and blast effects on structures – an overview. EJSE International 7, 76 – 91.

Nguyen, H.T., Li, K.Y., daGraca, R.L., Delphin, E., Xiong, M., and Ye, J.H. (2009). Behavior and cellular evidence for propofol-induced hypnosis involving brain glycine receptors. Anesthesiology 110(2), 326 – 332.

O'Keefe, J. and Conway, D.H. (1978). Hippocampal place units in the freely moving rat: why they fire where they fire. Exp Brain Res 31(4), 573 – 590.

Okie, S. (2005). Traumatic brain injury in the war zone. N Engl J Med 352(20), 2043 – 2047.

Olsen, K.S., Henriksen, L., Owen-Falkenberg, A., Dige-Petersen, H., Rosenørn, J., and Chraemmer-Jørgensen, B. (1994). Effect of 1 or 2 MAC isoflurane with or without ketanserin on cerebral blood flow autoregulation in man. Br J Anaesth 72(1), 66 – 71.

Oshima, T., Karasawa, F., Okazaki, Y., Wada, H., and Satoh, T. (2003). Effects of sevoflurane on cerebral blood flow and cerebral metabolic rate of oxygen in human beings: a comparison with isoflurane. Eur J Anaesthesiol 20(7), 543 – 547.

Østergaard, L., Engedal, T.S., Aamand, R., Mikkelsen, R., Iversen, N.K., Anzabi, M., Næss-Schmidt, E.T., Drasbek, K.R., Bay, V., Blicher, J.U., Tietze, A., Mikkelsen, I.K., Hansen, B., Jespersen, S.N., Juul, N., Sørensen, J.C., and Rasmussen, M. (2014). Capillary transit time heterogeneity and flow-metabolism coupling after traumatic brain injury. J Cereb Blood Flow Metab 34(10), 1585 – 1598.

Overgaard, J. and Tweed, W.A. (1974). Cerebral circulation after head injury. Part 1: Cerebral blood flow and its regulation after closed head injury with emphasis on clinical correlations. J Neurosurg 41(5), 531 – 541.

Owens, G.K., Kumar, M.S., and Wamhoff, B.R. (2004). Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 84(3), 767 – 801.

Owen-Smith, M.S. (1981). Explosive blast injury. Med Bull US Army Eur 38, 36 – 43.

Pang, Y., Fan, L.W., Zheng, B., Cai, Z., and Rhodes, P.G. (2006). Role of interleukin-6 in lipopolysaccharide-induced brain injury and behavioral dysfunction in neonatal rats. Neuroscience 141(2), 745 – 755.

Park, E., Ai, J., and Baker, A.J. (2007). Cerebellar injury: clinical relevance and potential in traumatic brain injury research. Prog Brain Res 161, 327 – 338.

Park, E., Gottlieb, J.J., Cheung, B., Shek, P.N., and Baker, A.J. (2011). A model of low-level primary blast brain trauma results in cytoskeletal proteolysis and chronic functional impairment in the absence of lung barotrauma. J Neurotrauma 28(3), 343 – 357.

Patel, P.M. and Drummond, J.C. (2010). Cerebral physiology and the effects of anesthetic drugs. 7th ed. R.D. Miller (ed). Churchill Livingston Elsevier: Philadelphia, PA pps. 305 – 339.

Pauca, A.L. and Dripps, R.D. (1973). Clinical experience with isoflurane (Forane): preliminary communication. Br J Anaesth 45(7), 697 – 703.

Paulson O.B., Strandgaard, S., and Edvinsson, L. (1990). Cerebral autoregulation. Cerebrovasc Brain Metab Rev 2(2), 161 – 192.

Perez-Polo, J.R., Rea, H.C., Johnson, K.M., Parsley, M.A., Unabia, G.C., Xu, G., Infante, S.K., DeWitt, D.S. and Hulsebosch, C.E. (2013). Inflammatory consequences in a rodent model of mild traumatic brain injury. J Neurotrauma 30(9), 727 – 740.

Perez-Polo, J.R., Rea, H.C., Johnson, K.M., Parsley, M.A., Unabia, G.C., Xu, G.Y., Prough, D., DeWitt, D.S., Spratt, H. and Hulsebosch, C.E. (2015). A rodent model of mild traumatic brain blast injury. J Neuros ci Res 93(4), 549 – 561.

Phillips, Y.Y. (1986). Primary blast injuries. Ann Emerg Med 15(12), 1446 – 1450.

Ping, P. and Johnson, P.C. (1994). Arteriolar network response to pressure reduction during sympathetic nerve stimulation in cat skeletal muscle. Am J Physiol 266(3 Pt 2), H1251 – H1259.

Polusny, M.A., Kehle, S.M., Nelson, N.W., Erbes, C.R., Arbisi, P.A., and Thuras, P. (2011). Longitudinal effects of mild traumatic brain injury and posttraumatic stress disorder comorbidity on postdeployment outcomes in National Guard soldiers deployed to Iraq. Arch Gen Psychiatry 68(1), 79 – 89.

Povlishock, J.T. and Kontos, H.A. (1992). The role of oxygen radicals in the pathobiology of traumatic brain injury. Hum Cell 5(4), 345 – 53.

Pun, P.B.L., Kan, M.E., Salim, A., Li, Z., Ng, K.C., Moochhala, S.M., Ling, E.A., Tan, M.H., and Lu, J. (2011). Low level primary blast injury in rodent brain. Front Neurol 2, 19.

Rafaels, K., Bass, C.R., Salzar, R,S., Panzer, M.B., Woods, W., Feldman, S., Cummings, T., and Capehart, B. (2011). Survival risk assessment for primary blast exposures to the head. J Neurotrauma 28(11), 2319 – 2328.

Rafaels, K.A., Bass, C.R., Panzer, M.B., Salzar, R.S., Woods, W.A., Feldman, S.H., Walilko, T., Kent, R.W., Capehart, B.P., Foster, J.B., Derkunt, B., and Toman. A. (2012). Brain injury risk from primary blast. J Trauma Acute Care Surg 73(4), 895 – 901.

Raghupathi, R. and Huh, J.W. (2007). Diffuse brain injury in the immature rat: evidence for an age-at-injury effect on cognitive function and histopathologic damage. J Neurotrauma 24(10), 1596 – 1608.

Raichle, M.E., Grubb, R.L., Gado, M.H., Eichling, J.O., and Ter-Pogossian, M.M. (1976). Correlation between regional cerebral blood flow and oxidative metabolism. In vivo studies in man. Arch Neurol 33(8), 523 – 526.

Readnower, R.D., Chavko, M., Adeeb, S., Conroy, M.D., Pauly, J.R., McCarron, R.M., and Sullivan, P.G. (2010). Increase in blood brain barrier permeability, oxidative stress, and activated microglia in a rat model of blast induced traumatic brain injury. J. Neurosci Res 88(16), 3530 – 3539.

Reener, D.V., Hisel, R.D., Hoffman, J.M., Kryscio, R.J., Lusk, B.T. and Geddes, J.W. (2011). A mulit-mode shock tube for investigation of blast-induced traumatic brain injury. J Neurotrauma 28(1), 95 – 104.

Reinstrup, P., Ryding, E., Algotsson, L., Messeter, K., Asgeirsson, B., and Uski, T. (1995). Distribution of cerebral blood flow during anesthesia with isoflurane or halothane in humans. Anesthesiology 82(2), 359 – 66.

Riha, M., Haskova, P., Martin, J., Filipsky, T., Vanova, K., Vavrova, J., Holeckova, M., Homola, P., Vitek, L., Palicka, V., Simunek, T. and Mladenka, P. (2016). Protective effects of D-penicillamine on catecholamine-induced myocardial injury. Oxid Med Cell Longev 2016(5213532), 1 – 10.

Rimel, R.W., Giordani, B., Barth, J.T., Boll, T.J., and Jane, J.A. (1981). Disability caused by minor head injury. Neurosurgery 9(3), 221 – 228.

Rimel, R.W., Giordani, B., Barth, J.T., and Jane, J.A. (1982). Moderate head injury: completing the clinical spectrum of brain injury. Neurosurgery 11(3), 344 – 351.

Risling, M. and Davidsson, J. (2012). Experimental animal models for studies on the mechanisms of blast-induced neurotrauma. Front Neurol 3(30), 1 – 9.

Risling, M., Plantman, S., Angeria, M., Rostami, E., Bellander, B.M., Kirkegaard, M., Arborelius, U., and Davidsson, J. (2011). Mechanisms of blast induced brain injuries, experimental studies in rats. Neuroimage 54 Suppl 1, S89 – S97.

Robinson, M.A., Baumgardner, J.E. and Otto, C.M. (2011). Oxygen-dependent regulation of nitric oxide production by inducible nitric oxide synthase. Free Radic Biol Med 51(11), 1952 – 1965.

Romba, J. and Martin, P. (1961). The propagation of air shock waves on a biophysical model. US Army Ordinance Technical Memorandum. pps. 17 – 61.

Rosenfeld, J.V., McFarlane, A.C., Bragge, P., Armonda, R.A., Grimes, J.B. and Ling, G.S. (2013). Blast-related traumatic brain injury. Lancet Neurol 12(9), 882 – 893.

Rossle, R. (1950). Pathology of blast effects. German Aviation Medicine (World War II) Washington, D.C., Department of the Air Force 2, 1260 – 1273.

Roszer, Tamás. (2012). The biology of subcellular nitric oxide. Springer: London, pps 1 – 210.

Rowe, R.K., Harrison, J.L., Thomas, T.C., Pauly, J.R., Adelson, P.D., and Lifshitz, J. (2013). Using anesthetics and analgesics in experimental traumatic brain injury. Lab Anim (NY) 42(8), 286 – 291.

Roy, C.S. and Sherrington, M.B. (1890). On the regulation of the blood-supply of the brain. J Physiol 11(1-2), 85 – 158.

Sajja, V.S.S.S., Galloway, M.P., Ghoddoussi, F., Thiruthalinathan, D., Kepsel, A., Hay, K., Bir, C.A., and VandeVord, P.J. (2012). Blast-induced neurotrauma leads to neurochemical changes and neuronal degeneration in the rat hippocampus. NMR Biomed 25(12), 1331 – 1339.

Sajja, V.S., Hubbard, W.B., Hall, C.S., Ghoddoussi, F., Galloway, M.P., and VandeVord, P.J. (2015). Enduring deficits in memory and neuronal pathology after blast induced traumatic brain injury. Sci Rep 5, 15075.

Sajja, V.S., Perrine, S.A., Ghoddoussi, F., Hall, C.S., Galloway, M.P., and VandeVord, P.J. (2014). Blast neurotrauma impairs working memory and disrupts prefrontal myo-inositol levels in rats. Mol Cell Neurosci 59, 119 – 126.

Sakabe, T., Maekawa, T., Fujii, S., Ishikawa, T., Tateishi, A., and Takeshita, H. (1983). Cerebral circulation and metabolism during enflurane anesthesia in humans. Anesthesiology 59(6), 532 – 536.

Säljö, A., Svensson, B., Mayorga, M., Hamberger, A., and Bolouri, H. (2009). Low-level blasts raise intracranial pressure and impair cognitive function in rats. J Neurotruama 26(8), 1345 – 1352.

Sato, M., Chang, E., Igarashi, T., and Noble, L.J. (2001). Neuronal injury and loss after traumatic brain injury: time course and regional variability. Brain Res 917(1), 45 – 54.

Scheff, S.W., Baldwin, S.A., Brown, R.W., and Kraemer, P.J. (1997). Morris water maze deficits in rats following traumatic brain injury: lateral controlled cortical impact. J Neurotrauma 14(9), 615 – 627.

Schenk, F. and Morris, R.G. (1985). Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. Exp Brain Res 58(1), 11 – 28.

Schlünzen, L., Cold, G.E., Rasmussen, M., and Vafaee, M.S. (2006). Effects of dose-dependent levels of isoflurane on cerebral blood flow in healthy subjects studied using positron emission tomography. Acta Anaesthesiol Scand 50(3), 306 – 312.

Schmuck, G., Ahr, H.J. and Schluter, G. (2000). Rat cortical neuron cultures: an in vitro model for differentiating mechanisms of chemically induced neurotoxicity. In Vitr Mol Toxicol 13(1), 37 – 50.

Schmuck, G. and Kahl, R. (2009). The use of Fluoro-Jade in primary neuronal cell cultures. Arch Toxicol 83(4), 397 – 403.

Schmued, L.C., Albertson, C., and Slikker, W.Jr. (1997). Fluoro-Jade: a novel fluorochrome for the sensitive and reliable histochemical localization of neuronal degeneration. Brain Res 751(1), 37 – 46.

Schmued, L.C. and Hopkins, K.J. (2000). Fluoro-Jade: novel fluorochromes for detecting toxicant-induced neuronal degeneration. Toxicol Pathol 28 (1), 91 – 99.

Schmued, L.C., Stowers, C.C., Scallet, A.C. and Xu, L. (2005). Fluro-Jade C results in ultra high resolution and contrast labeling of degenerating neurons. Brain Res 1035(1), 24 – 31.

Schwinn, D.A., McIntyre, R.W., and Reves, J.G. (1990). Isoflurane-induced vasodilation: role of the alpha-adrenergic nervous system. Anesth Analg 71(5), 451 – 459.

Sedy, J., Urdzikova, L., Jendelova, P., and Sykova, E. (2008). Methods for behavioral testing of spinal cord injured rats. Neurosci Biobehav Rev 32(3), 550 – 580.

Segars, R.A. and Carboni, M.G. (2008). A shocktube for downselecting material concepts for blast protection part 1: description of the shock tube and comparison of flush mounted and recess mounted pressure sensors. US Army Technical Report Natick/TR-09/010, 1 – 27.

Sell, S.L., Johnson, K., DeWitt, D.S., and Prough, D.S. (2016). Persistent behavioral deficits in rats after parasagittal fluid percussion injury. J Neurotrauma 33, 1 – 11.

Shanker, T. (2007). Iraqi bombers thwart efforts to shield G.I.'s. *The New York Times*, June 2.

Singh, A.K., Ditkofsky, N.G., York, J.D., Abujudeh, H.H., Avery, L.A., Brunner, J.F., Sodickson, A.D., and Lev, M.H. (2016). Blast injuries: from improvised explosive device blasts to the Boston Marathon bombing. Radiographics 36(1), 295 – 307.

Singh, I.N., Sullivan, P.G., and Hall, E.D. (2007). Peroxynitrite-mediated oxidative damage to brain mitochondria: Protective effects of peroxynitrite scavengers. J Neurosci Res 85(10), 2216 – 23.

Singleton, R.H., Stone, J.R., Okonkwo, D.O., Pellicane, A.J., and Povlishock, J.T. (2001). The immunophilin ligand FK506 attenuates axonal injury in an impact-acceleration model of traumatic brain injury. J Neurotrauma 18(6), 607 – 614.

Sloan, T.B. (2002). Anesthetics and the brain. Anesthesiol Clin North America 20(2), 265 – 292.

Soehle, M., Czosnyka, M., Pickard, J.D., and Kirkpatrick, P.J. (2004). Continuous assessment of cerebral autoregulation in subarachnoid hemorrhage. Anesth Analg 98(4), 1133 – 1139.

Sosa, M.A., De Gasperi, R., Paulino, A.J., Pricop, P.E., Shaughness, M.C., Maudlin-Jeronimo, E., Hall, A.A., Janssen, W.G., Yuk, F.J., Dorr, N.P., Dickstein, D.L., McCarron, R.M., Chavko, M., Hof, P.R., Ahlers, S.T. and Elder, G.A. (2013). Blast overpressure induces shear-related injuries in the brain of rats exposed to a mild traumatic brain injury. Acta Neuropathol Comm 1, 51 – 78.

Squadrito, G.L. and Pryor, W.A. (1995). The formation of peroxynitrite in vivo from nitric oxide and superoxide. Chem Biol Interact 96(2), 203 – 206.

Stahl, Saul. (2003). Geometry from Euclid to Knots. Pearson Education: Upper Saddle River, NJ.

Statler, K.D., Alexander, H., Vagni, V., Dixon, C.E., Clark, R.S., Jenkins, L., and Kochanek, P.M. (2006). Comparison of seven anesthetic agents on outcome after experimental traumatic brain injury in adult, male rats. J Neurotrauma 23(1), 97 – 108.

Steiner, L., Coles, J., Johnston, A., Chatfield, D., Smielewski, P., Fryer, T., Aigbirhio, F., Clark, J., Pickard, J., Menon, D., and Czosnyka, M. (2003). Assessment of cerebrovascular autoregulation in head-injured patients: a validation study. Stroke 34(10), 2404 – 2409.

Stern, M.D., Lappe, D.L., Bowen, P.D., Chimosky, J.E., Holloway, G.A., Keiser, H.R., and Bowman, R.L. (1977). Continuous measurement of tissue blood flow by laser-Doppler spectroscopy. A Journal of Physiol 232(4), H441 – H448.

Stewart, J.B. and Pecora, C. (2015). Explosively driven air blast in a conical shock tube. Rev Sci Instrum 86(3), 035108.

Stiefel, M.F., Tomita, Y., and Marmarou, A. (2005). Secondary ischemia impairing the restoration of ion homeostasis following traumatic brain injury. J Neurosurg 103(4), 707 – 714.

Stuehr, D.J., Cho, H.J., Kwon, N.S., Weise, M.F. and Nathan, C.F. (1991). Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD- and FMN-containing flavoprotein. Proc Natl Acad Sci USA 88(17), 7773 – 7777.

Stuss, D.T., Ely, P., Hugenholtz, H., Richard, M.T., Larochelle, S., Poirier, C.A., and Bell, I. (1985). Subtle neuropsychological deficits in patients with good recovery after closed head injury. Neurosurgery 17(1), 41 – 47.

Sun, Z., Liu, Y.D., and Zhong, R.G. (2012). Reactions of amine and peroxynitrite: evidence for hydroxylation as a predominant reaction and new insight into the modulation of CO_2. J Phys Chem A 116(30), 8058 – 8066.

Sweis, B.M., Bachour, S.P., Brekke, J.A., Gewirtz, J.C., Sadeghi-Bazargani, H., Hevesi, M., and Divani, A.A. (2016). A modified beam-walking apparatus for assessment of anxiety in a rodent model of blast traumatic brain injury. Behav Brain Res 296, 149 – 156.

Szabo, C., Zingarelli, B., O'Connor, M. and Salzman, A.L. (1996). DNA strand breakage, activation of poly (ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite. Proc Natl Acad Sci USA 93(5), 1753 – 1758.

Tanielian, T. and Jaycox, L.H. (2008). Invisible Wounds of War: Psychological and Cognitive Injuries, Their Consequences, and Services to Assist Recovery. Rand Corporation: Santa Monica.

Taylor, P. and Ford, C. (2009). Simulation of blast-induced early time intracranial wave physics leading to traumatic brain injury. J Biomech Eng 131(6), 061007.

Terrio, H., Brenner, L.A., Ivins, B.J., Cho, J.M., Helmick, K., Schwab, K., Scally, K., Bretthauer, R., and Warden, D. (2009). Traumatic brain injury screening: preliminary findings in a US Army Brigade Combat Team. J Head Trauma Rehabil 24(1), 14 – 23.

Todd, N.M. and Weeks, J. (1996). Comparative effects of propofol, pentobarbital, and isoflurane on cerebral blood flow and blood volume. J Neurosurg Anesthesiol 8(4), 296 – 303.

Toklu, H.Z., Muller-Delp, J., Yang, Z., Oktay, S., Sakarya, Y., Strang, K., Ghosh, P., Delp, M.D., Scarpace, P.J., Wang, K.K., and Tumer, N. (2015). The functional and structural changes in the basilar artery due to overpressure blast injury. J Cereb Blood Flow Metab 35(12), 1950 – 1956.

Tompkins, P., Tesiram, Y., Lerner, M., Gonzalez, L.P., Lightfoot, S., Rabb, C.H. and Brackett, D.J. (2013). Brain injury: neuro-inflammation, cognitive deficit and magnetic resonance imaging in a model of blast induced traumatic brain injury. J Neurotrauma 30(22), 1888 – 1897.

Tran-Ho, L-C., May, P.M. and Hefter, G.T. (1997). Complexation and copper(I) by thioamino acids. Implications for copper speciation in blood plasma. J Inorg Biochem 68(3), 225 – 231.

Tung, A., Herrera, S., Fornal, C.A., and Jacobs, B.L. (2008). The effect of prolonged anesthesia with isoflurane, propofol, dexmedetomidine, or ketamine on neural cell proliferation in the adult rat. Anesth Analg 106(6), 1772 – 1777.

Twedt, D.C., Sternlieb, I. and Gilbertson, S.R. (1979). Clinical, morphologic, and chemical studies on copper toxicosis of Bedlington terriers. J Am Vet Med Assoc 175(3), 269 – 275.

Van Aken, H., Fitch, W., Graham, D.I., Brussel, T., and Themann, H. (1986). Cardiovascular and cerebrovascular effects of isoflurane-induced hypotension in the baboon. Anesth Analg 65(6), 565 – 574.

Vorhees, C.V. and Williams, M.T. (2014). Value of water mazes for assessing spatial and egocentric learning and memory in rodent basic research and regulatory studies. Neurotoxicol Teratol 45, 75 – 90.

Wada, K., Chatzipanteli, K., Busto, R., and Dietrick, W.D. (1998). Role of nitric oxide in traumatic brain injury in the rat. J Neurosurg 89(5), 807 – 818.

Wald, S.L., Shackford, S.R. and Fenwick, J. (1993). The effect of secondary insults on mortality and long-term disability after severe head injury in a rural region without a trauma system. J Trauma 34(3), 377 – 381.

Walshe, J.M. (1956). Penicillamine, a new oral therapy for Wilson's disease. Am J Med 21(4), 487 – 495.

Wang, J., Jahn-Eimermacherb, A., Brucknera, M., Werner, C., Engelhard, K., and Thala, S.C. (2015). Comparison of different quantification methods to determine hippocampal damage after cerebral ischemia. J Neurosci Methods 240, 67 – 78.

Wang, J.M. and Cheng, J. (2016). Damage of vascular endothelial barrier induced by explosive blast and its clinical significance. Chin J Traumatol 19(3), 125 – 128.

Wang, Y., Arun, P., Wei, Y., Oguntayo, S., Gharavi, R., Valiyaveettil, M., Nambiar, M.P., and Long, J.B. (2014). Repeated blast exposures cause brain DNA fragmentation in mice. J Neurotrauma 31(5), 498 – 504.

Warden, D. (2006). Military TBI during the Iraq and Afghanistan wars. J Head Trauma Rehabil 21(5), 398 – 402.

Wei, E.P., Dietrich, W.D., Povlishock, J.T., Navari, R.M., and Kontos, H.A. (1980a). Functional, morphological, and metabolic abnormalities of the cerebral microcirculation after concussive brain injury in cats. Circ Res 46(1), 37 – 47.

Wei, E.P., Kontos, H.A., and Beckman, J.S. (1996). Mechanisms of cerebral vasodilation by superoxide, hydrogen peroxide, and peroxynitrite. Am J Physiol 271(3 Pt 2), H1262 – H1266.

Wei, E.P., Kontos, H.A., and Patterson, J.L. (1980b). Dependence of pial arteriolar response to hypercapnia on vessel size. Am J Physiol 238(5), 697 – 703.

Weiss, K.H. and Stremmel, W. (2012). Evolving perspectives in Wilson's disease: diagnosis, treatment and monitoring. Curr Gastroenterol Rep 14(1), 1 – 7.

Werner, C. and Engelhard, K. (2007). Pathophysiology of traumatic brain injury. Br J Anaesth 99(1), 4 – 9.

Wojcik, B.E., Stein, C.R., Bagg, K., Humphrey, R.J. and Orosco, J. (2010). Traumatic brain injury hospitalizations of U.S. Army soldiers deployed to Afghanistan and Iraq. Am J Prev Med 38(1 Suppl), S108 – S116.

Wolf, J.A. and Koch, P.F. (2016). Disruption of network synchrony and cognitive dysfunction after traumatic brain injury. Front Syst Neurosci 10, 43.

Wolf, S.J., Bebarta, V.S., Bonnett, C.J., Pons, P.T., and Cantrill, S.V. (2009). Blast injuries. Lancet 374(9687), 405 – 415.

Yamakami, I. and McIntosh, T.K. (1989). Effects of traumatic brain injury on regional cerebral blood flow in rats as measured with radiolabeled microspheres. J Cereb Blood Flow Metab 9(1), 117 – 124.

Yeoh, S., Bell, E.D., and Monson, K.L. (2013). Distribution of blood-brain barrier disruption in primary blast injury. Ann Biomed Eng 41(10), 2206 – 2214.

Yu, G.X., Mueller, M., Hawkins, B.E., Mathew, B.P., Parsley, M.A., Vergara, L.A., Hellmich, H.L., Prough, D.S., and DeWitt, D.S. (2014). Traumatic brain injury in vivo and in vitro contributes to cerebral vascular dysfunction through impaired gap junction communication between vascular smooth muscle cells. J Neurotrauma 31(8), 739 – 748.

Zhang, X., Chen, J., Graham, S.H., Du, L., Kochanek, P.M., Draviam, R., Guo, F., Nathaniel, P.D., Szabo, C., Watkins, S.C., and Clark, R.S. (2002). Intranuclear localization of apoptosis-inducing factor (AIF) and large scale DNA fragmentation after traumatic brain injury in rats and in neuronal cultures exposed to peroxynitrite. J Neurochem 82(1), 181 – 191.

Zhao, K.S., Liu, J., Yang, G.Y., Jin, C., Huang, Q. and Huang, X. (2000). Peroxynitrite leads to arteriolar smooth muscle cell membrane hyperpolarization and low vasoreactivity in severe shock. Clin Hemorheol Microcirc 23(2-4), 259 – 267.

Zingarelli, B., Day, B.J., Crapo, J.D., Salzman, A.L. and Szabo, C. (1997). The potential role of peroxynitrite in the vascular contractile and cellular energetic failure in endotoxic shock. Br J Pharmacol 120(2), 259 – 267.

Zunic, G., Romic, P., Vueljic, M., and Jovanikic, O. (2005). Very early increase in nitric oxide formation and oxidative cell damage associated with the reduction of tissue oxygenation is a trait of blast casualties. Vojnosanit Pregl 62(4), 273 – 280.

Zweifach, B.W. (1974). Quantitative studies of microcirculatory structure and function. I. Analysis of pressure distribution in the terminal vascular bed in cat mesentery. Cir Res 34(6), 843 – 857.

VITA

Uylissa Ann Rodriguez was born in McAllen, Texas on December 29, 1981, the daughter of Armando Salome Rodriguez and Lydia Muñoz Rodriguez. The oldest of three, she has one younger sister, Marissa Jill Rodriguez-Ramirez, one younger brother, Armando Joel Rodriguez and two nieces, Kadence Saige Rodriguez and Solara Paradox Ramirez. Ms. Rodriguez entered South Texas Community College during her sophomore year in high school and the University of Texas-Pan American during her junior year in high school as part of the Concurrent Enrollment program sponsored by her school, Donna High School, of which she graduated valedictorian in 2000. She obtained a Bachelor's of Science degree *summa cum laude* from the University of Texas-Pan American in 2004 then a Master's of Science degree *magna cum laude* in 2009 before moving to Galveston to start the PhD program under the Graduate School of Biomedical Science's Neuroscience and Cell Biology Program at University of Texas Medical Branch. She entered candidacy in 2012 and began working on her dissertation work under the mentorship of Dr. Douglas S. DeWitt. Ms. Rodriguez has received awards from the TIRR Foundation's Mission Connect Annual Scientific Symposium and an Advances in Translational Cell Biology award from the Cell Biology Symposium for Graduates.

Education

B.S. Biology, May 2004, *The University of Texas-Pan American, Edinburg, Texas*
M.S. Biology, May 2009, *The University of Texas-Pan American, Edinburg, Texas*

Submitted Publications

Rodriguez, U.A., Zeng, Y., Deyo, D.J., Parsley, M.O., Hawkins, B.E., Prough, D.S. and DeWitt, D.S. Peroxynitrite contributes to cerebral vascular dysfunction after mild blast TBI. *Journal of Neurotrauma*, May 2017.

Abstracts

1.) Rodriguez, U.A., Parsley, M.O., Zeng, Y., Prough, D.S. and DeWitt, D.S. The effects of blast-induced neurotrauma on cerebral vascular reactivity. Mission Connect, Houston, TX, December 2013.
2.) Rodriguez, U.A., Parsley, M.O., Zeng, Y., Prough, D.S. and DeWitt, D.S. The effects of blast-induced neurotrauma on cerebral perfusion. Cell Biology Symposium, Galveston, TX, March 2014.
3.) Rodriguez, U.A., Parsley, M.O., Zeng, Y., Prough, D.S. and DeWitt, D.S. The effects of blast-induced neurotrauma on neuronal injury. Mission Connect, Houston, TX, December 2014.
4.) Bolding, I.J., Ruppert, K., Rodriguez, U.A., Zeng, Y., Prough, D.S. and DeWitt, D.S. A comparison of the cerebral vascular effects of Vandenberg or Advanced Blast Simulator blast injury in rats. Mission Connect, Houston, TX, December 2014.
5.) Rodriguez, U.A., Parsley, M.O., Zeng, Y., Prough, D.S. and DeWitt, D.S. The effects of blast-induced neurotrauma on cerebral perfusion, cerebral vascular reactivity and neuronal injury. Cell Biology Symposium, Galveston, TX, April 2015.
6.) Rodriguez, U.A., Parsley, M.O., Zeng, Y., Prough, D.S. and DeWitt, D.S. The effects of blast-induced neurotrauma on behavioral outcomes. National Student Research Forum, Galveston, TX, May 2015.
7.) Rodriguez, U.A., Parsley, M.O., Zeng, Y., Prough, D.S. and DeWitt, D.S. The effects of blast-induced neurotrauma on cerebral vascular, histopathological and behavioral outcomes. National Neurotrauma Society Symposium, Santa Fe, NM, July 2015.
8.) Bolding, I.J., Ruppert, K., Rodriguez, U.A., Zeng, Y., Prough, D.S. and DeWitt, D.S. A comparison of the cerebral vascular effects of Vandenberg or Advanced Blast Simulator blast injury in rats. National Neurotrauma Society Symposium, Santa Fe, NM, July 2015.
9.) Rodriguez, U.A., Parsley, M.O., Zeng, Y., Prough, D.S. and DeWitt, D.S. The effects of blast-induced neurotrauma on cerebral vascular, histopathological and behavioral outcomes. Mission Connect, Houston, TX, December 2015.
10.) Rodriguez, U.A., Deyo, D.J., Parsley, M.O., Prough, D.S. and DeWitt, D.S. The effects of peroxynitrite scavenger penicillamine methyl ester (PenME) after bTBI on cerebral perfusion and arterial pressure. National Neurotrauma Society Symposium, Lexington, KY, July 2016.
11.) Rodriguez, U.A., Deyo, D.J., Parsley, M.O., Prough, D.S. and DeWitt, D.S. The peroxynitrite scavenger penicillamine methyl ester (PenME) prevents blast-induced increases in cerebral vascular resistance. Mission Connect, Houston, TX, December 2016.

Summary of Dissertation

Traumatic brain injuries (TBI) caused by blast have been called the “signature wound” of the latest conflicts in the Middle East with estimates of the prevalence of blast-induced TBI (bTBI) as high as 19% - 23%. Of the four currently accepted classifications of injury due to blast, primary blast injury due to blast wave over- and underpressure exposure is the least understood. Recent evidence indicates that bTBI is associated with some degree

of cerebral vascular injury and alterations in cerebral perfusion. However, the degree to which blast-related cerebral vascular dysfunction contributes to the pathophysiology of bTBI is unknown. This research was aimed at investigating the effects of primary blast injury on cerebral perfusion and cerebral vascular reactivity in rats using a compressed air driven shock tube in which only the animal's head was exposed to the shock wave, avoiding the controversial effects of torso protection in whole body blast exposure. I tested the hypothesis that bTBI, like non-blast TBI, results in cerebral hypoperfusion and impaired cerebral vascular reactivity by measuring relative cerebral perfusion *in vivo* and dilatory responses to reduced intravascular pressure in middle cerebral arterial segments harvested from rats subjected to mild bTBI. Additionally, I wanted to determine if cognitive and vestibulomotor dysfunction and neuronal injury resulted after mild bTBI. Lastly, we assessed the effects of peroxynitrite scavenging on relative cerebral perfusion, mean arterial blood pressure and cerebral vascular resistance in rats treated with penicillamine methyl ester after mild bTBI. These studies revealed that mild bTBI reduced cerebral perfusion without reducing blood pressure, indicating that mild bTBI significantly increased cerebral vascular resistance. Blast-induced impairment of cerebral dilator responses to reduced intravascular pressure might contribute to these significant reductions in cerebral perfusion, especially in the presence of arterial hypotension, a common occurrence in combat situations when blast exposure is accompanied by hemorrhagic hypotension. Additionally, these results indicate that low level shock wave exposure resulted in neuronal injury and cognitive and vestibulomotor dysfunction. Lastly, a trend towards increased cerebral perfusion along with significant reductions in cerebral vascular resistance after treatment with penicillamine methyl ester suggest that blast-induced cerebral vascular dysfunction may be due, in part, to the actions of peroxynitrite.

Permanent address: 2905 DeLeon St., Donna, TX 78537

This dissertation was typed by Uylissa Ann Rodriguez.