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Traumatic Brain Injury Induced Cerebral Blood Flow Changes - A Potential Role For Caffeine

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TRAUMATIC BRAIN INJURY INDUCED CEREBRAL BLOOD FLOW CHANGES – A POTENTIAL ROLE FOR CAFFEINE

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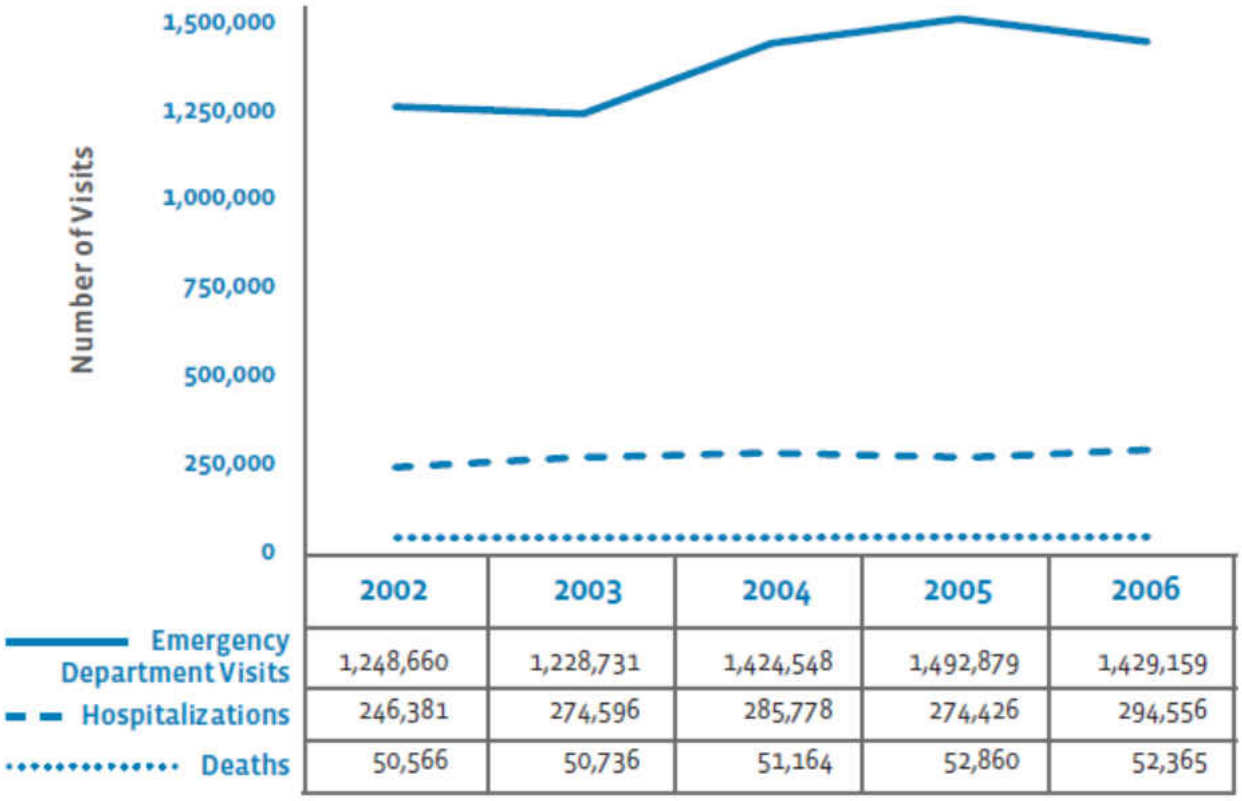
CHAPTER 1 INTRODUCTION

Traumatic brain injury (TBI) is a serious health problem both in the United states and across the globe. The Centers for Disease Control and Prevention (CDC) defined TBI as "*an occurrence of injury to the head that can arise from blunt/penetrating trauma or acceleration-deceleration forces which may be associated with symptoms like decreased level of consciousness, amnesia, other neuropsychological abnormalities, intracranial lesions, or death*" (Thurman et al., 1994).

1.1 Epidemiology of TBI

TBI is the leading cause of death and life-long disability in the people of the United States. It was estimated that each year approximately 1.5 million Americans sustain a TBI (at least 3 TBIs/minute) (Sosin et al., 1995), from which, around 230,000 patients who seek medical attention are hospitalized (CDC, 1998). As per recent estimation, 124,000 (43.1%) of persons discharged with TBI from acute hospitalization develop TBI-related long-term disability (Selassie et al., 2008). Annually 51,000 Americans die from TBI, which accounts for one-third of all the injury related deaths (Sosin et al., 1996). In addition, some cases of TBI may go misdiagnosed or even unreported. An estimated 2% of the US population, and a staggering 57 million individuals worldwide sustain TBI related disabilities (Ashman et al., 2006; Langlois et al., 2006). Between the years 2002 - 2006, TBI-related emergency department visits in the United States increased by 14.4%, hospitalizations increased by 19.5%, and deaths increased by 3.5% (Figure 1-1) (Faul et al., 2010).



	2002	2003	2004	2005	2006
Emergency Department Visits	1,248,660	1,228,731	1,424,548	1,492,879	1,429,159
Hospitalizations	246,381	274,596	285,778	274,426	294,556
Deaths	50,566	50,736	51,164	52,860	52,365

Figure 1-1: Estimated number of TBI related emergency department visits, hospitalizations, and deaths per annum in the United States between 2002-2006. Adapted from (Faul et al., 2010).

TBI is generally caused by a sudden, violent blow or any other traumatic injury to the head. The common causes of TBI include: falls, vehicle-related collisions, struck by/against events, assaults, non motor vehicle crashes and others (Figure 1-2) (Faul et al., 2010; Langlois et al., 2006). Falls result in the majority of TBI-related emergency department visits (523,043) and also hospitalizations (62,334) (Faul et al., 2010). Motor vehicle crashes are the leading cause of TBI-related deaths, with the highest rates in age group of 20 to 24 years (Faul et al., 2010). TBI is commonly seen in all age groups, but TBI rates vary by age and sex. The highest incidence of TBI is generally seen in children (0 to 4 years), followed by older adolescents (15 to 19 years), young adults (15

to 24 years) and older individuals (65 years or above) (Chua et al., 2007; Corrigan et al., 2010). Adults aged 75 years and above are found to have the highest rate of TBI-related hospitalization and death (Faul et al., 2010). In all age groups, TBI rates are higher for males compared to females, because of males risk taking tendency and engagements with high-risk activities (Corrigan et al., 2010; Faul et al., 2010).

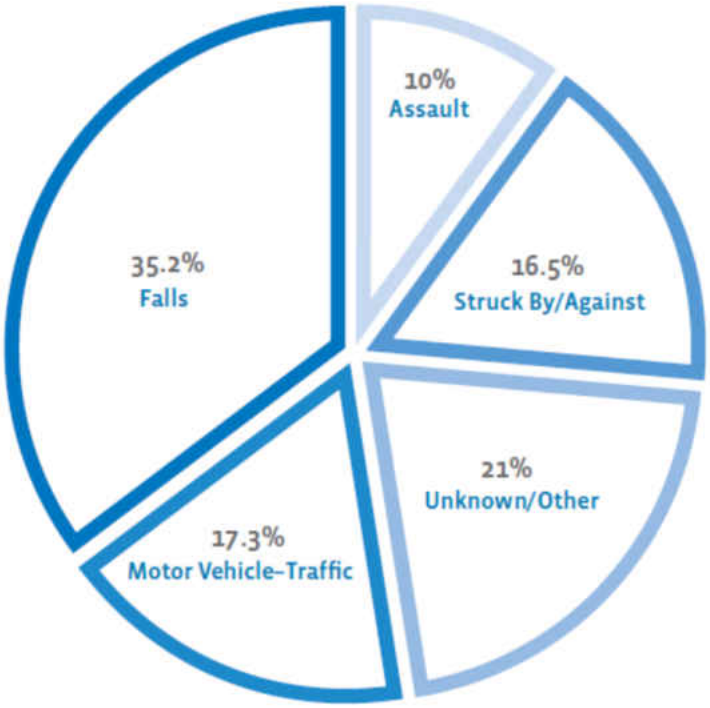


Figure 1-2: Estimated percentage of various causes of TBI per annum in the United States between 2002-2006. Adapted from (Faul et al., 2010).

1.2 Burden of TBI on the Nation's Economy

The majority of TBIs occur in young adults who are in their prime working years. TBI survivors, particularly those who suffered from severe TBI may not return to work from months to years or forever, depending on the severity of the injury and disabilities it has caused. As a result, TBIs tend to have a huge negative effect on the economic future of the country. TBI costs can be broadly divided into two categories: direct medical costs

for the acute treatment and indirect costs such as loss of productivity during the period of disability caused by TBI. It is estimated that the annual cost of TBI in the United States is $60.4 billion, of which productivity losses account for $51.2 billion (Finkelstein, 2006). However, the annual loss from TBI could be much greater than the estimated $60.4, since the estimate is based on medical treatment alone and does not include the cost of rehabilitation and other services required for TBI patients with long-term disabilities (Corrigan et al., 2010).

1.3 Rationale of the Proposed Study

TBI is a major health concern in the United States. In spite of several extensive studies on developing an effective treatment for TBI, to date there are no effective neuroprotective therapies. One of the most common consequences observed in TBI patients is alterations in cerebral blood flow (CBF), because autoregulatory mechanisms of cerebral perfusion is often impaired following TBI. CBF is often found to be decreased following TBI; a reduced CBF has been associated with unfavorable neurological outcome and can render the brain vulnerable to secondary damage (delayed pathological events resulting from primary traumatic insult). Therefore, it is important to monitor and maintain adequate CBF following TBI. Although, several studies have attempted to understand the CBF changes following TBI, these studies are limited to only one or two anatomical regions of the brain. CBF changes in different regions of the brain following TBI still needs to be explored and a precise neuroprotective therapy needs to be developed to address deleterious alterations in CBF after TBI.

Previous studies have shown that caffeine acts as a neuroprotectant in several neurological disorders through various mechanisms and can play a potential role in altering CBF. Few studies have even demonstrated the neuroprotective effects of caffeine in TBI. Thus, the exact role of caffeine on CBF changes following TBI and its effects on outcome are still not clear. Hence, this study was undertaken to better understand the effects of caffeine on CBF changes following TBI in different regions of the brain using Arterial Spin Labeling (ASL) imaging, a magnetic resonance imaging (MRI) technique. *The rationale of the proposed study is discussed in detail in Chapter 2.*

1.4 Specific Aims

The specific aims of this study are:

1. To determine the changes in CBF following TBI in a rat model using ASL-MRI imaging.
2. To determine the effects of acute and chronic caffeine treatment on changes in CBF following TBI in a rat model using ASL-MRI imaging.
3. To assess group-wise differences in CBF of rats following TBI with and without caffeine (chronic and acute) pretreatment.

1.5 Hypothesis

Following severe TBI, derangement of CBF often occurs, rendering the brain at risk of secondary damage. Caffeine may play a role to reduce the extent of such secondary damage by altering CBF following TBI. Acute caffeine produces vasoconstriction by antagonizing adenosine receptors. But chronic caffeine consumption results in an adaptation of the vascular adenosine receptor system and creates a tolerance to this

effect. It was reported that acute caffeine withdrawal from chronic caffeine ingestion leads to vasodilation and thus increased CBF, by binding of adenosine (Ade) to its receptors. It is thus hypothesized that chronic caffeine treatment prior to TBI and caffeine withdrawal after TBI results in favorable CBF changes post-TBI and may reduce the extent of secondary brain damage, potentially by optimizing CBF.

CHAPTER 2 TRAUMATIC BRAIN INJURY AND CEREBRAL BLOOD FLOW

2.1 Types of TBI

TBI can be broadly classified into two types based on the intactness of the dura mater after the traumatic event. A penetrating head injury, commonly known as open head injury (OHI) is a head injury in which the dura matter is penetrated/pierced following TBI (Silver et al., 2005). Open head injury can be caused by high-velocity projectiles (e.g. a bullet shot from gun) as well as objects with low velocities (e.g. knives). Closed head injury (CHI) is defined as an injury to the head that does not penetrate the skull and the dura remains intact (Silver et al., 2005). CHI is caused when the head suddenly and violently hits an object such as in a car crash when the head strikes the dashboard. In general, most people think an OHI is more severe than a CHI. But a CHI can be more devastating than an OHI, as there is no opening to the skull and the fluids that build-up in the brain after CHI can increase intracranial pressure and may cause further damage.

2.1.1 Closed Head Injury (CHI)

Most common types of CHI include: concussion (temporary dysfunction of normal brain function), cerebral contusion (a bruise in brain tissue), diffuse axonal injury and intracranial hematoma. CHIs primarily occur during traffic accidents, falls, sports injuries, and acts of violence (CDC, 1997). Blast-related TBIs in the war-field also often result in CHIs to the soldiers; due to the objects dislodged by the blast which may hit the head severely, or people being thrown into rapid motion by the blast wave (Vasterling et al., 2009). From an estimated 17 million TBIs that occur annually in the United States, CHIs and other forms of mild TBIs account for around 75% of TBIs (Faul et al., 2010). CHI is the leading cause of death in children under the age of 4 years (Ibrahim et al.,

2010). Also, it is one of major causes of physical disability and cognitive disorder in young adults (Cossa and Fabiani, 1999). Hence, CHIs are of greatest concern with regards to public health.

2.1.2 Grading TBI Severity

The grading of TBI severity from the clinical assessment of the TBI patient is critical (Whitfield et al., 2009). Severity of TBI refers to the amount of acute disruption of brain physiology or anatomical structures following TBI (Zasler et al., 2007). The level of injury severity is measured using various scoring methods, such as, Abbreviated Injury Severity (AIS) scale, Glasgow Coma Scale (GCS), Loss of Consciousness (LOC) and posttraumatic amnesia (PTA). These injury scoring methods of TBI classify the injury severity as mild TBI (or minor TBI), moderate TBI and severe TBI (Table 1-1) (Rimel et al., 1982; Zuercher et al., 2009).

Table 2-1: Scoring methods for grading TBI severity. Adapted and modified from PhD thesis (Zakaria, 2011).

Classification of TBI based on injury severity				
Scoring Method	**Measure**	**Mild TBI**	**Moderate TBI**	**Severe TBI**
AIS	Anatomical/structural damage	1-2	3	4-6
GCS	Neurological deficit/mental status	13-15	9-12	3-8
LOC	Alertness/mental status	< 20 min	20 min to 36 hrs	> 36 hrs
PTA	Mental status/memory and recall	< 24 hrs	1-7 days	> 7 days

2.2 Effects of TBI

TBIs can lead to massive brain damage or even death. The pathological mechanisms involved in neuronal cell death following TBI involve a complex interaction of acute and delayed anatomic, molecular, biochemical, and physiological events (Vink and Nimmo, 2009). TBI is known to cause neurological deficits through both primary and secondary (delayed) events (Graham et al., 2000). Primary damage to the brain cells and tissues is complete at the time of impact/insult. Primary brain damage includes: skull fractures, contusions/bruises, hematomas, lacerations and nerve damage. Following the primary mechanical insult TBI results in delayed secondary brain damage due to neurometabolic and cellular changes that account for most of the neurological deficits observed after TBI. Secondary brain damage includes: hypoxia-ischemia, swelling/edema, raised intracranial pressure and associated vascular changes, diffuse axonal injury (DAI), and meningitis/abscess.

Secondary damage evolves from days to years from the time of primary injury and is reversible (Kumar and Loane, 2012; Li et al., 2008). The development of secondary injury provides an opportunity for therapeutic intervention to prevent progressive neuronal damage and loss of function after injury. Despite several studies on understanding the pathological mechanisms of secondary brain damage and its treatment, no effective neuroprotective therapy is currently available for TBI and there is a growing need for therapeutic agent(s). More focused research aimed to better understand the poorly studied secondary injury events of TBI is very critical for the development of neuroprotective therapy for TBI. One such secondary injury event that is

poorly studied is cerebral blood flow (CBF) changes and their role in the progression of the secondary injury following TBI.

2.3 Cerebral Blood Flow (CBF)

CBF is defined as the arterial blood supply to the brain tissue in a given time. It is measured by the amount of blood supplied to a part of brain in a minute (e.g. 30 ml/100g/min). The brain is one organ of the body which weighs only 2% of the body weight, but receives 15% to 18% of the total cardiac output and utilizes 20% of the total body oxygen and 25% of total body glucose (Zauner and Muizelaar, 1997). Brain requires continuous blood supply for both survival and normal functioning. The high metabolic demand of the brain and lack of substantial energy reserves makes it more vulnerable to altered CBF, as observed in most of the cerebral insults. The primary energy source of the brain comes from adenosine triphosphate (ATP) and other high energy phosphates.

2.3.1 Cerebral Hemodynamics

Normal functioning of the brain requires ample supply of oxygen and nutrients through a complex and dense network of blood vessels (Figure 2-1). Blood is supplied to the brain (also face and scalp) through two major sets of blood vessels: the right and left common carotid arteries and the right and left vertebral arteries. Both the carotid arteries together contribute approximately 80% of the total cerebral perfusion and the remaining 20% comes from the two vertebral arteries, which fuse to form a basilar artery (Zauner and Muizelaar, 1997). The carotid arteries and vertebrobasilar arteries forms a circle of communicating arteries at the base of the brain, known as Circle of Willis (Figure 2-2).

Arteries including the anterior cerebral artery, middle cerebral artery and posterior cerebral artery arise from the Circle of Willis and travel to other parts of brain.

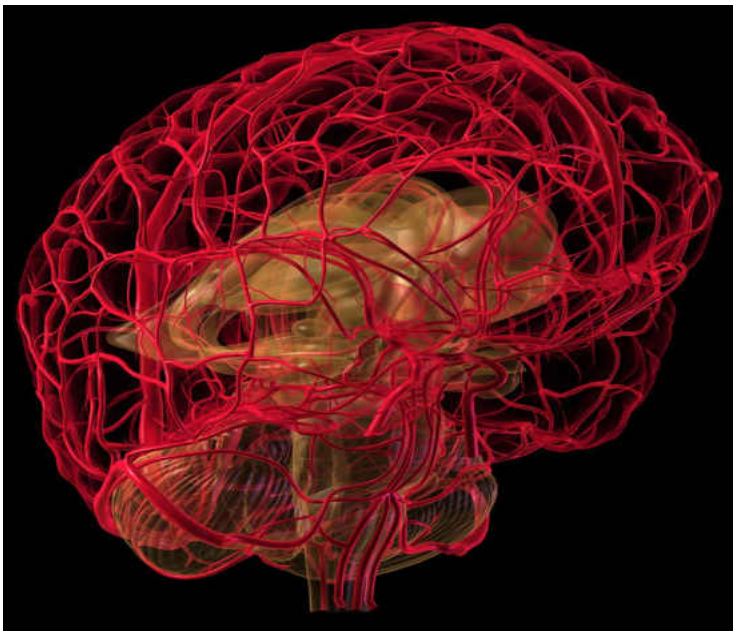


Figure 2-1: Figure showing the dense and complex network of arterial vessels that supply blood to the brain. Adapted from (http://www.3fx.com/Our-Work/Illustration.aspx#prettyPhoto/1/)

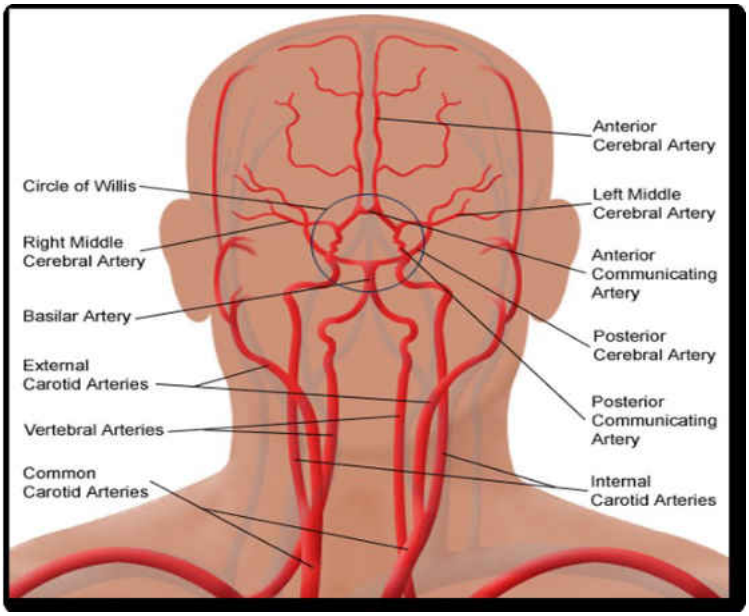


Figure 2-2: Diagram illustrating the location of different arteries of the human brain (https://mynyp.org/images/my_health/ei_2397.gif).

In general, as the arteries travel further away from the heart, they become smaller in size (diameter) and are known as arterioles. They have a relatively thick and strong wall for their size, containing high percentage of smoothe muscle. They receive oxygenated blood from the arteries and supply to different parts of the brain and other organs of the body. Arterioles being the most highly regulated blood vessels in the body, play a crucial role in blood pressure regulation by the contraction and relaxation of their smooth muscle in the walls of the vessel. Arterioles serve to collect blood from arteries and deliver it to the capillaries, where important exchanges (CO_2, O_2, nutrients, waste, etc) take place. Unlike arteries and arterioles, the capillaries do not have smooth muscle lining surrounding them and are of single cell diameter; which aids tin fast and easy transfer/exchange of gases, nutrients, wastes, etc between blood and tissue.

2.3.2. Cerebral Blood Flow Regulation

The regulation of blood flow to the brain is extremely complex as there are multiple overlapping mechanisms (Peterson et al., 2011). CBF is influenced and regulated by a number of factors, such as: intracranial pressure (ICP), arterial blood pressure (ABP), venous outflow, blood viscosity, partial pressure of carbon dioxide ($PaCO_2$), partial pressure of oxygen (PaO_2), cerebrovascular reactivity, collateral flow, and the cerebral autoregulation status (Zauner and Muizelaar, 1997). CBF regulation has long-been a topic of debate. Though a great deal of progress has been made, the interaction between these different components is not fully understood. However, the most commonly agreed regulatory mechanism involved in CBF regulation is cerebral pressure autoregulation.

2.3.3. Cerebral Pressure Autoregulation (CPA)

The ability of the cerebral arteries (arterioles in specific) to maintain a constant CBF over a range of cerebral perfusion pressure (CPP) is generally defined as cerebral pressure autoregulation (CPA) (Peterson et al., 2011; Rangel-Castilla et al., 2008). It is given by the equation,

$$CBF = CPP/CVR$$

where, CVR = Cerebrovascular Resistance. CPP is the net driving force of the cerebral perfusion; it is given by the equation (Zauner and Muizelaar, 1997),

$$CPP = MABP - CVP \text{ (or) } CPP = MABP - ICP$$

Where, MABP = Mean Arterial Blood Pressure, CVP = Cerebral Venous Pressure and ICP = Intracranial Pressure.

CBF is maintained relatively constant across CPP of 50 - 150 mm Hg. In a normally functioning brain, only minimal CBF changes are produced within this CPP range (Rangel-Castilla et al., 2008). A constant CBF is maintained across this CPP range by an increase or decrease in vascular resistance of the vessels that supply blood to the brain. If CPP pressure goes beyond this autoregulation range, CBF clearly varies with CPP and cerebral vasodilation or vasoconstriction cannot maintain a normal CBF (Peterson et al., 2011). Following cerebral insult (ischemia, TBI or subarachnoid hemorrhage), the normal autoregulatory mechanism is lost or its range is disturbed (Figure 2-3) (Rangel-Castilla et al., 2008).

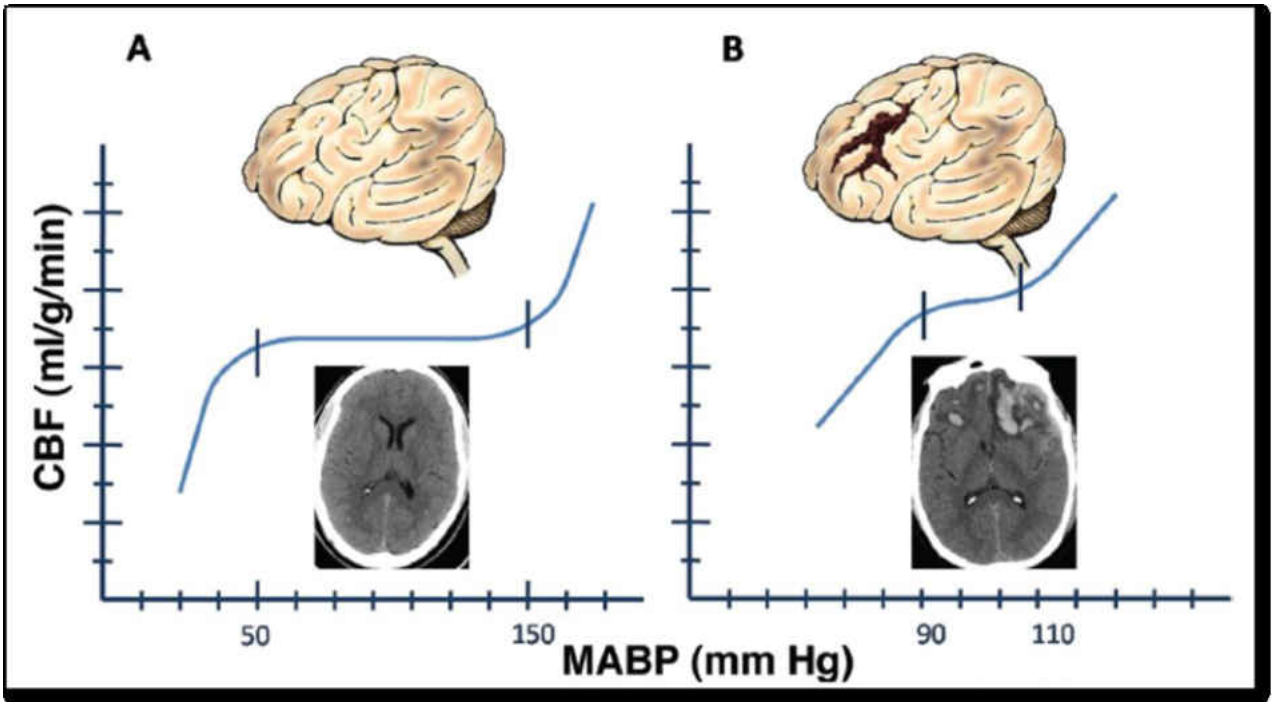


Figure 2-3: Graphs showing CPA curves in normal (A) and in TBI (B) brain (Rangel-Castilla et al., 2008).

Three main mechanisms that control cerebral autoregulatory response have been postulated to be metabolic regulation, myogenic regulation, and neurogenic regulation (Peterson et al., 2011). However, the exact mechanism controlling the autoregulatory response is still not clear. Metabolic regulation has been well established for more than a century (Roy and Sherrington, 1890). CBF varies with cerebral metabolism and the same was recently shown using advanced functional imaging techniques like PET or BOLD fMRI (Chen et al., 2001; Daly and Fredholm, 1998). Regional CBF to different parts of the brain is heterogeneous and is mainly determined by the cerebral metabolism. Normally, a tight coupling exists between the regional metabolic demand for energy substrates (oxygen and glucose) generated from local neuronal activity and the amount of blood supplied to that part of cerebral tissue. This is termed flow-metabolism coupling (Peterson et al., 2011). However, the coupling between CBF

autoregulation and vasoactive metabolites is not well defined. Molecules like CO_2, O_2, K^+, Ca^{2+}, H^+ and metabolites like adenosine are thought to play a role in metabolic coupling (Kuschinsky and Wahl, 1978).

The short latency of cerebral autoregulatory response is considered to be an argument in favor of the myogenic mechanism (Zauner and Muizelaar, 1997). As per myogenic hypothesis, the smooth muscle cells (SMC) surrounding the small arteries and arterioles constrict or dilate in response to changes in transmural pressure (Zauner and Muizelaar, 1997). In an experimental study, it was found that rapid change in intravascular pressure alters the state of the actin and myosin filaments within the SMCs. Another regulatory mechanism emphasizes the extensive arborization of perivascular nerves to play a role in CBF regulation; this is termed neurogenic regulation (Zauner and Muizelaar, 1997). Most of the recent studies have predominantly shown the role of adenosine in the regulation of CBF (Arnaud, 1993; Fredholm et al., 1999; Gilbert, 1984; Verbeeck, 2008) which is discussed in Section 2.5.

2.4 Effects of TBI on CBF

Cerebral autoregulatory mechanisms of blood circulation are impaired following TBI, which could be a critical factor in the secondary injury progression (Kennedy and Haskell, 2011; Sedlacik et al., 2008). It is commonly found that TBI results in reduction of CBF in both the clinical and experimental settings (Sedlacik et al., 2008; Shen et al., 2007; Yuan et al., 1988). Severe TBI often results in CBF disruption that may lead to ischemia (inadequate blood supply) or hyperemia (excessive blood supply) (Marion et al., 1991). It was proposed that cerebral ischemia is the most important cause of secondary brain damage after severe TBI (Marion et al., 1991). Ischemic cell changes

have been observed in around 90% of the patients who die following TBI (Ngai et al., 2001). In humans, ischemic cell death is likely to occur when the CBF drops below 15 - 20 ml/100g/min. A histological study found that cerebral ischemia induced brain damage is common in most head-injured patients who die (Graham et al., 1978). CBF changes in the first few days after TBI are complex, and crucial factors like hypotension and hypoxia at the time of injury definitely play a crucial role on the outcome (Lobato et al., 1988). It was also reported that global CBF was significantly reduced in TBI patients compared against aged matched control individuals (Barclay et al., 1985). A study on humans found that low CBF in the first 24 hrs after TBI was associated with poor a outcome, although it was not significantly different (Marion et al., 1991). Some experimental studies on CBF changes following CHI are discussed below.

2.4.1 Experimental Studies on CBF changes following CHI

Although CHI is the most common type of TBI, CBF changes following CHI are poorly understood. Several studies have attempted to determine the changes in CBF following a CHI (Table 2-2), but no conclusive results have been found. TBI often causes brain swelling resulting in the compression of ventricles (decreases CSF volume) and also leads to vasoconstriction (decreased blood flow) as the volume within the intact skull is fixed (Lunt et al., 2004). This is one of the reasons for the reductions in CBF following CHI, as observed in both clinical and experimental setting (Field et al., 2003; Sedlacik et al., 2008; Shen et al., 2007). Different experimental studies and their experimental model attempted to understand the CBF changes following CHI are shown below (Table 2-2).

Table 2-2: Literature review on experimental studies in CBF changes following TBI.

Reported by	Weight Drop Model Specifications	CBF Measured Using	Regions Analyzed	Post-TBI Time Points
Ito et al, 1996.	SD Rats Height: 2 m Weight: 450 g	Laser-Doppler Flowmetry	Probe in cerebral cortex	30 mins to 4 hrs
Prat et al, 1997	SD Rats Height: 1 m Weights: 350 g, 400 g, 450 g	Laser-Doppler Flowmetry	Probe in right frontal lobe	Up to 8 hrs
Petrov et al, 2001	SD Rats Height: 2 m Weight: 450 g	Laser-Doppler Flowmetry	Probe in cerebral cortex	60 mins to 4 hrs
Shen et al, 2007	SD Rats Height: 2 m Weight: 450 g	Arterial Spin Labeling Imaging, 1 Slice	L & R medial dorsal cortex, L & R hippocampus	4 hrs 24 hrs 48 hrs

Ito et al., reported a decrease in CBF in the cortex during posttraumatic hypotension and then a hyperemic reaction with a maximum value at 2 hours post recovery followed by a decreasing trend from 2 hrs to 4hrs post-TBI (Ito et al., 1996). Prat et al., reported a stable and normal CBF in the frontal lobe during the first three hours, followed by a decrease from 4 hrs to 8 hrs post-TBI, with an increase at 6 hrs post-TBI (Prat et al., 1997). It was also found that a significant loss of autoregulation was found in traumatized rats compared to controls and CBF was consistently lower during this period (Sedlacik et al., 2008). Petrov et al, found a reduced (up to 50%) cortical perfusion during the second hour following TBI, but a brief increase was observed during the third and fourth hour and then followed by a substantial decrease at the end

of 4 hrs post-TBI (Petrov and Rafols, 2001). Three of the above studies reviewed here have used Laser-Doppler Flowmetry (LDF) for measuring the CBF which was limited to measurement from only one region where the probe was inserted, unlike recent studies that are using advanced MRI perfusion imaging techniques such as ASL which enables CBF measurement in multiple cerebral regions.

Shen et al., have used ASL imaging technique to determine post-traumatic CBF changes in cortex and hippocampus (Shen et al., 2007). Medial dorsal cortex has shown up to a 28% reduction in CBF at 4 hrs post-TBI and some recovery at 24 hrs and 48 hrs, but not complete recovery as compared to the baseline. Hippocampal blood flow also showed similar trends as in the medial dorsal cortex, but the CBF reduction was only 12% at 4 hrs post-TBI (Shen et al., 2007). However, this study was done on a small sample size (n=5). All the four studies discussed above are limited to CBF changes only from one or two different anatomical regions of the brain. CBF changes in different regions of the brain following CHI, including the regions that are not directly under the impact location needs to be explored; as the impaired global CBF may affect regional CBF throughout the brain. Also, most of the previous experimental studies have analyzed CBF changes only up to 2 days post-TBI. CBF changes up to 7 days post-TBI needs to be studied.

From the above discussion it is clear that CBF changes following TBI are extremely complex. However, the majority of the studies have shown a reduction in CBF at different post-traumatic time points and it was also mentioned before that post-traumatic ischemia following TBI is associated with poor prognosis. Therefore, the main aim in the treatment of TBI patients is prevention of secondary damage due to post-traumatic

ischemia. A study even suggested to optimize cerebral perfusion and blood flow in head injury treatment (Ribeiro and Sebastiao, 2010). Thus, the current study was focused on optimizing CBF following TBI, which may reduce the extent of secondary injury. As mentioned previously, adenosine and its interaction on adenosine receptors which influence cerebrovascular smooth muscle tone plays a crucial role in CBF regulation. Targeting the adenosine receptor mechanism to make favorable CBF changes, can be attained using a therapeutic agent (caffeine) which has structural similarity and affinity to adenosine. This is discussed below in detail.

2.5 Adenosine and its Receptors

Adenosine (Ade) is a purine endogenous nucleoside, present in all tissues of a mammalian organism and plays a central role as a structural element in nucleic acid energy metabolism of all living organisms (Al Moutaery et al., 2003). Ade plays a prominent role in biochemical processes like energy transfer (ATP - adenosine triphosphate, ADP - adenosine diphosphate) and in signal transduction (cAMP - cyclic adenosine monophosphate). It is also an inhibitory neurotransmitter, that has a role in promoting sleep and also in suppressing arousal; with increased levels of Ade following prolonged period of wakefulness. Ade is not a classical neurotransmitter produced and released vesicularly during axonal firing (Pelligrino et al., 2010). Low concentrations of Ade are usually present in the extracellular fluid, but the levels increase radically during enhanced neural activity, stress or in ischemic conditions by acting as a cytoprotective modulator (Addicott et al., 2009). Ade levels in the CNS range between 30 and 300 nM. Ade also acts as an extracellular signaling molecule by binding to its receptors, that are virtually found on every cell in the body (Washington et al., 2005).

Ade receptors belong to the family of G protein coupled receptors (GPCR), one of the biggest protein families with 50% of all the therapeutics targeting these receptors (Li et al., 2008). Four different types of Ade receptors have been characterized: A_1, A_{2A}, A_{2B} and A3 (Fredholm et al., 1994). Characteristics of each of these receptors and their distribution in the central nervous system (CNS) is shown in Table 2-3 and Figure 2-4. It was found that A_1 and A_{2A} have high affinity for Ade compared to A_{2B} and A_3 receptor subtypes, and hence they have more physiological importance (Ribeiro and Sebastiao, 2010).

Table 2-3: Types of adenosine receptors and their distribution in the CNS. Adapted from (Wardas, 2002).

Receptor subtype	Second messengers	Distribution in the CNS	Selective agonists	Selective antagonists
A_1	G_i, G_o AC PLC, PLD K^+, Ca^{2+}	cerebral cortex, hippocampus, striatum, thalamus, cerebellum	CPA CCPA CHA R-PIA	DPCPX CPT
A_{2A}	G_s, G_{olf} AC	striatum, n.accumbens, olfactory tubercle, globus pallidus, cerebral cortex, hippocampus	CGS 21680 2-HE-NECA	SCH 58261 KF 17837 KW6002 ZM 241385, CSC
A_{2B}	G_s AC	low level in the brain hippocampus, thalamus, hypothalamus, striatum	not available	not available
A_3	G_i, G_q AC	low level in the brain hippocampus, thalamus, cerebral cortex, cerebellum	2-Cl-IB-MECA	MRS 1220, MRE 3008F20

AC – adenylyl cyclase; Ca^{2+} – calcium channel; G – G-protein inhibiting AC (Gi, Go, Gq) and stimulating AC (Gs, Golf); K^+ – potassium channel; PLC – phospholipase C; PLD – phospholipase D; – stimulation; – inhibition

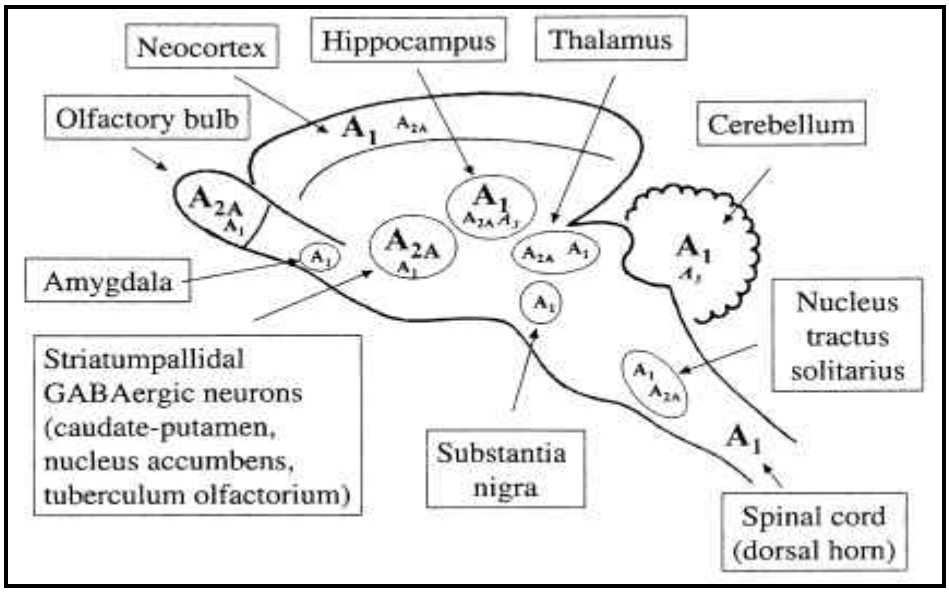


Figure 2-4: Location of different adenosine receptors in the brain (http://www.benbest.com/health/caffeine.html)

2.5.1 Role of Adenosine in the Regulation of CBF

Adenosine (Ade) is a potent dilator of cerebral blood vessels and was found to have an important role in regulation of CBF (Lusardi et al., 2012). Ade receptors on the vascular smooth muscle (VSM) cause vasodilation by relaxing VSM (Blaha et al., 2009; Washington et al., 2005). Adenosine was found to induce vasodilation when bound to A_{2A} and A_{2B} subtypes of Ade receptors (Blaha et al., 2009; Lusardi et al., 2012), by opening ATP-dependant K^+ channels and decreasing the conductance of Ca^{2+}; while other mechanisms of actions may also have a role (Addicott et al., 2009). The half maximal effective concentration (EC50) values for Ade are far greater for A_{2A} receptor compared to A_{2B} receptor, suggesting that A_{2A} receptors are likely to be of greater physiological importance (Nawrot et al., 2003). Though some studies indicate the role of A_1 receptors in vasodilation, Ade acting through A_{2A} receptor on cerebrovascular smooth muscle (CVSM) to induce dilation of intracerebral arterioles has been widely

accepted (Blaha et al., 2009; Sachse et al., 2008; Washington et al., 2005). A similar mechanism of vasodilation is also shown by the Ade receptors on the endothelial cells (Blaha et al., 2009), by releasing nitric oxide (NO) (Echeverri et al., 2010). NO derived from endothelial cells elicits vasodilation (Echeverri et al., 2010). It was also reported that Ade stimulates the production of NO in the endothelial cells, to cause vasodilation (Chen and Parrish, 2009; Echeverri et al., 2010). Furthermore cerebral endothelium may serve to regulate extracellular Ade concentrations and VSM may have a more complex role; Ade binds to its receptors leading to vasodilation (Johansson et al., 1997).

2.6 Caffeine Consumption and Metabolism

Caffeine (1,3,7-trimethylxanthine) (Figure 2-5) is the most popular and most highly consumed psychoactive drug, with an estimated 70% of the world population taking caffeine in some form. The majority of the Americans consume caffeine on a daily basis in various forms (Richard, 2005). Caffeine is present in different doses in several dietary sources like tea, coffee, chocolate bars, energy drinks, and others as shown in the Table 2-4. Coffee and tea account for about 54% and 43% of all caffeine consumption, respectively (Gilbert, 1984). Medicinally, caffeine is used as a stimulant, or added to analgesics in over the-counter formulations. It acts as a central nervous system (CNS) stimulant, temporarily warding off drowsiness and restoring alertness by blocking A_1 and A_{2A} receptors in the brain (Daly and Fredholm, 1998).

Figure 2-5: Chemical structure of caffeine (www.chemistry.about.com).

Table 2-4: Caffeine concentration in various foods and beverages (Barone and Roberts, 1996; Debry, 1994; Fredholm et al., 1999).

Product	Volume or weight	Caffeine content
		mg
Roasted and ground coffee		
Percolated	150 ml	40–170
Drip	150 ml	60–180
Decaffeinated	150 ml	2–5
Instant coffee		
Caffeinated	150 ml	40–180
Decaffeinated	150 ml	2–8
Tea		
Bagged	150 ml	28–44
Leaf	150 ml	30–48
Instant	150 ml	24–50
Iced	150 ml	28–32
Cocoa	150 ml	2–7
Chocolate bar		
Milk	28 g	1–15
Sweet	28 g	5–36
Dark	28 g	5–35
Baking chocolate	28 g	18–118
Soft drinks		
Regular cola	180 ml	15–24
Caffeine-free cola	180 ml	0
Diet cola	180 ml	13–29

Caffeine consumption from all sources per person around the world is estimated to be around 70 to 76 mg/day (Gilbert, 1984). However, caffeine consumption is much higher in some countries as listed in Figure 2-6, compared to the rest of the world. In the United States daily caffeine consumption is estimated to be around 168-220 mg/day/person and a much higher consumption of 390–410 mg/day/person in some Scandinavian countries (Fredholm et al., 1999). As per a recent market survey, the total number of coffee shops in the Unites States drastically increased by about 70% between 2000 - 2005, making a total of around 21,400 coffee houses around the country (http://www.marketresearchworld.net). This increasing demand for coffee may serve as an indirect indicator of increasing caffeine intake among Americans. Therefore, better understanding of caffeine mediated neuroprotection or any other potential health benefits is of great importance for the United States and other high caffeine consuming countries.

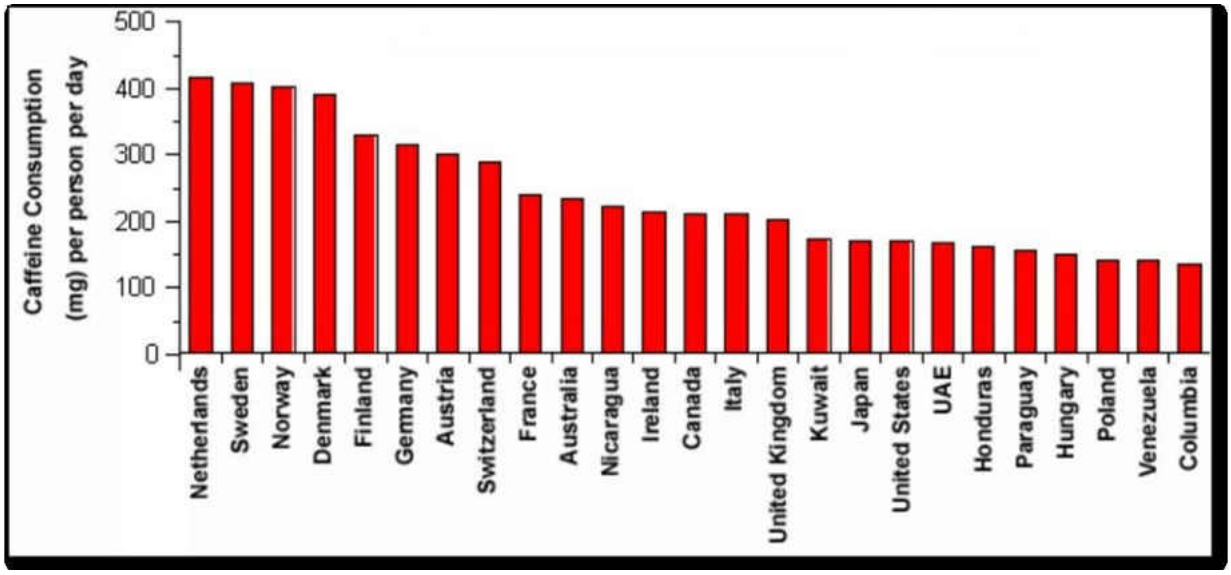


Figure 2-6: Caffeine consumption (mg) per person per day in some high caffeine consuming countries (http://faculty.washington.edu/chudler/caff.html).

The consumption of moderate amounts of caffeine was found to have beneficial effects like increased energy availability, enhanced physical performance, increased ability to make correct decisions, enhanced physical and motor performance and increased alertness (Glade, 2010). But, when very large doses of caffeine is ingested orally, toxic effects are observed with a median lethal dose (LD_{50}) of about 200mg/kg in rats (Eichler, 1976). LD_{50} of caffeine for humans (average adult) is estimated to be around 150 to 200 mg/kg of body mass; roughly about 10 g caffeine or nearly 100 cups of coffee ingestion) (Fredholm et al., 1999). Daily caffeine intake was reported to be safe if it falls below the following limits: healthy adults (400-450 mg/kg), pregnant woman (300 mg/kg) and children in the age group of 4-6 years (45 mg/kg) (Nawrot et al., 2003). It is metabolized in the liver to form several metabolites like dimethyl and monomethylxanthines, dimethyl and monomethyl uric acids, trimethyl- and dimethyl allantoin, and uracil derivatives (Arnaud, 1993) and has half-life of around 4.9 hrs to 6 hrs in healthy adults (Verbeeck, 2008).

2.6.1 Role of Caffeine in Neuroprotection against TBI

Caffeine is both water and lipid soluble. Its hydrophobic properties makes it pass through all the biological membranes. This makes it readily pass through the blood-brain-barrier (BBB) which separates the blood stream from the interior of the brain within 30 mins after ingestion (Fredholm et al., 1999). Any therapeutic agent against cerebral disorders, has to pass through the BBB to exert its effects upon drug delivery. Having this ability makes caffeine one of the potential therapeutic agents for the treatment of several neurological disorders, including TBI.

In experimental TBI, caffeine and adenosine receptor agents can be neuroprotective or neurotoxic, depending on the dose, model, and timing (Chen and Chern, 2011). Recent studies have shown that caffeine can exert a neuroprotective effect in brain injury models such as Parkinson's disease, ischemic brain injury, stroke, etc (Chen et al., 2001; Riksen et al., 2006). Few studies reported caffeine mediated neuroprotection against TBI through different mechanisms. In rat models, acute administration of extremely large doses of caffeine (100-150 mg/kg) immediately before injury worsened the outcome (Al Moutaery et al., 2003). On the other hand, in mice, chronic pretreatment with caffeine (5-50 mg/kg) reduced hippocampal neuronal cell death after experimental TBI (Washington et al., 2005). Similarly, other beneficial effects of chronic caffeine pretreatment were reported. It was demonstrated that at 24 hrs after a cortical impact brain injury, neurological deficits, cerebral edema, and inflammatory cell infiltration were all significantly attenuated in mice pretreated chronically for 3 weeks with caffeinated drinking water (0.1 g/L, 0.25 g/L and 5.0 g/L) (Li et al., 2008). But acute caffeine pretreatment (5 mg/kg, 15 mg/kg and 50 mg/kg; i.p. injection, 30 min before TBI,) was not effective in offering neuroprotection (Li et al., 2008) or has exacerbated TBI induced brain injury (Al Moutaery et al., 2003). However, acute caffeine treatment (i.p. injection, 20 mg/kg) 12 - 18 hrs following TBI was found to be beneficial in decreasing the intracranial pressure (ICP) post-trauma (Blaha et al., 2009). Dash et al., have reported that early administration of caffeinol (10 mg/kg caffeine plus 0.65 g/kg alcohol) is beneficial in reducing some of the deficits and cortical tissue loss following TBI using controlled cortical impact model of brain injury (Dash et al., 2004). Another recent study using rat fluid percussion injury found acute caffeine treatment (25 mg/kg,

i.p. injection within a min after impact) can prevent lethal apnea following TBI (Lusardi et al., 2012).

Although acute caffeine treatment following TBI is beneficial to some extent, as discussed earlier (Blaha et al., 2009; Lusardi et al., 2012), acute caffeine pretreatment adversely affects TBI outcome as evidenced by significant increase in righting latency, neurological deficits and mortality after closed head injury (Al Moutaery et al., 2003; Li et al., 2008). But, chronic treatment pretreatment provides a consistent neuroprotective effect following TBI as demonstrated by decreased neurological deficits, reduced inflammation, reduction in hippocampal neuronal cell death and modulation of glutamate release (Li et al., 2008; Washington et al., 2005). Also, a clinical study on humans found that the caffeine levels in CSF correlated with prognosis, with higher caffeine level in CSF predicting better clinical outcome (Sachse et al., 2008). From this discussion it is clear that very limited research has been done in finding the potential neuroprotective effects of caffeine following TBI. Substantial research has been done to understand both the effects of caffeine on CBF and also CBF changes following TBI, but the effects of caffeine on CBF changes following TBI still needs to be better understood.

2.6.2 Caffeine Effects on CBF through its action on Adenosine Receptors

Caffeine is a weak non-specific adenosine (Ade) receptor antagonist and causes most of its biological effects via antagonizing all types of adenosine receptors (Addicott et al., 2009). Caffeine has structural similarity to Ade and tends to bind to Ade receptors. It exerts vasoconstrictive effects by antagonizing Ade receptors on vascular cells (e.g. smooth muscle, endothelium) (Pelligrino et al., 2010). Ade binding to the A_{2A} and A_{2B} receptor subtypes located on cerebrovascular smooth muscle causes vasodilation as

discussed in previously (Ngai et al., 2001). But caffeine, at normal dietary concentrations, acts as a competitive antagonist of A_{2A} and A_{2B} receptors located on vascular smooth muscle and prevents the vasodilatory effects of Ade (Addicott et al., 2009). A 250 mg caffeine dose has been shown to reduce in resting CBF between 22% and 30% (Field et al., 2003; Lunt et al., 2004).

However, evidence from receptor binding, and physiology studies suggest an adaptation (tolerance) to the effects of caffeine following chronic intake. This adaptation presumably accounts for the development of tolerance to the vasoconstrictive and neurostimulant effects of caffeine (Addicott et al., 2009). Some receptor binding studies on rodents suggest that the majority of this adaptation is due to receptor upregulation (e.g. increase in the number of Ade receptors) (Johansson et al., 1997) and also from the sensitization (e.g. conversion to high affinity state) of the Ade receptors following chronic caffeine intake (Green and Stiles, 1986). Tolerance to caffeine effects develops very quickly, especially after heavy doses, e.g. tolerance to sleep disruption (400 mg of caffeine 3 times a day for 7 days) and tolerance to subjective effects of caffeine (300 mg 3 times per day for 18 days (Fredholm et al., 1999). A study reporting upregulation of A_{2A} receptors on human platelets following 2 weeks of chronic caffeine treatment also supports this hypothesis (Varani et al., 1999). Another study on development of tolerance to effects of caffeine on blood pressure and heart rate also serve as an indirect evidence for Ade receptor upregulation following chronic intake (Denaro et al., 1991).

The withdrawal effects of chronic caffeine following a period of abstention also indicates the role of Ade receptors adaptation. Headache, impaired concentration, irritability,

drowsiness, insomnia and fatigue are commonly observed caffeine withdrawal symptoms that emerge from 12 to 24hrs following caffeine cessation (Fredholm et al., 1999; Lane and Phillips-Bute, 1998; Nehlig, 1999). These effects peak at ~48 hrs, and usually lasting from 1 to 7 days and occasionally beyond 7 days (Fredholm et al., 1999; JHU, 2003). Chronic caffeine use of even small doses (100 mg/kg) was found to result in withdrawal symptoms (Griffiths et al., 1990). It is very important to note that headaches are most commonly observed following caffeine withdrawal from chronic ingestion, and this corresponds with increased CBF after 20 - 24 hrs of caffeine abstention (Couturier et al., 1997; Jones et al., 2000). This may be due to the Ade binding to an increased number of Ade receptors on vascular smooth muscle which are normally antagonized by chronic caffeine levels in the body (Addicott et al., 2009). However, the extent of Ade receptor upregulation depends on the amount of caffeine intake per day, and therefore it is unique to every individual.

2.7 Hypothesis: Chronic Caffeine mediated CBF changes following TBI

Despite extensive research, no effective neuroprotective therapy is currently available for TBI. From the above discussions, it is very clear that reduction in CBF is the single most important secondary damage caused to the brain following TBI and it has been associated with unfavorable neurological outcome. Therefore, restoring CBF during the acute phase following TBI may improve neurological outcome. One such compound that can potentially play a role in such restoration is caffeine, following chronic intake. Caffeine exerts its action on Ade receptor present on cerebrovascular smooth muscle. We hypothesize that chronic caffeine treatment prior to TBI and caffeine withdrawal following TBI may result in favorable CBF changes and thus reduce the extent of

secondary injury damage. In addition, adaptation of the Ade receptors on the cerebrovascular smooth muscle may also play a role in CBF restoration following TBI, in chronic caffeine pretreated rats. This hypothesis (Figure 2-7) if proved will serve as a stepping stone in CBF management following TBI.

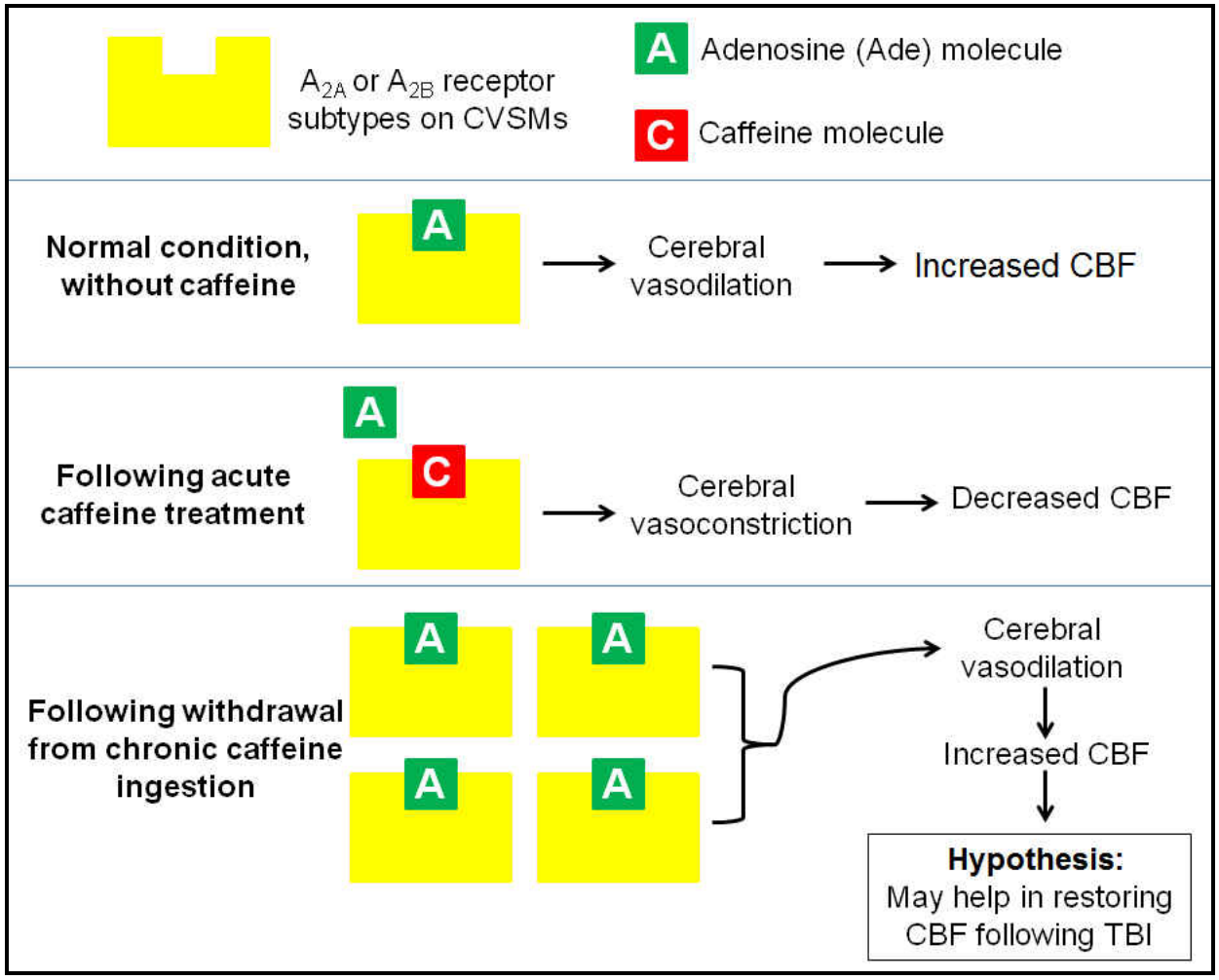


Figure 2-7: Demonstration of hypothesis of this study.

To confirm the hypothesis that chronic caffeine pretreatment can restore CBF following TBI, effects of acute caffeine pretreatment on CBF following TBI also needs to be studied. It is hypothesized that acute caffeine pretreatment will demonstrate

exacerbated reduction in CBF during acute period following TBI. Therefore, unlike chronic caffeine pretreated rats, acute caffeine pretreated rats may not provide any beneficial effects on CBF following TBI.

Given its widespread use, it is very important to know the status of caffeine withdrawal in TBI patients, before giving any drug to alter the CBF. Increased CBF as a result of overnight caffeine withdrawal, accompanied by any vasodilatory drug given for maintaining CBF may worsen the outcome by leading to hyperemia. Therefore, it is essential to understand the caffeine withdrawal effects following TBI to minimize differences in CBF in a subject sample that includes chronic caffeine consumers.

CHAPTER 3 CEREBRAL BLOOD FLOW MEASUREMENT USING ARTERIAL SPIN LABELING IMAGING

3.1 Cerebral Blood Flow Measurement Techniques

CBF and metabolism are highly controlled processes in a normal functioning brain, but they are frequently impaired following TBI and in other neurological diseases. An impaired CBF has been found to trigger secondary brain damage (especially in early phases), and consequently worsening of the patient's outcome. Therefore it is essential to measure and understand CBF changes following TBI, as this will enable an improved and more effective planning of therapeutic strategies to reduce the extent of secondary brain damage (Bor-Seng-Shu et al., 2012; Coles, 2006). The technologies available to measure CBF have evolved over time, and a number of advanced perfusion imaging techniques are currently available. The main imaging techniques dedicated to cerebral hemodynamics are positron emission tomography (PET), single photon emission computed tomography (SPECT), xenon-enhanced computed tomography (XeCT), dynamic perfusion computed tomography (PCT), MRI dynamic susceptibility contrast (DSC), perfusion weighted imaging (PWI), arterial spin labeling (ASL), and Doppler ultrasound (Wintermark et al., 2005). Although the underlying principles of each of these techniques are different, all these techniques provide similar information about cerebral hemodynamic parameters like CBF and cerebral blood volume (CBV). A comparison on some important aspects of some of the perfusion imaging techniques are shown in Table 3-1. Each technique has its own advantage and drawbacks. As part of our efforts to understand CBF changes following TBI we used an ASL imaging technique that is becoming more accessible with the availability of high field MR units (Monet et al.,

2009). The main advantage ASL imaging is its ability to obtain repeated measurements of CBF, both noninvasively and rapidly. However, the clinical applications of ASL are still under evaluation.

Table 3-1: Comparison of commonly used perfusion imaging techniques. Modified from (Wintermark et al., 2005).

Property	Perfusion Imaging Technique				
	PET	**SPECT**	**Doppler**	**PWI**	**ASL**
Contrast agent requirement	Yes	Yes	No	Yes	No
Data acquisition	5–9 mins	10–15 mins	10–20 mins	5-15 mins	5–10 mins
Data processing	5–10 mins	5 mins	None	20 mins	5 mins
Assessed parameters	CBV, CBF, rOEF, glucose metabolism	CBF	Internal carotid artery BFV	CBV, CBF	CBF
Reproducibility	5%	10%	5%		10%
Minimal time interval between 2 successive exams	10 mins	10 mins	0 mins	30 min	0 mins
Brain coverage	Whole brain	Whole brain	One measurement for each hemisphere	Whole brain	Whole brain
Spatial resolution	4–6 mm	4–6 mm	NA	4 mm	2 mm
Clinical applications	Dementia and psychiatric diseases, epilepsy, brain tumors, brain activation studies	Trauma, dementia and psychiatric diseases, epilepsy, brain activation studies	Trauma, vasospasm	Stroke, ischemia and trauma	Trauma, neurodegenerative disorders, brain tumors, brain activation studies

CBV=cerebral blood volume, rOEF=regional oxygen extraction fraction, BFV=blood flow volume.

3.2 Arterial Spin Labeling Imaging

Principle of ASL: ASL is a magnetic resonance imaging (MRI) technique that provides highly repeatable and quantitative measures of CBF (Liu and Brown, 2007). It is performed non-invasively by magnetically tagging (by inverting or saturating) the water nuclei in arterial blood (to serve as a diffusible tracer) before it enters into the plane of interest in the designated tissue. Following certain transit time through this plane of interest, the amount of labeling is measured. This labeling is then compared to a control image which is obtained without spin labeling. By subtracting the labeled image from control image, the tagged spins are identified because of their difference in magnetization (Figure 3-1) (Monet et al., 2009). The intensity of the signal on the resulting (subtracted) image is proportional to perfusion, thus allowing the measurement of CBF.

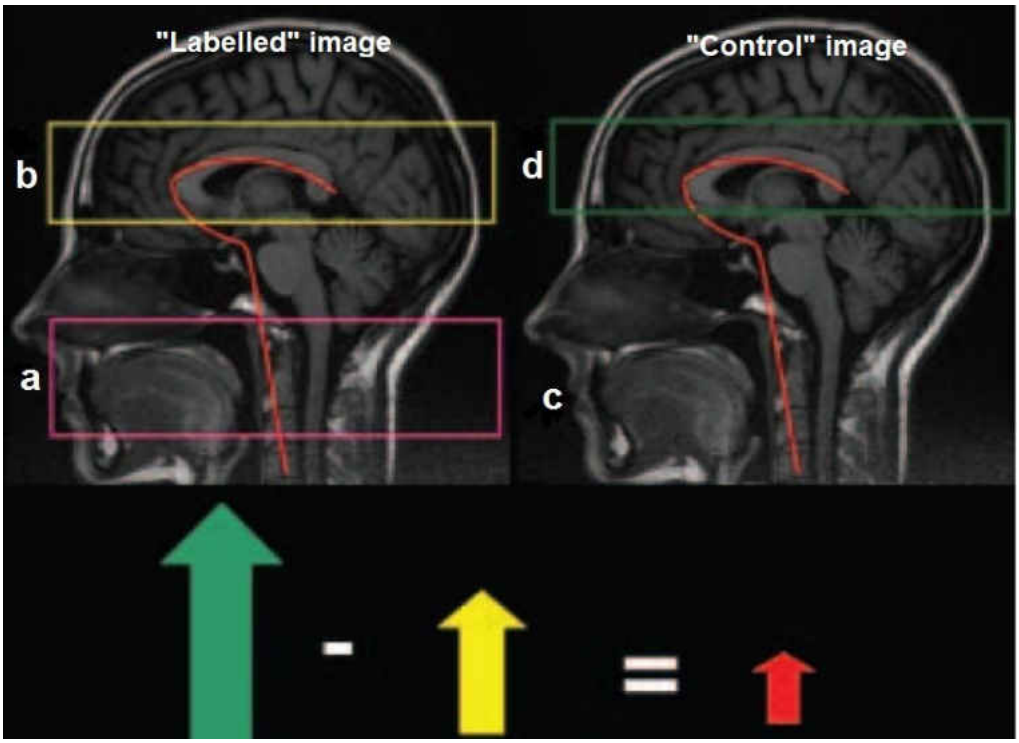


Figure 3-1: Diagram showing the principle of ASL imaging technique. The difference between labeled and control images acquired is proportional to CBF. a) magnetic labeling of arterial blood, b) acquisition of labeled image in the plane of interest, c) no labeling of arterial blood, and d) acquisition of the control image in the plane of interest. Adapted and modified from (Monet et al., 2009).

The local CBF can be related to the signal by the equation (Luh et al., 1999; Wang et al., 2003) given below:

$$CBF = \frac{\lambda\ \Delta M}{2\alpha\ M_0\ TI_1\ \exp(-TI_2/T_{1a})}$$

Where, M = signal intensity, ΔM = difference in signal intensity between control and tag acquisitions, λ = blood/tissue water partition coefficient, α = inversion efficiency, TI_1 = duration between the inversion and saturation pulses, TI_2 = image acquisition starting time, T_{1a} = longitudinal relaxation time of blood. At the 7T scanner, CBF values were obtained by assuming λ = 0.9 mL/g, α=0.95, $TI_1/TI1_s/TI_2/T_{1a}$(seconds) = 0.5/0.8/1/1.5, and the equilibrium magnetization M_0 image was acquired.

Features of ASL: The most important feature of ASL perfusion imaging is that, it does not require any contrast media like PET, SPECT, XeCT scanning. ASL uses endogenous water as a tracer (Wintermark et al., 2005). It takes 5-10 minutes for ASL image acquisition, depending on the quality of the scanner. Several pairs of label and control images are averaged to obtain the desired signal-to-noise ratio (SNR). ASL perfusion imaging is very sensitive to the movement of the subject during scanning, because ASL is a subtraction technique. With recent advancements , this sensitivity to motion is greatly reduced using motion correction algorithms (Wintermark et al., 2005). Recent technical advances have also helped improve the signal from ASL and its acquisition. Perfusion monitoring using ASL requires a high SNR, because there is only

1% difference between the two (label and control) image acquisitions (Wintermark et al., 2005).

3.2.1 Pulsed ASL vs Continuous ASL

After the initial developments of ASL technique in 1992 (Detre et al., 1992; Williams et al., 1992), the ASL imaging modalities are classified into two categories: continuous ASL (CASL) and pulsed ASL (PASL), based on how the spin labeling is performed. Both these techniques require labeling of a certain amount of blood magnetization and acquisition of a control image is necessary. Also, both these techniques have their own advantages and limitations (Table 6).

Table 3-2: Comparison of ASL perfusion imaging techniques. Adapted and modified from (Monet et al., 2009).

PASL		**CASL**	
Advantages	Limitations	Advantages	Limitations
Reduced magnetization transfer effects, shorter image acquisition time	Sensitive to transit times, reduced coverage	Good SNR	Sensitive to transit times, magnetization transfer effect, application of a long off-resonance radio frequency (RF) pulse before image acquisition, loss of spin by blood water as it travels from labeling plane to imaging slice

CASL imaging is based on an adiabatic inversion pulse. It uses continuous low intensity pulse to tag arterial blood at the neck until tissue magnetization reaches a steady state (Monet et al., 2009). But CASL has some limitations as mentioned in the Table 6. PASL can be used to improve blood flow quantification, by using a short labeling RF pulse 5-

20 ms radio frequency and by minimizing the imaging slice distance from the labeling region (Barbier et al., 2001; Martirosian et al., 2010). Since PASL is less effected by magnetization transfer effect, compared to CASL, it is more advantageous in clinical practice and is the most commonly used method (Monet et al., 2009). In this study, we have used PASL perfusion imaging for the relative measurement of CBF following TBI.

3.2.2 Clinical Applications of ASL

ASL perfusion imaging can be performed on any patient that can tolerate normal MRI scanning. However, clinical usage of ASL techniques is not as widespread as traditional MRI imaging. However, the utility of ASL has been demonstrated for a range of cerebrovascular diseases, both acute and chronic (Wintermark et al., 2005). Some studies on stroke and transient ischemic attack have demonstrated the feasibility of acquiring CBF measurements using ASL (Detre et al., 1998; Wolf et al., 2003). ASL has also been used for studying CBF changes in several pathological situations such as temporal lobe epilepsy, cerebral tumor perfusion, malignant gliomas and to evaluate functional brain activation. However, studies related to CBF measurement following TBI in animal models are limited, especially those using ASL imaging. The most important advantage of ASL is its ability to perform repeated measurements non-invasively, which is often needed before and after giving any vasoactive drug for neuroprotection through therapeutic intervention and also in case of neurointerventional procedures like stenting or carotid endarterectomy (Wintermark et al., 2005). Most importantly, ASL CBF maps can help neurosurgeons in planning neuro-surgical procedures. In the recent years, ASL is being widely used to understand CBF changes following TBI in both clinical and

experimental settings (Bir et al., 2012; Hayward et al., 2011; Kim et al., 2010; Shen et al., 2007).

CHAPTER 4 TO DETERMINE CEREBRAL BLOOD FLOW CHANGES FOLLOWING TBI IN A RAT MODEL USING ASL-MR IMAGING (SPECIFIC AIM 1)

4.1 Introduction

As discussed previously (Chapter 2), several studies have attempted to understand the CBF changes following TBI. But, very limited information is available on the CBF changes in various regions (right parietal cortex, left parietal cortex, right striatum, left striatum, right hippocampus, left hippocampus, bilateral thalamus and brainstem) of the brain following TBI. Therefore, specific aim 1 of this study attempts to analyze relative changes in regional and global CBF following TBI, at 4hrs, 24 hrs, 3 days and 7 days post-TBI.

4.1.1 Rationale for using Marmarou Impact Acceleration Model

Various animals models of TBI are currently in use to understand the pathological events underlying human TBI and to develop effective therapies to reduce the extent of cellular damage (Cernak, 2005). These models are capable of reproducing almost all the injury events observed in human brain trauma. The most commonly used experimental models to study traumatic axonal injury (TAI), the most common consequence of TBI, and its implications are shown in Table 4-1. TBI induction in smaller animals came into existence when Marmarou and colleagues developed a simple inexpensive weight-drop model using rats (Marmarou et al., 1994). Since then it is well known as "Marmarou head impact model" or "impact acceleration model" and became one of the most widely used models (Cernak, 2005; Kallakuri et al., 2012; Wang and Ma, 2010). The selection of this experimental model is based on the research goals and specific objectives of the current study. This study aims to

understand CBF changes (a potential contributor of secondary brain damage when CBF falls drastically) following CHI. It was reported that TBI induced through the Marmarou impact acceleration model is capable of producing both vascular and neural changes (Foda and Marmarou, 1994). Changes in arterial blood pressure resulting from TBI induced by this model may lead to vasoconstriction of cerebral vessels, leading to decreased CBF (Foda and Marmarou, 1994; Marmarou et al., 1994). Significant changes in the CBF of cortical regions following TBI induced by the weight drop model have already been reported (Ito et al., 1996; Petrov and Rafols, 2001; Prat et al., 1997; Shen et al., 2007). Therefore, the Marmarou impact acceleration model is an appropriate choice for both understanding CHI mediated CBF changes and for the development of possible therapeutic agents which can offer neuroprotection by limiting cellular injury potentially mediated by CBF changes.

Table 4-1: Experimental *in vivo* models of TAI (Wang and Ma, 2010).

Model type	Main pathological changes	Advantages	Disadvantages	Main lesion (TAI) location
In vivo models				
Instant rotational injury model	Diffuse TAI	Reproducible, reliable, similar to clinical situation, widespread lesion	Usually need large animals, high cost, technical demands	Widespread
Impact acceleration injury model	Diffuse brain injury associated diffuse TAI	Economical, simple, practical, widespread lesion	Biomechanics not fully and strictly controlled	Widespread
Lateral fluid percussion injury model	Combined focal and diffuse brain injury associated TAI	Reproducible, reliable, suitable for most species	High morbidity, complex pathological changes, not suited for detailed biomechanical analyses	Central white matter
Controlled cortical impact model	More focused injury compared to FPI	Highly reproducible, reliable, strict biomechanical control	Focused injury, lack of brain stem deformation in lateral CCI	White matter adjacent to the injury site
Nerve stretch injury model	Isolated TAI	Reproducible, reliable, strict biomechanical control, facilitate observation	Isolated injury, difficulty applying to brain	Elongation site
In vitro models	Neuron injury associated TAI or only TAI	Reproducible, reliable, simple, very controlled conditions, facilitate observation	Neglect many factors associated with human TAI	Varies with different *in vitro* models

Injury Device

The injury device used for this study was slightly modified from the original Marmarou model (Figure 4-1) in which a plexiglass tube (inner diameter 19 mm, outer diameter 25 mm, height 2.5 m) was attached to a stand using three clamps. Rigidity of the plexiglass tube may not be assured when fixed to a stand as it is prone to undergo vibrations as the impactor falls freely. So as to improve the structural stability, we secured the plexiglass to a steel building column . The impactor consisted of a column of brass weights (50 g each) together to form a 450 g impactor (18mm diameter). A steel disc (helmet) (10 mm diameter) was fixed to the exposed rat skull, positioned midline between lambda and bregma using cyanoacrylate (Figure 4-2). This helmet prevents skull fractures at the site of impact and also distributes the impact energy; but does not compromise in achieving high kinetic energy levels at the site of impact delivery (Marmarou et al., 1994). The rat was positioned in the foam bed (12x12x43 cm) under the plexiglass tube with the helmet centered just below the plexiglass tube. TBI will be induced by releasing the impactor from a height of 2 m on to the helmet.

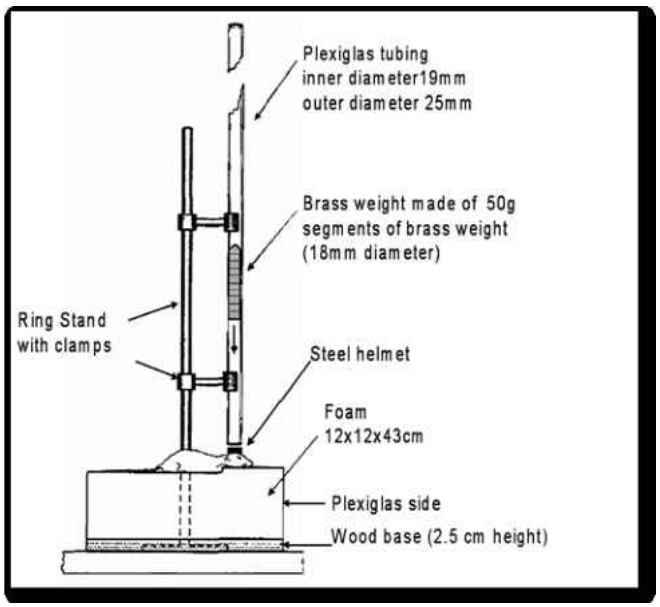


Figure 4-1: Schematic representation of the Marmarou impact acceleration model. Adapted from (Marmarou et al., 1994).

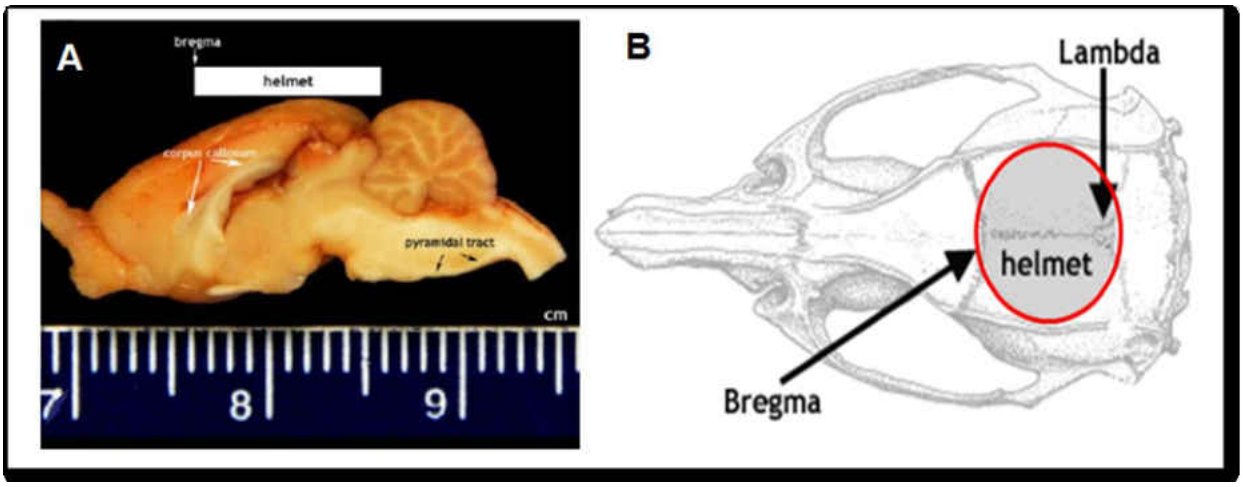


Figure 4-2: Demonstration of the helment placement on rat brain in sagittal view (A) (Kallakuri et al., 2012) and in axial view on the exposed skull, between bregma and lambda (B).

4.2 Methods

4.2.1 Animal Handling

All surgical and animal handling procedures were approved by the Institutional Animal Care and Use Committee (IACUC), Wayne State University, Detroit. A total of 16 adult male Sprague Daley rats (Harlan, Indianapolis, IN) weighing 350-425 g were utilized as a part of specific aim 1. The rats were randomly assigned into two groups: sham (n=7) and TBI-only (n=9). All the rats had free access to food and water.

4.2.2. Surgical Procedures and TBI Induction

The rats were anesthetized initially by a mixture of 3% isoflurane and 0.6L/min oxygen in an anesthesia chamber. After 3 mins of induction, anesthesia was maintained at 2-3% isoflurane and 0.6L/min oxygen via a nose cone. The eyes of the animal were lubricated with sterile ophthalmic ointment to prevent corneal drying. The scalp and surrounding area was shaved with electric clippers to gain access to the surgical site. The skin was cleaned and disinfected alternatively with Betadine and 70% alcohol 3 times. After ensuring that the rats were unresponsive to noxious stimulation, a midline skin incision of ~3cm was made exposing the periosteum covering the vertex of the skull. The periosteum was reflected and a 10 mm diameter steel disc with a thickness of 3 mm was affixed using cyanoacrylate (Elmer's Products, Columbus, OH) at the midline, between bregma and lambdoid sutures. The rat was positioned in a prone position on a foam bed contained in a Plexiglas box and secured in place with tape to prevent any movement of the rat before the impact and to prevent falling off the foam bed after TBI. Just prior to the induction of TBI, the nose cone supplying anesthesia was taken off. Then, the 450 g cylindrical brass weight (18 mm diameter) was dropped from 2 m on to

the steel helmet affixed to the exposed skull. To avoid a second impact, the Plexiglas box was quickly removed after impact. Immediately after the impact, rats were laid on their back and were monitored for latency to surface right (elaborated in next paragraph). After surface righting, the steel helmet was removed and the skull was examined for any fractures. The wound was cleaned and disinfected with Betadine solution (1:10 dilution) and the skin was closed with sterile suture (4.0). The rats were returned to their cages in a supine position and allowed to recover. For sham rats, all surgical procedures including placement on the foam bed were performed but the rats were not subjected to TBI.

4.2.3 Surface Righting Reflex

Surface righting (SR) is the ability of an animal to roll over from a supine position (on its back) to prone position (Figure 4-3). It is also known as reflex righting. SR time is the time taken by an animal to come back to its natural normal ventral position after being placed on its back following head impact. TBI results in loss of consciousness from minutes to hours following impact, depending on the severity of the injury. Therefore, SR time serves as an indirect indicator of duration of unconsciousness of rats following impact (Adams, 1986).

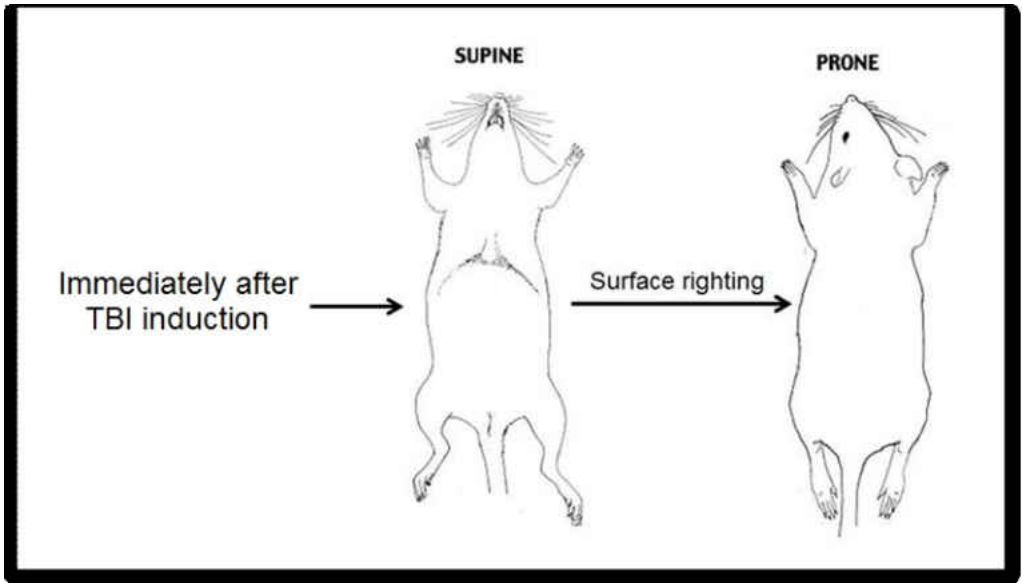


Figure 4-3: Diagram of a rat in supine position turning over to prone position, demonstrating the surface righting reflex immediately after TBI. Modified from (Yang et al., 2002).

In this study, SR duration from all the rats in both sham and TBI rats was recorded. For TBI group rats, SR duration is the time taken to do surface righting following head impact. For sham rats, SR duration was taken as the time to surface right following sham surgery.

4.2.4 Perfusion and Termination

All the rats used in this study were sacrificed 7 days post-TBI or sham surgery following completion of the last MRI scanning. This study having an imaging component, rats were scanned several times before and after TBI. In case of sham rats, they were pre-scanned twice, i.e., 21 days before water treatment and 1 day before sham surgery. In the case of TBI rats, they were pre-scanned only 1 day before TBI. Post-sham surgery or post-TBI scanning's were at 4 hrs, 24 hrs, 3 days and 7 days (Table 4-2).

At the conclusion of 7 day post-TBI MRI scanning procedure, rats were anesthetized (50 mg/kg sodium pentobarbital) and CSF was then harvested by placing the rats in sternal recumbency with the chest and front legs placed over a rolled surgical towel (approximately 1.5 - 2 inches in diameter). CSF was collected by inserting a TB syringe with 26x1/2" gauge needle in the atlanto-occipital membrane and then into the underlying dura to reach cisterna magna. About 50-100 µl of CSF was collected from each rat. Then the lateral chest wall and overlying skin was rapidly cut open with blunt scissors on both sides of the sternum exposing the heart. About 1.5 mL of blood was collected from the heart using a 25 gauge needle and was centrifuged to separate the serum. The serum and CSF sample were then stored at -80ºC for future biomarker studies. To perfuse the brain, a 22 gauge needle was introduced into aorta to perfuse with normal saline (100 ml). Venous return was drained by opening the right atrium, followed by perfusion with cold 4% paraformaldehyde in phosphate buffered saline (400 ml) to fix the brain. The perfused brain was harvested and post fixed in cold 4% paraformaldehyde with 20% sucrose for future histological assessment.

Table 4-2: Demonstration of the step wise procedures performed on rats from sham and TBI groups.

Step wise procedure	Sham group (n=7)	TBI-only group (n=9)
21 days before TBI	MRI	NA
3 Weeks pure water treatment	✔	NA
1 day before sham surgery or TBI	MRI	MRI
On the day of surgery/TBI impact	skin incision (sham surgery)	TBI impact (2 m height, 450 g weight)
4 hrs post-TBI	MRI	MRI
24 hrs post-TBI	MRI	MRI
3 days post-TBI	MRI	MRI
7 days post-TBI	MRI	MRI
CSF, serum samples and brain harvest on 7th day	✔	✔

NA = not applicable and ✔ = Performed as described in left most column.

4.2.5 ASL Image Acquisition

At the designated imaging time points, each rat was initially anesthetized in a anesthesia chamber by a mixture of 3% isoflurane and 0.6L/min oxygen for 3 mins. Anesthesia was maintained by 0.75-1.75% isoflurane and 0.6 L/min of oxygen via a nose cone encompassing the duration of entire imaging procedure. Rats were secured with stereotaxic ear bars to minimize movement during MRI scanning. The rats head was positioned in such a way that it is aligned at the isocenter of the magnet.

All MRI measurements were performed in a 7T horizontal-bore magnetic resonance spectrometer (Figure 4-4) (ClinScan, Bruker, Karlsruhe, Germany) with a 30 cm bore actively shielded gradient coil capable of producing a magnetic field of up to 290 mT/m. A whole-body birdcage radiofrequency (RF) coil (inner diameter, 7 cm) was used as the transmitter for homogeneous RF excitation, and a surface coil was used as the receiver, with active RF decoupling to avoid signal interference. Though only pulsed ASL perfusion imaging data (for measuring CBF) was required for this study, several other MRI sequences (T1 and T2 imaging, Susceptibility Weighted Imaging-SWI, Diffusion Tensor Imaging-DTI, Magnetic Resonance Spectroscopy-MRS) data were acquired for future analysis. The total duration of image acquisition for all sequences for a single rat brain at a given time point was around 2 hrs. At the conclusion of the scanning, the rat was placed on a water circulating heat pad to maintain adequate body temperature.

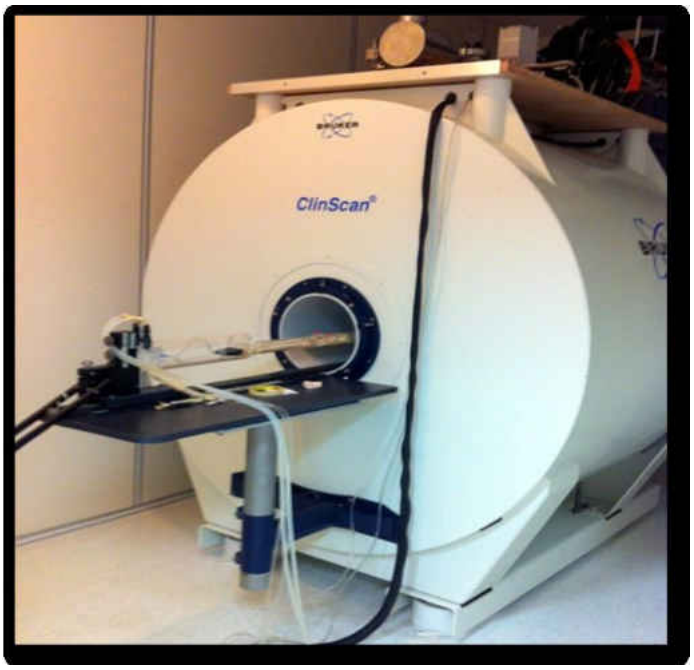


Figure 4-4: An image of the 7T MRI scanner from which the MRI data in this study was acquired.

The ASL image acquisition parameters used for this study were: repetition time (TR) = 3000 ms, echo time (TE) = 16 ms, number of slices = 8, slice thickness = 2 mm, inter-slice gap = 0.5 mm and number of acquisitions = 51. Eight 2D coronal slice images covering the entire brain (Figure 4-5) were obtained for each rat at each time point. The field of view (FoV) was 35x28.44 mm^2 with a matrix size of 128 x 104. The voxel size was 0.273x0.273x2 mm^3. At first, the data from the brainstem region (slices 1 to 4) was acquired and then the grids were moved to acquire data from the cortical and subcortical regions (slices 5 to 8), as shown in Figure 14. The time of acquisition (TA) of ASL data for all slices was around 6 mins.

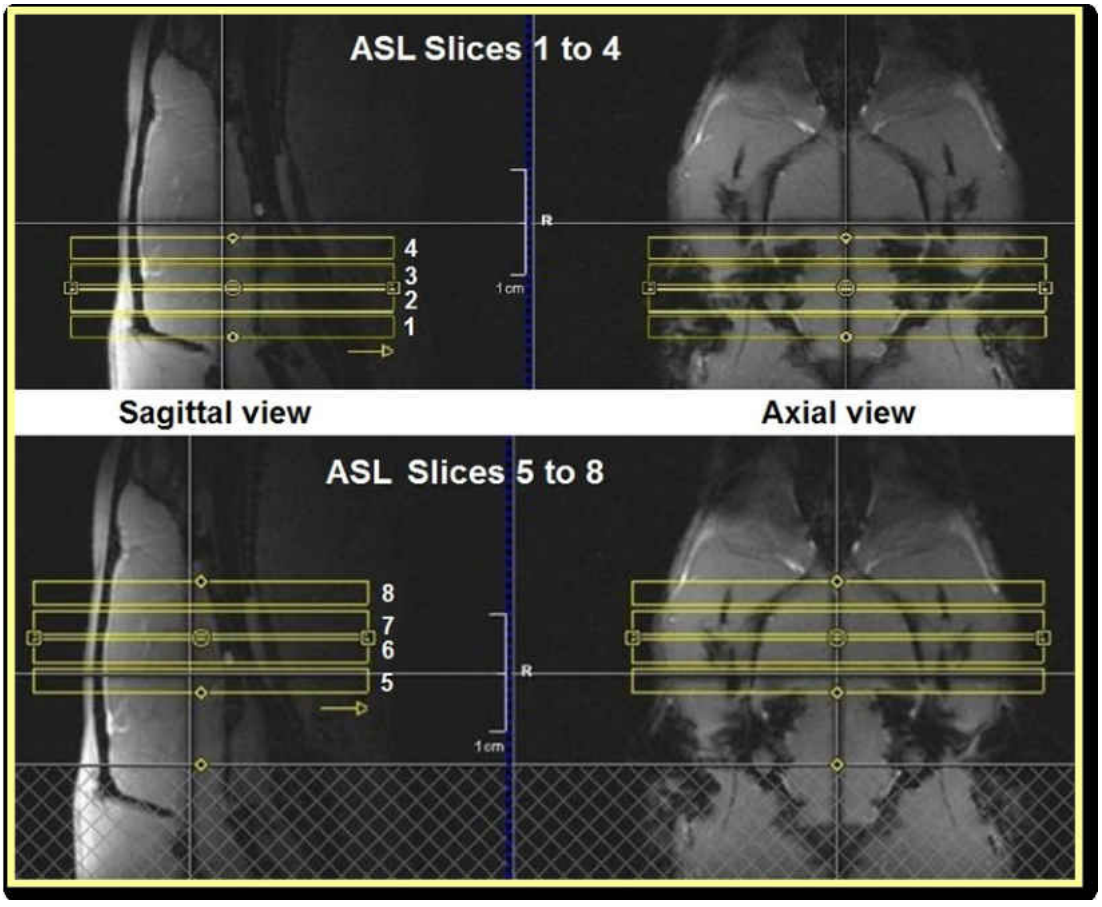


Figure 4-5: ASL image acquisition: Slice locations from where the CBF data was collected. Slices 1 to 4 covered brainstem regions and slices 5 to 8 covered cortical and subcortical regions of the brain.

4.2.6 ASL Data Analysis

ASL data was analyzed from sham group (n = 7) and TBI group (n = 6). The CBF maps generated by the MRI scanner were used for the measurements of CBF. The scanner generated maps were imported into signal processing in Nmri (SPIN) software (version 2045), developed here at Wayne state University (http://www.mrc.wayne.edu/) for MRI data analysis. The imported ASL images were amplified (up to three times) for an accurate region of interest (ROI) delineation. The ROI around brain regions was drawn using the "polygon selection" in SPIN software. Brain regions were identified and delineated by using Paxinos rat brain atlas (Figures 15, 16 and 17) (Paxinos and Watson, 2007). The mean value of the signal intensity obtained from each ROI served as the arbitrary CBF value for that region. Since we measured only relative CBF changes and not the absolute CBF, the CBF mean values do not have a measurement unit. We measured CBF in various regions and termed these measurements as regional CBF (rCBF). The regions included: right parietal cortex (Rt PCx), left parietal cortex (Lt PCx), average of left and right parietal cortex (Avg PCx), right striatum (Rt St), left striatum (Lt St), average of left and right striatum (Avg St), right hippocampus (Rt Hp), left hippocampus (Lt Hp), average of left and right hippocampus (Avg Hp), bilateral thalamus (Th) and brainstem (Bs) (Figures 4-6, 4-7 and 4-8). The ROI drawn for parietal cortex (PCx) consisted of areas that corresponded to primary and secondary somatosensory cortex (Paxinos, 1985). Average CBF values from all these regions (Rt PCx, Lt PCx, Rt St, Lt St, Rt Hp, Lt Hp, Th and Bs) were then used to calculate a combined regional CBF (combined rCBF). In addition, average global CBF (gCBF) was also measured by drawing a single ROI around the complete brain in each of the 8 ASL slices. Table 4-3, shows the number of slices that were used to measure CBF for each

region. For each group of rats (sham and TBI) at any given time point, CBF values obtained from a brain region from all the rats in that group was averaged to represent a mean CBF value for that region.

Table 4-3: Different brain regions from where CBF was measured and the number of ASL slices from which CBF were measured.

Brain regions where CBF was measured	Number of ASL slices from which CBF was measured
Right parietal cortex (Rt PCx)	2
Left parietal cortex (Lt PCx)	2
Average of left and right parietal cortex (Avg PCx)	2
Right striatum (Rt St)	1
Left striatum (Lt St)	1
Average of left and right striatum (Avg St)	1
Right hippocampus (Rt Hp)	1
Left hippocampus (Lt Hp)	1
Average of left and right hippocampus (Avg Hp)	1
Bilateral thalamus (Th)	1
Brainstem (Bs)	3
Combined rCBF	5
Global CBF	8

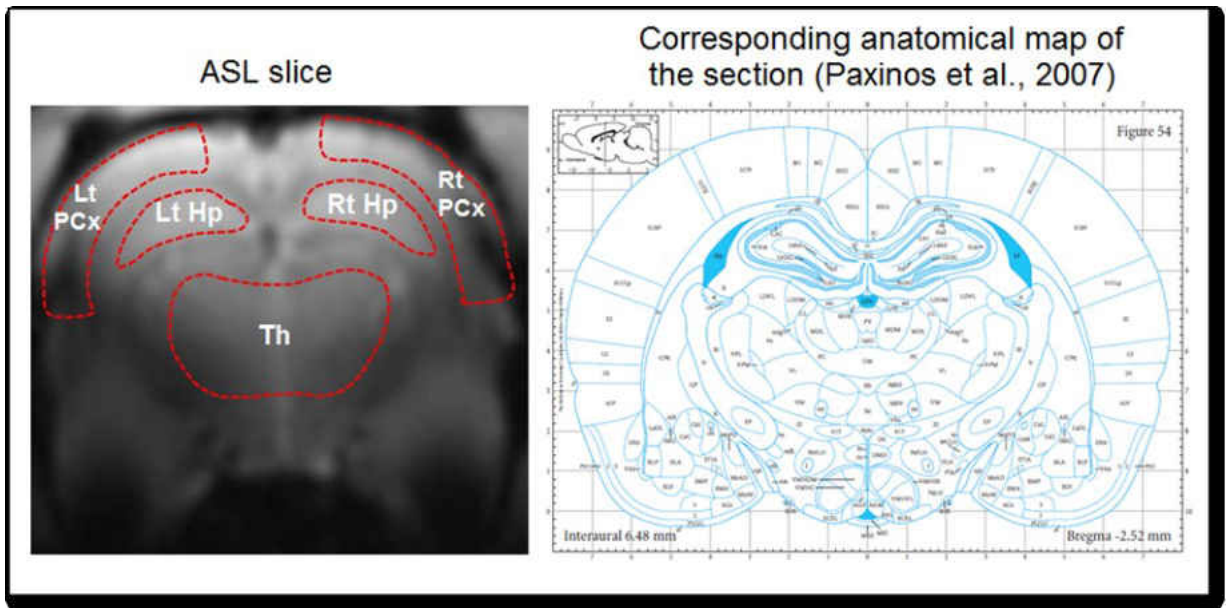


Figure 4-6: ASL image of a rat brain showing regions of interest (ROIs) for CBF measurement and the corresponding anatomical map (Paxinos and Watson, 2007). Left parietal cortex = Lt PCx, right parietal cortex = Rt PCx, left hippocampus = Lt Hp, right hippocampus = Rt Hp and bilateral thalamus = Th.

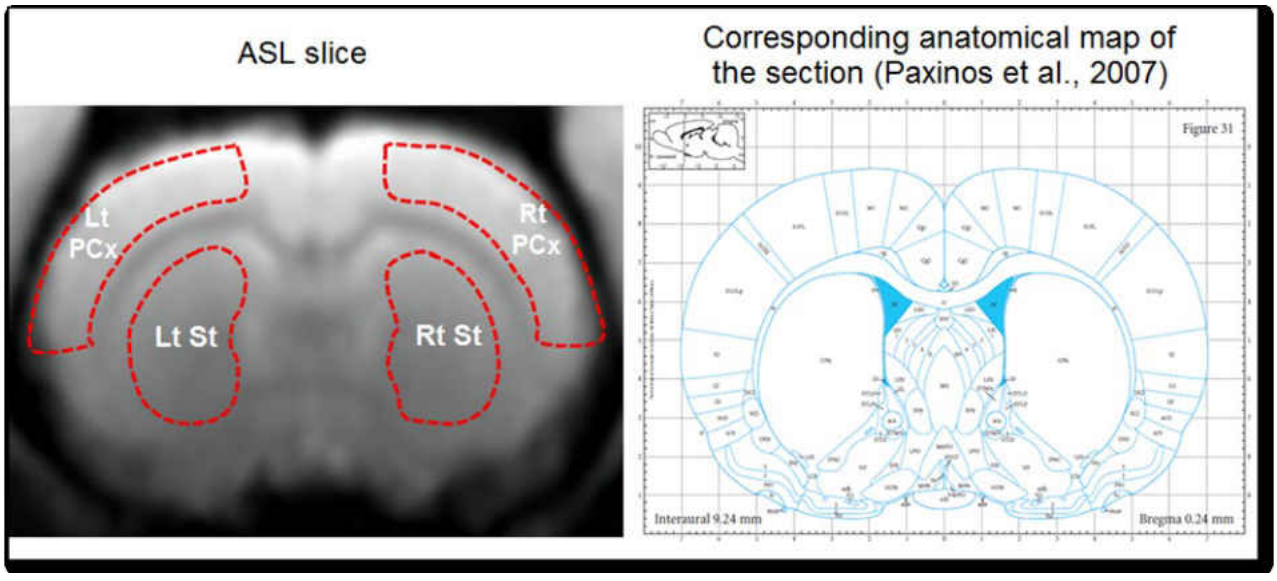


Figure 4-7: ASL image of a rat brain showing regions of interest (ROIs) for CBF measurement and the corresponding anatomical map (Paxinos and Watson, 2007). Left parietal cortex = Lt PCx, right parietal cortex = Rt PCx, left striatum = Lt St and right striatum = Rt St.

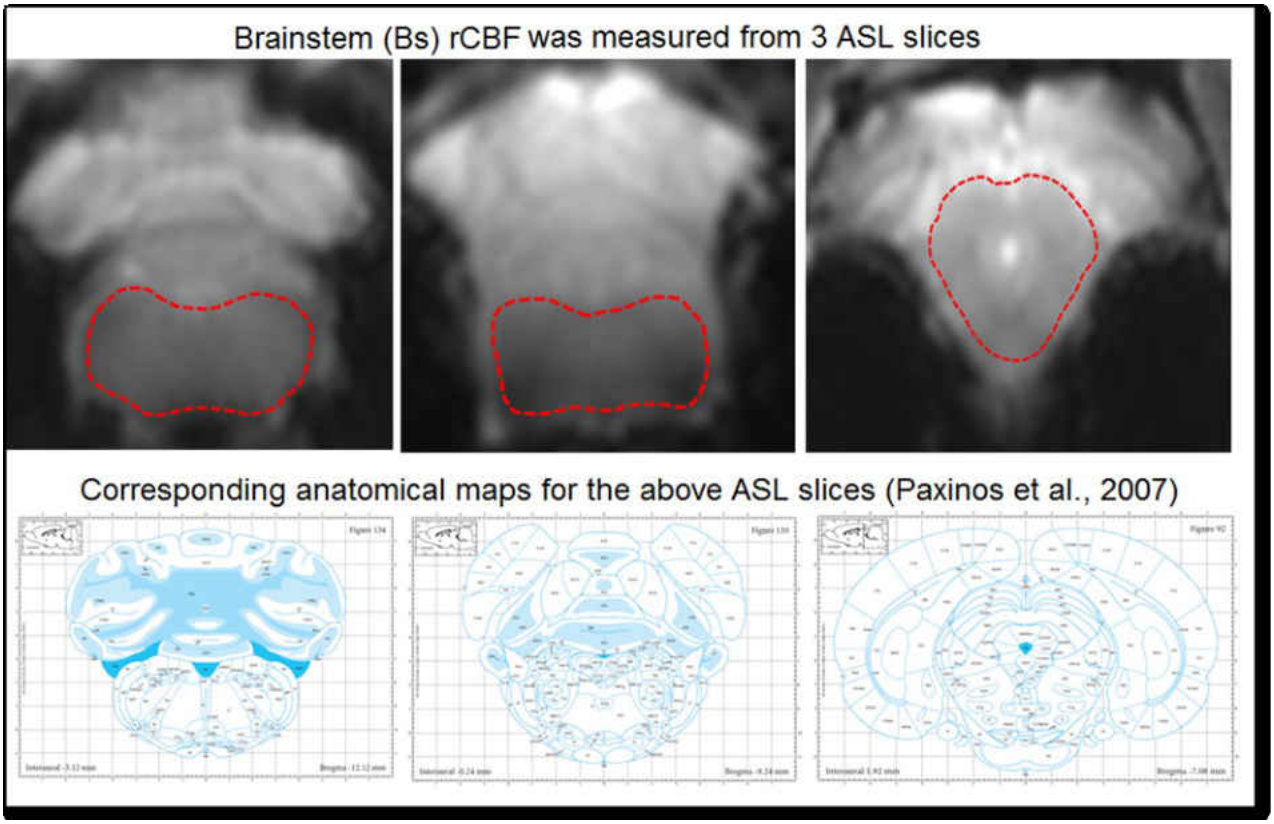


Figure 4-8: ASL images of a rat brain showing regions of interest (ROIs) for brainstem (Bs) in three different slices from where CBF was measured and the corresponding anatomical maps (Paxinos and Watson, 2007).

4.3 Statistical Analysis

All data were analyzed for statistical significance using SPSS (version 20, Chicago, IL). The CBF value obtained from ASL images was expressed as the mean value ± standard error of the mean (SEM). The data were first tested for reliability by Cronbach's alpha test with a Cronbach's alpha value ≥ 0.7 being considered as reliable. Reliability of the data at each time point for all the rats was combined and tested (Table 4-4).

Table 4-4: Cronbach's alpha reliability test values of the CBF mean values (ASL data) for all rats combined at each MRI scanning time point.

Time point of ASL data	Cronbach's alpha test value
Pre-Rx	0.89
Pre-scan	0.94
4 hrs post-TBI	0.90
24 hrs post-TBI	0.86
3 Days post-TBI	0.93
7 Days post-TBI	0.94

Group-wise differences were analyzed by repeated measures analysis of variance (ANOVA). Data was further analyzed for within group differences by one-way ANOVA using Least Significant Difference (LSD) post-hoc test. A p value of <0.05 was considered as significant.

4.4 Results (Sham and TBI-only groups)

4.4.1 Surface Righting of Sham and TBI rats

Rats in TBI group showed a significantly prolonged SR duration compared to sham rats (Figure 4-9). Compared to SR duration of 162±25 sec in sham rats, SR duration in TBI rats was significantly higher ($p<0.05$) with an average duration of 355±65 sec.

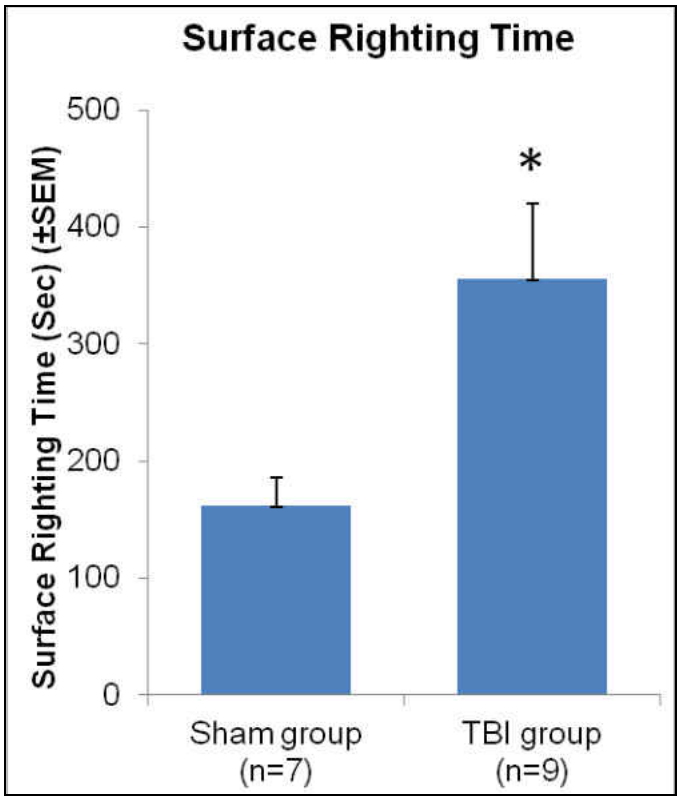


Figure 4-9: Plot showing mean SR duration of sham and TBI-only group rats. * indicates significant difference (p<0.05) in SR duration of TBI group rats compared to sham rats.

4.4.2 CBF Changes in Sham rats

As mentioned previously, CBF changes in sham rats were measured at various time points: pre-water treatment (pre-Rx), 1 day before sham surgery (pre-scan), 4 hrs, 24 hrs, 3 days and 7 days post-sham surgery.

Parietal cortex CBF changes in sham rats

In PCx of sham rats, no apparent CBF changes were observed. No significant differences were observed between and within CBF values of Rt and Lt PCx until 7 days post-sham surgery (Figure 4-10). At 4 hour and 24 hour post-sham surgery, some

reduction in CBF was observed which was not significant. Furthermore, no differences were observed between Avg PCx and Rt and Lt PCx.

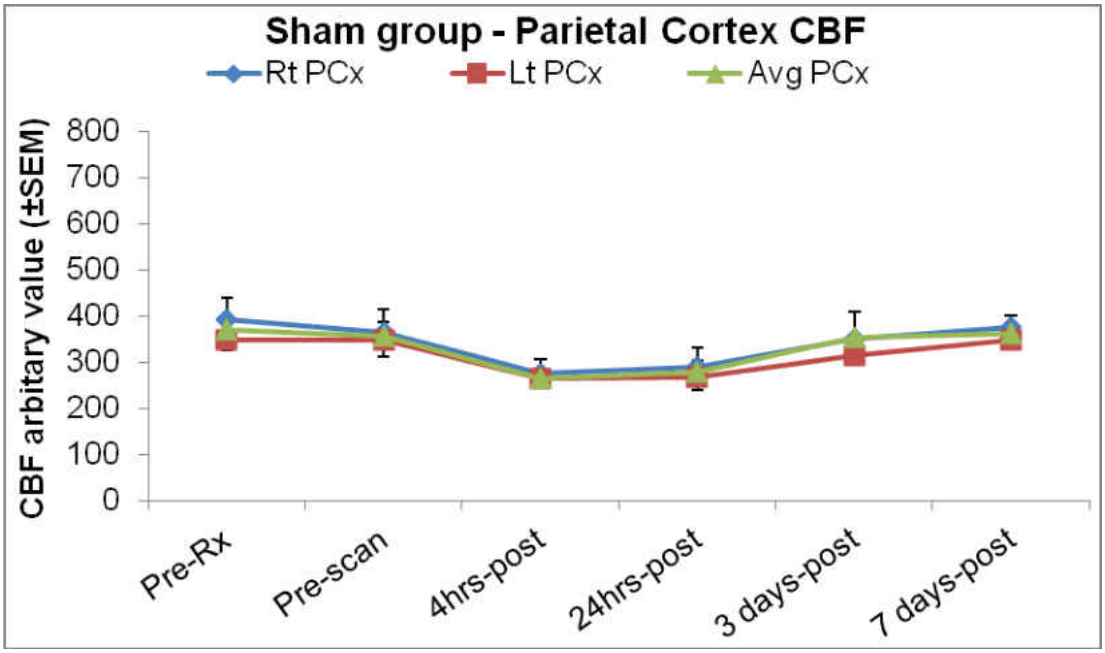


Figure 4-10: Plot showing CBF changes in the right parietal cortex (Rt PCx), left parietal cortex (Lt PCx) and average of right and left parietal cortex (Avg PCx) of sham rats at various time points (pre-Rx=pre-water treatment, pre-scan=1 day before sham surgery, 4 hrs, 24 hrs, 3 days and 7 days post-sham surgery).

Striatum CBF changes in sham rats

In striatum of sham group rats, no apparent CBF changes were observed. In both, Lt St and Rt St, no significant temporal differences in CBF were observed between any time points (Figure 4-11). At 24 hour post-sham surgery, some insignificant reduction in CBF was observed in striatum. CBF of average of striatum also did not show any significant differences at any time point.

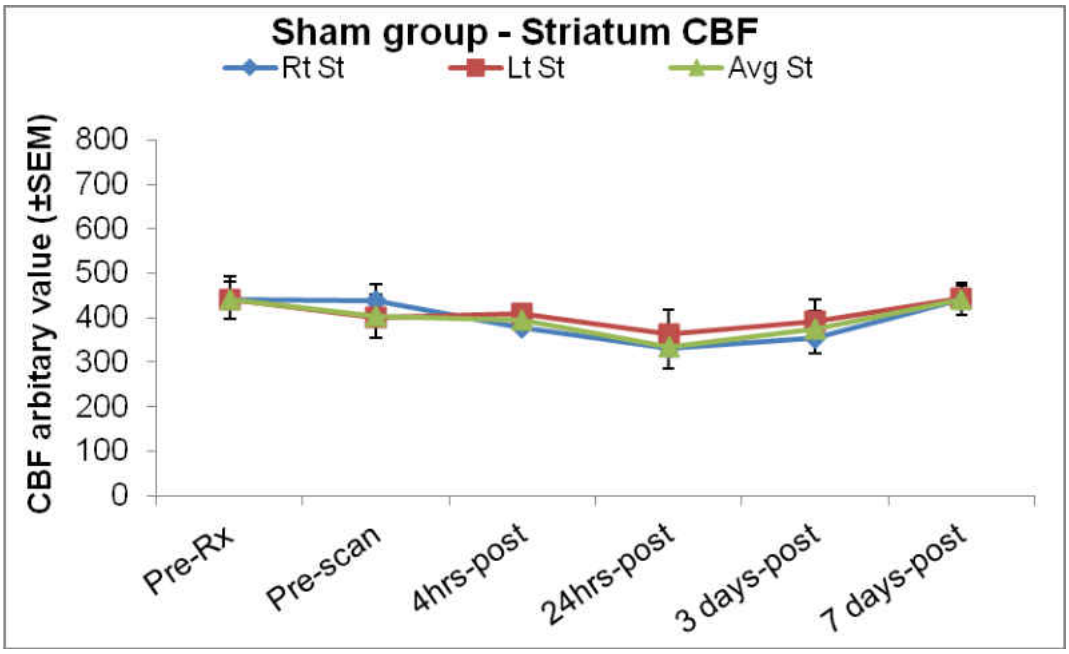


Figure 4-11: Plot showing CBF changes in the right striatum (Rt St), left striatum (Lt St) and average of right and left striatum (Avg St) of sham rats at various time points (pre-Rx=pre-water treatment, pre-scan=1 day before sham surgery, 4 hrs, 24 hrs, 3 days and 7 days post-sham surgery).

Hippocampus CBF changes in sham rats

In hippocampus (left, right and average) of sham group rats, no apparent changes in CBF were observed (Figure 4-12). Pre-sham surgery level of CBF was maintained till 7 days post-sham surgery with no significant changes between the time points.

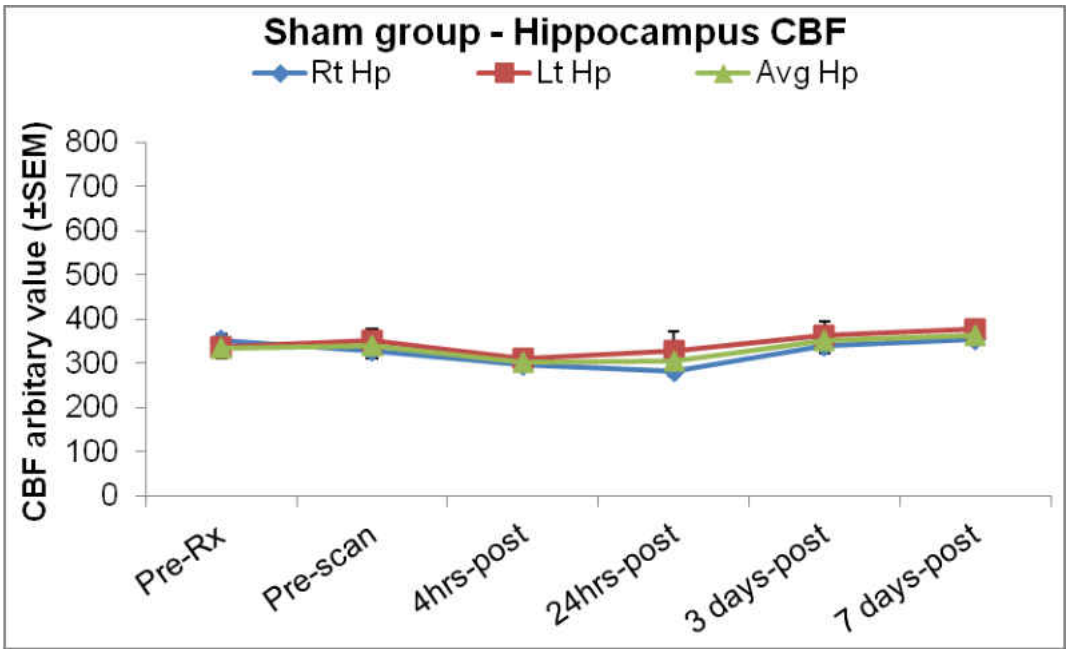


Figure 4-12: Plot showing CBF changes in the right hippocampus (Rt Hp), left hippocampus (Lt Hp) and average of right and left hippocampus (Avg Hp) of sham rats at various time points (pre-Rx=pre-water treatment, pre-scan=1 day before sham surgery, 4 hrs, 24 hrs, 3 days and 7 days post-sham surgery).

Bilateral thalamus and brainstem CBF changes in sham rats

In thalamus of sham rats, there was a slight decrease in CBF at 4 hrs and 24 hrs post-surgery with recovery by 3 days post-surgery (Figure 4-13). Brainstem also showed a slight reduction in CBF at 4 hrs post-surgery, but recovered by 24 hrs post-surgery. However, no significant differences in CBF were found between time points for both thalamus and brainstem in sham group rats.

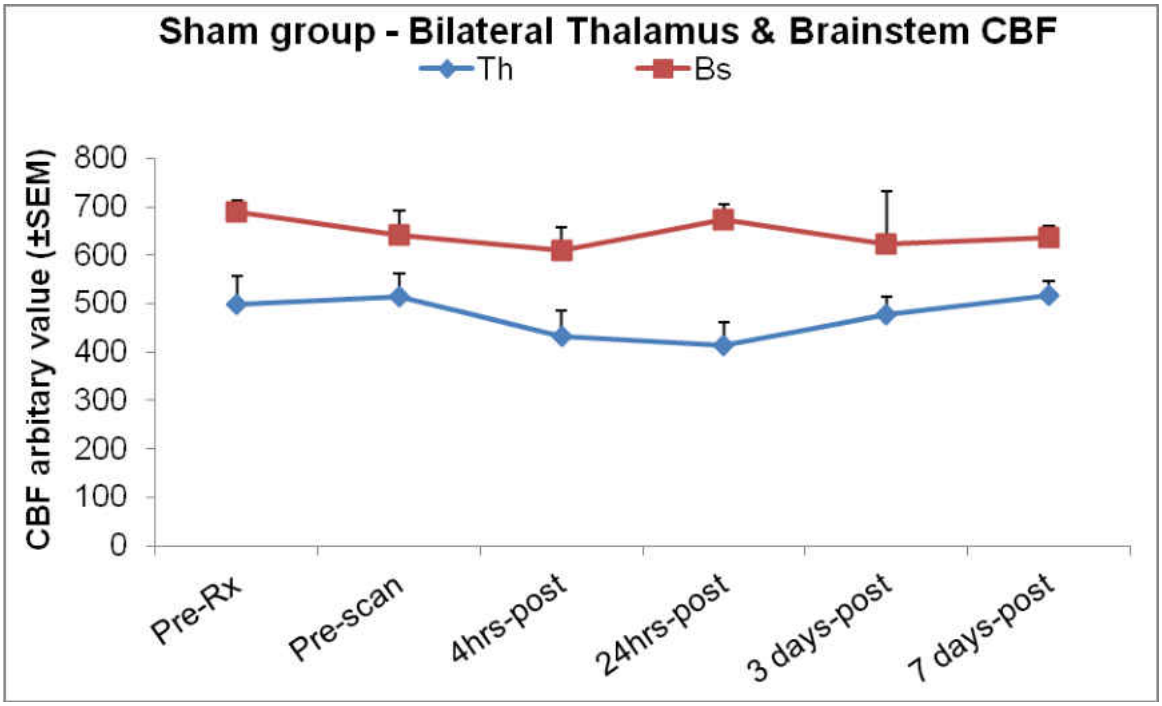


Figure 4-13: Plot showing CBF changes in the bilateral thalamus (Th) and brainstem (Bs) of sham at various time points (pre-Rx=pre-water treatment, pre-scan=1 day before sham surgery, 4 hrs, 24 hrs, 3 days and 7 days post-sham surgery).

Combined regional CBF and global CBF changes in sham rats

Both, the combined rCBF and global CBF levels of the sham group rats remained at the same levels as their respective pre-scan levels throughout the measured time points without any significant differences between any time points (Figure 4-14).

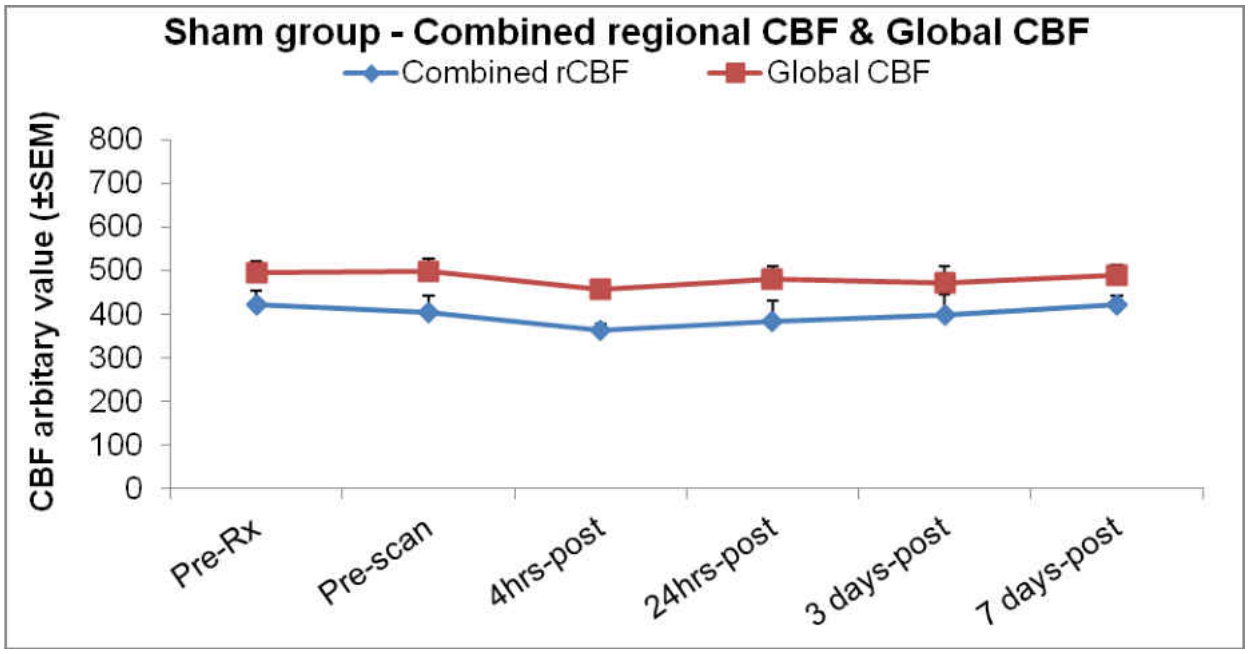


Figure 4-14: Plot showing changes in combined regional CBF (rCBF) and global CBF of sham rats at various time points (pre-Rx=pre-water treatment, pre-scan=1 day before sham surgery, 4 hrs, 24 hrs, 3 days and 7 days post-sham surgery).

4.4.3 CBF Changes in TBI-only rats

As mentioned previously, CBF changes in different brain regions (Rt PCx, Lt PCx, Rt St, Lt St, Rt Hp, Lt Hp, Th and Bs) following TBI were also analyzed at several time points: pre-scan (1 day before TBI), 4 hrs post-TBI, 24 hrs post-TBI, 3 days post-TBI and 7 days post-TBI.

Parietal cortex CBF changes in rats subjected to TBI-only

In rats subjected to TBI alone, CBF in Rt and Lt PCx was observed to show a trend of an insignificant reduction by 4 hrs post-TBI (p=0.210 and 0.133 respectively) with a period of recovery by 24hrs and 3 days post-TBI (Figure 4-15). However, by 7 days post-TBI, CBF in Rt and Lt PCx significantly decreased (p=0.014 and 0.013

respectively) compared to their pre-TBI levels. This decrease in CBF at 7 days post-TBI was also significantly different from that at 24hrs and 3 days post TBI, but not from 4 hrs post-TBI. Avg PCx (Rt and Lt PCx average) CBF also showed a similar trend with a significant reduction at 7 days post-TBI (p=0.011).

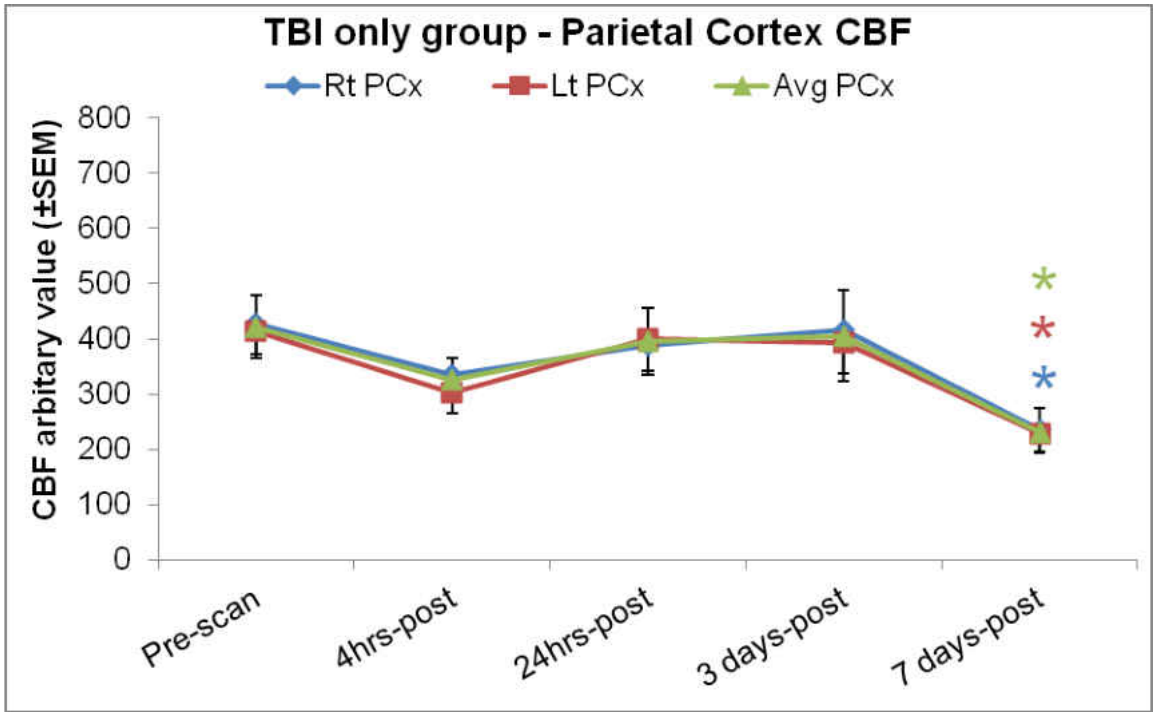


Figure 4-15: Plot showing CBF changes in the right parietal cortex (Rt PCx), left parietal cortex (Lt PCx) and average of right and left parietal cortex (Avg PCx) in rats subjected to TBI. CBF was measured at various time points: pre-scan=1 day before TBI, 4 hrs post-TBI, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

Striatum CBF changes in rats subjected to TBI-only

In TBI-only rats, CBF changes of both right and left striatum showed a trend similar to CBF changes of Avg St. CBF measurements at post-TBI time points 4 hrs, 24 hrs and 3 days were not significantly different from pre-TBI CBF level (Figure 4-16). However right, left and average striatum CBF measurements decreased at 7 days post-TBI and

were significantly different (p=0.001 for all respectively) from their pre-TBI levels as well as from the rest of the time points (4 hrs, 24 hrs and 3 days post-TBI) (p<0.05). No other significant differences were observed.

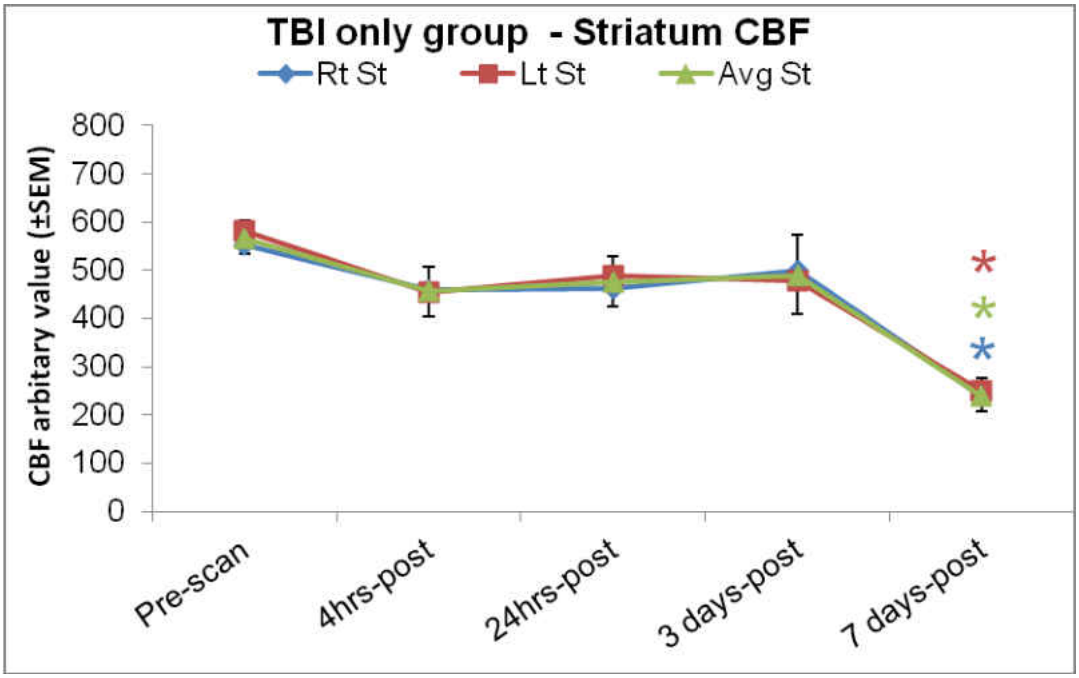


Figure 4-16: Plot showing CBF changes in the right striatum (Rt St), left striatum (Lt St) and average of right and left striatum (Avg St) in rats subjected to TBI. CBF was measured at various time points: pre-scan=1 day before TBI, 4 hrs post-TBI, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

Hippocampus CBF changes in rats subjected to TBI-only

In rats subjected to TBI, a significant reduction (p<0.05) in CBF of the hippocampus (left, right and average) was observed at 7 days post-TBI compared to their pre-TBI levels (Figure 4-17). Furthermore, CBF decreased at 7 days post-TBI in Rt Hp and Avg Hp was also significantly different (p<0.05) from their respective CBF values at 24 hrs and 3 days post-TBI.

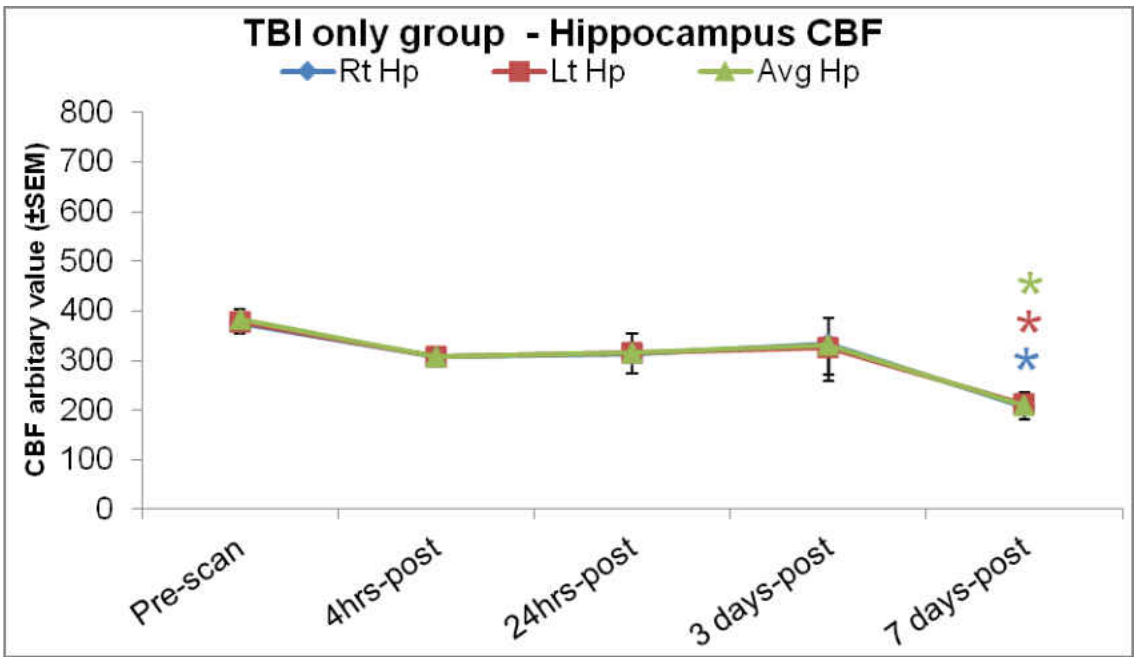


Figure 4-17: Plot showing CBF changes in the right hippocampus (Rt Hp), left hippocampus (Lt Hp) and average of right and left hippocampus (Avg Hp) in rats subjected to TBI. CBF was measured at various time points: pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

Bilateral thalamus and brainstem CBF changes in rats subjected to TBI-only

Thalamus CBF also showed a similar trend up to 3 days post-TBI, but at 7 days post-TBI a decrease in CBF was observed (Figure 4-18). However, no significant changes in CBF of thalamus and brainstem were observed at any time point. In rats subjected to TBI, brainstem showed a slight decrease in CBF at 4 hrs post-TBI, recovered at 24 hrs post-TBI, again showed a slight decrease at 3 days post-TBI and recovery at 7 days post-TBI.

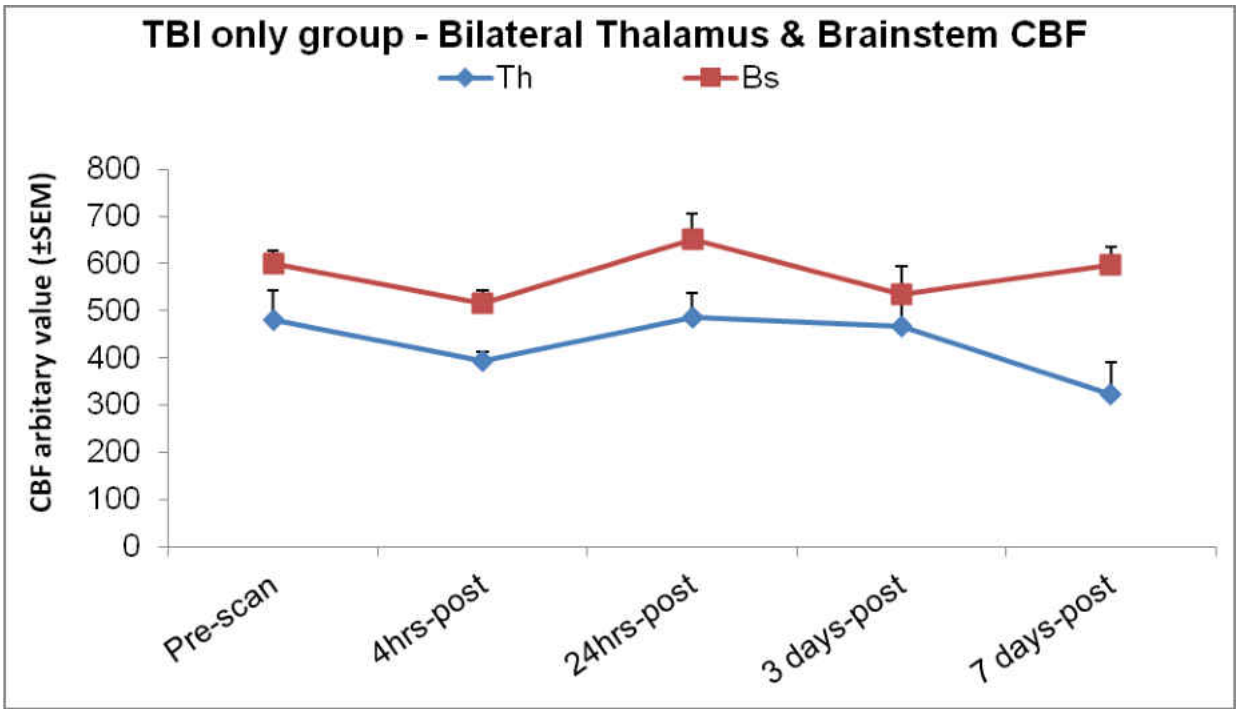


Figure 4-18: Plot showing CBF changes in the bilateral thalamus (Th) and brainstem (Bs) in rats subjected to TBI. CBF was measured at various time points: pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

Combined regional CBF and global CBF changes in rats subjected to TBI-only

In rats subjected to TBI, a significant reduction in combined rCBF and global CBF was observed at 7 days post-TBI compared to their pre-TBI levels (p<0.05), as well as to their 24 hrs post-TBI and 3 days post-TBI levels (Figure 4-19). Although a slight reduction in combined rCBF and global CBF was observed at 4 hrs post-TBI, it was not found to be significant. Both combined rCBF and global CBF changes showed a similar trend across all time points.

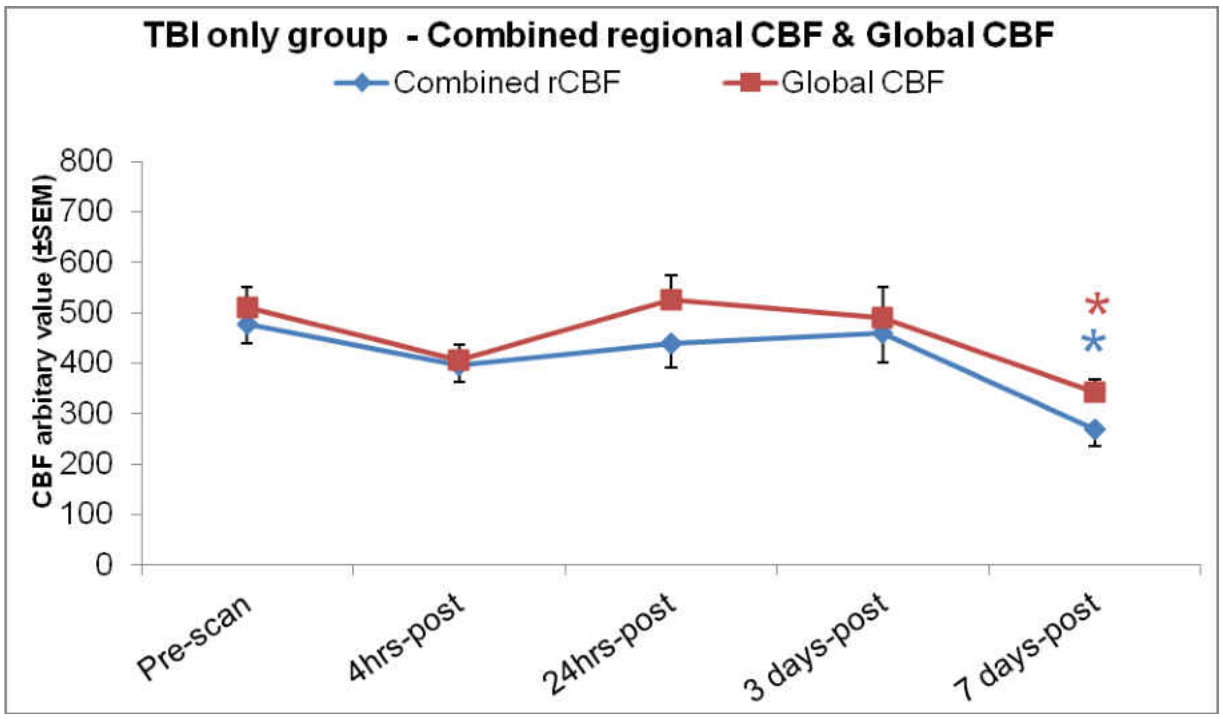


Figure 4-19: Plot showing changes in combined regional CBF (rCBF) and global CBF in rats subjected to TBI. CBF was measured at various time points: pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

4.5 Discussion (Specific Aim 1)

4.5.1 Surface righting (SR) Duration: Sham group Vs TBI-only group

Loss of consciousness for a brief duration is commonly observed immediately following TBI. This is often indirectly measured by monitoring SR duration in rodent experimental studies (Adams, 1986; Li et al., 2011). In our study, TBI rats demonstrated significantly prolonged SR duration compared to rats that underwent sham surgery. Although rats in both sham and TBI groups were exposed to a similar anesthesia levels, the prolonged SR duration in TBI group can be attributed to TBI insult. This supports monitoring SR duration as an indirect indicator of loss of consciousness, a common phenomenon observed following TBI.

4.5.2 CBF Changes in Sham rats

No significant changes in regional and global CBF following sham surgery

In this study, CBF changes were measured in different brain regions: right parietal cortex (Rt PCx), left parietal cortex (Lt PCx), average of left and right parietal cortex (Avg PCx), right striatum (Rt St), left striatum (Lt St), average of left and right striatum (Avg St), right hippocampus (Rt Hp), left hippocampus (Lt Hp), average of left and right hippocampus (Avg Hp), bilateral thalamus (Th) and brainstem (Bs). No significant changes in regional and global CBF were observed before and 21 days after pure-water treatment. This indicates the reproducibility of the CBF measurements made using pulsed ASL imaging. The CBF in all these regions followed a trend of a slight insignificant indication of reduction at 4 and 24 hrs post-sham surgery followed by a trend of recovery at 3 days post-sham surgery with no significant differences in any of these regions. Furthermore, both combined regional and global CBF were measured to evaluate how accurately global CBF changes reflect regional CBF changes. Both combined rCBF and global CBF also showed an insignificant reduction at 4 hrs post-sham surgery and recovered at 24 hrs post-sham surgery. From these it is clear that there were no significant changes in regional and global CBF following sham surgery. Furthermore it also reveals that rCBF changes can be a reflection of global CBF changes.

4.5.3 CBF Changes in TBI rats

CBF changes following a closed head injury in an experimental model of TBI is poorly studied. The few available studies have demonstrated CBF changes in cortical regions only for up to 4 hrs and 8 hrs post-TBI using laser-Doppler flowmetry (Ito et al., 1996;

Petrov and Rafols, 2001; Prat et al., 1997). Using a 450 g impactor dropped from 2m, Ito et al. observed a severe elevation in ICP resulting in reduced CBF (< 30% of control value) in cerebral cortex 2 hours post trauma (Ito et al., 1996). They have studied CBF changes only in cerebral cortex until 4 hrs post-TBI. Prat et al., demonstrated no change in frontal lobe CBF in the first 3 hours after trauma (1 m, 450 g), but was found to decrease from 4-8 hrs following TBI. They proposed that this decrease in CBF or increase in ICP 4-8 hrs following TBI is associated with loss of cerebral autoregulation (Prat et al., 1997). However, weight drop from 1 m height does not induce as severe injury as weight drop from 2 m height. Moreover, Prat et al. have studied CBF changes in only one region and only up to 8 hrs following TBI. Petrov et al., attempted to study the effect of closed head injury on microcirculation in a cortical region following trauma (2m, 400 g) and observed a decrease by 2-3 hrs after trauma, followed by trend towards normalization by 4 hrs post-TBI (Petrov and Rafols, 2001). These studies have analyzed CBF changes only up to 4 hours (Ito et al., 1996; Petrov and Rafols, 2001) and 8 hours (Prat et al., 1997) following TBI. They have measured the CBF using laser-Doppler flowmetry, which can make only one measurement for each hemisphere. Our study demonstrated that CBF in the cortex and subcortical structures such as St and Hp showed an acute reduction of up to 19%, albeit insignificant, at 4 hrs post-TBI and recovery by 24 hrs post-TBI, as reported in these studies.

Another study using MRI based perfusion imaging (ASL) measured CBF changes up to 2 days post-TBI (2 m, 450 g) in two different regions (hippocampus and parietal cortex) (Shen et al., 2007). The authors reported reduction in hippocampal CBF at 4 hrs (8-14%), 24 hrs (8%) and 48 hrs post-TBI (18%) compared to their pre-TBI levels. Our

study also demonstrated that CBF in the hippocampus reduced by 4 hrs (19%) and 24 hrs post-TBI (17%). Furthermore, they reported reduction if the CBF of parietal cortex using ASL data from one slice at 4 hrs (~26%), 24 hrs (23%) and 48 hrs post-TBI (17%) compared to their pre-TBI levels. But, the authors of the study did not analyze the CBF data statistically. Our study using ASL data from two slices, also demonstrated reduction in the CBF of parietal cortex at 4 hrs post-TBI (23%), but showed a temporal pattern of recovery at 24 hrs (only 6% reduction) and 3 days post-TBI (4% reduction) compared to their pre-TBI levels. But, Shen et al, measured CBF in only limited structures for only up to 48 hrs after trauma. However, in the current study CBF measurement encompassed diverse cortical, subcortical and brainstem regions extending up to 7 days post TBI using data from multiple ASL slices. To the best of our knowledge, no other experimental study has extensively measured both regional and global CBF changes up to 7 days following a closed head injury induced using the Marmarou weight drop model.

TBI induces region specific CBF changes

Our study demonstrated a decrease in both regional and global CBF following closed head injury. In rat brains subjected to TBI, CBF in the cortical and subcortical regions such as PCx, St, Hp and Th was observed to be more affected compared to the brainstem region. This variation in rCBF changes between cortical and brainstem regions may be related to the location of the impact. CBF of Avg PCx, St and Hp showed an insignificant reduction (~19%) at 4 hrs post-TBI and then recovered by 24 hrs post-TBI. But a significant reduction in cortical and subcortical regions CBF was

observed on 7 days post-TBI (~49%) compared to their pre-TBI levels, as well as to their 24 hrs post-TBI and 3 days post-TBI levels. But thalamus, as well as brainstem did not show any significant changes in CBF following TBI. Therefore cortical region CBF was found to be more effected following TBI induced by an impact acceleration injury model compared to brainstem. Although CBF in the brainstem, when analyzed separately, did not show significant changes until 7 days post-TBI, combined rCBF and global CBF changes which included brainstem showed a significant reduction by 7 days post-TBI.

The combined regional CBF (rCBF) also showed an insignificant reduction of about 16% compared to baseline at 4 hrs post-TBI and a significant reduction of ~44% compared to baseline at 7 days post-TBI. Global CBF also showed an insignificant reduction of about 20% compared to baseline at 4 hrs post-TBI and a significant reduction ~32% at 7 days post-TBI. In general, it clearly indicates reduction in both regional and global CBF at 4 hrs (~19%) and 7 days (~39%) after closed head injury. It was previously reported that reduction in CBF following TBI in the first few hours after injury may reach ischemic levels. In humans, ischemic (CBF 15 -20 ml/100g/min) cell death has been observed in 30% of all TBI cases within the first 6 hrs after injury (Bouma and Muizelaar, 1995) and was found to cause early death (Schroder et al., 1996). This decreased CBF may even trigger secondary injury events. Also, cerebral ischemia have been observed in around 90% of the patients who die following TBI (Greve and Zink, 2009). However, the occurrence of ischemic cell death at 7 days post-TBI is still not established. To the best of our knowledge, no other experimental studies have reported rCBF (Rt PCx, Lt PCx, Rt St, Lt St, Rt Hp, Lt Hp, Th and Bs) and global

CBF changes up to 7 days post-TBI following closed head injury. Ischemic cell death occurrence after 7 days post-TBI still needs further investigation. Our results show that CBF changes can take place even 7 days after severe TBI. Furthermore, this dynamic profile of CBF changes following TBI in different regions of the brain showed the existence of some regional differences in brain response to trauma, revealing the spatial and temporal dynamics of cerebral perfusion following TBI.

Although the reduction in regional or global CBF at 4 hrs post-TBI was not significant, it may still play a role in inducing injury changes. This acute drop in CBF following TBI may be one of the reasons for secondary (delayed) brain damage following TBI. Some studies even suggested prevention and early treatment to maintain CBF may lead to better prognosis and functional recovery following TBI (Pace et al., 2006; Ribeiro and Sebastiao, 2010). Impaired cerebral autoregulation may be one of the reasons for the abnormal CBF changes following TBI and it was suggested to be associated with various cerebrovascular abnormalities following head injury (Sviri and Newell, 2010). It was further suggested that impaired cerebral autoregulation might increase the vulnerability to cerebral ischemic insult (Sviri and Newell, 2010). However, it is still not clear whether posttraumatic reduction in cerebral perfusion is due to intracranial hypertension or it was due to impaired autoregulation mediated by the pathological changes in cerebral vasculature. As the volume within the intact skull is fixed, post-traumatic inflammation leading to vasoconstriction (decreased CBF) may be one of the reasons for sub-acute (7 days post-TBI) reduction in CBF (Lunt et al., 2004). Therefore it is important to monitor, understand and maintain CBF for the long term (at least for one week after head injury) in TBI patients to prevent ischemic cell death or any other

secondary pathological mechanisms that may be triggered by reduced CBF. Potential therapeutic agents which can offer neuroprotection by making favorable CBF changes need to be developed.

CHAPTER 5 TO DETERMINE THE EFFECTS OF CHRONIC AND ACUTE CAFFEINE ON CEREBRAL BLOOD FLOW CHANGES FOLLOWING TBI IN A RAT MODEL USING ASL-MR IMAGING (SPECIFIC AIM 2)

5.1 Introduction

CBF regulation mediated by adenosine receptor mechanisms is often impaired following TBI. We observed that both regional and global CBF were found to be affected following TBI induced by an impact acceleration device. Considering the potential implications of a reduced CBF, there is a need to identify potential therapeutic agent(s) that can restore or maintain CBF. This, motivated us to study the role of chronic and acute caffeine in offering such benefit (restoration or maintenance of CBF) following TBI. Caffeine utilizes adenosine receptors to exert its effects on CBF and thus specific aim 2 of this study attempts to analyze relative changes in regional and global CBF following TBI in rats pretreated with chronic and acute caffeine.

5.2 Methods

5.2.1 Animal Handling

All surgical and animal handling procedures were approved by the Institutional Animal Care and Use Committee (IACUC), Wayne State University, Detroit. A total of 17 adult male Sprague Daley rats (Harlan, Indianapolis, IN) weighing 350-400 g were utilized as a part of specific aim 2. Rats were randomly assigned to two pretreatment groups: chronic caffeine+TBI (CC+TBI) (n=9) and acute caffeine+TBI (AC+TBI) (n=8). All the rats had free access to food.

5.2.2 Caffeine Treatment

For chronic caffeine pretreatment, rats were given drinking water mixed with caffeine (caffeinated water) (1.5 g/L, caffeine cat# C0750, Sigma Aldrich, St. Louis, MO) for three weeks before TBI and acute caffeine treatment rats were given a single dose of caffeine (150 mg/kg) 30 mins before TBI. During caffeine pretreatment, rats were housed individually with their own water bottle for 3 weeks after which they were subjected to TBI. Following TBI, they were given regular water with no caffeine. AC+TBI group rats had free access to regular drinking water throughout. Caffeinated water was prepared by adding caffeine to purified drinking water, and mixed for 4 mins using magnetic stir bar to avoid formation of lumps and ensuring that all caffeine dissolved completely. Caffeinated water was changed three times a week to maintain an appropriate caffeine concentration. The amount of caffeinated water consumed by each rat was monitored by measuring the caffeinated water left in the bottle before giving known volume of fresh caffeinated water, when changing the caffeinated water. Table 11, briefly demonstrates the step wise procedures performed on rats from both the groups.

Table 5-1: Demonstration of the step-wise procedures performed in rats from chronic caffeine+TBI and acute caffeine+TBI groups.

Step Wise Procedure	Chronic Caffeine+TBI	Acute Caffeine+TBI
21 days before TBI induction	MRI	NA
Caffeinated water (1.5 g/L) treatment for 3 weeks (no treatment after TBI)	✔	NA
1 day before TBI	MRI	MRI

Caffeine (150 mg/kg) i.p. injection, 30 mins before TBI	NA	✔
On the day of TBI induction	TBI impact (2 m height, 450 g weight)	TBI impact (2 m height, 450 g weight)
4 hrs post-TBI	MRI	MRI
24 hrs post-TBI	MRI	MRI
3 days post-TBI	MRI	MRI
7 days post-TBI	MRI	MRI
CSF, serum samples and brain harvest on 7th day	✔	✔

NA = not applicable and ✔ = Performed as described in left most column.

5.2.3 Surgical Procedures and TBI Induction

All surgical and animal handling procedures followed for these two groups (CC+TBI and AC+TBI) were similar to surgical procedures followed for the TBI-only group as discussed in Chapter 4 (section 4.2.1 and 4.2.2). TBI was induced by the Marmarou impact acceleration injury device as described before for TBI-only group in Chapter 4 (section 4.1.1). The only difference between TBI-only group and these groups (CC+TBI and AC+TBI) is: chronic caffeine treated rats underwent 3 weeks of caffeine pretreatment before TBI induction and acute caffeine rats were pretreated with a single dose of caffeine 30 mins before TBI.

5.2.4 Surface Righting Reflex

SR duration for all the rats from CC+TBI and AC+TBI groups was recorded immediately after TBI induction in the manner described previously in Chapter 4 (section 4.2.3).

5.2.5 ASL Image Acquisition

CBF changes were measured using arterial spin labeling (ASL) perfusion imaging with same image acquisition parameters as described in Chapter 4 (section 4.2.5). For chronic caffeine treated rats, ASL images were acquired at time points: pre-Rx (pre-caffeine treatment, 21 days before TBI), pre-scan (1 day before TBI), 4 hrs, 24 hrs, 3 days and 7 days post-TBI. For acute caffeine treated rats, ASL images were acquired at time points: pre-scan (1 day before TBI), 4 hrs, 24 hrs, 3 days and 7 days post-TBI.

5.2.6 ASL Data Analysis

For specific aim 2, ASL data was analyzed from 13 rats (CC+TBI group = 6 and AC+TBI group = 7) by drawing regions of interest (ROIs) using SPIN software as explained in Chapter 4 (section 4.2.6). CBF changes were measured in different brain regions: right parietal cortex (Rt PCx), left parietal cortex (Lt PCx), average of left and right parietal cortex (Avg PCx), right striatum (Rt St), left striatum (Lt St), average of left and right striatum (Avg St), right hippocampus (Rt Hp), left hippocampus (Lt Hp), average of left and right hippocampus (Avg Hp), bilateral thalamus (Th) and brainstem (Bs) (Figures). Also, combined regional CBF (rCBF) and global CBF were measured in a similar method as described in Chapter 4 (4.2.6).

5.3 Statistical Analysis

All data were analyzed for statistical significance using SPSS (version 20, Chicago, IL). The CBF value obtained from ASL images was expressed as the mean value ± standard error of the mean (SEM). The data were first tested for reliability by

Cronbach's alpha test (Table 10). Group-wise differences were analyzed by repeated measures of analysis of variance (ANOVA) (Chapter 6). Data was further analyzed for within group differences by one-way ANOVA using Least Significant Difference (LSD) post-hoc test. A p value of <0.05 was considered as significant.

5.4 Results (CC+TBI and AC+TBI groups)

Following chronic caffeine pretreatment (1.5 g/L) for 21 days or after acute caffeine pretreatment 30 mins before TBI, rats did not exhibit any abnormal behavior. However following TBI, one acute caffeine pretreated rat that exhibited severe distress was sacrificed 2 hrs post-trauma.

5.4.1 Surface righting of chronic and acute caffeine pretreated rats in comparison to sham and TBI-only rats

Rats in TBI group showed a significantly prolonged SR duration compared to sham rats. Compared to SR duration of 162±25 sec in sham rats, SR duration in TBI rats was significantly higher (p=0.004) with an average duration of 355±65 sec (Figure 5-1). Average SR duration in chronic caffeine pretreated rats was 269±24 sec compared to a surface righting duration of 309±36 sec in acute caffeine pretreated rats. Acute caffeine pretreated rats showed prolonged surface righting duration compared to chronic caffeine pretreated rats, but not at a level of significance. However, acute caffeine pretreated rats took significantly increased (p=0.030) SR duration (309±36 sec) compared to sham rats. No other significant differences were observed.

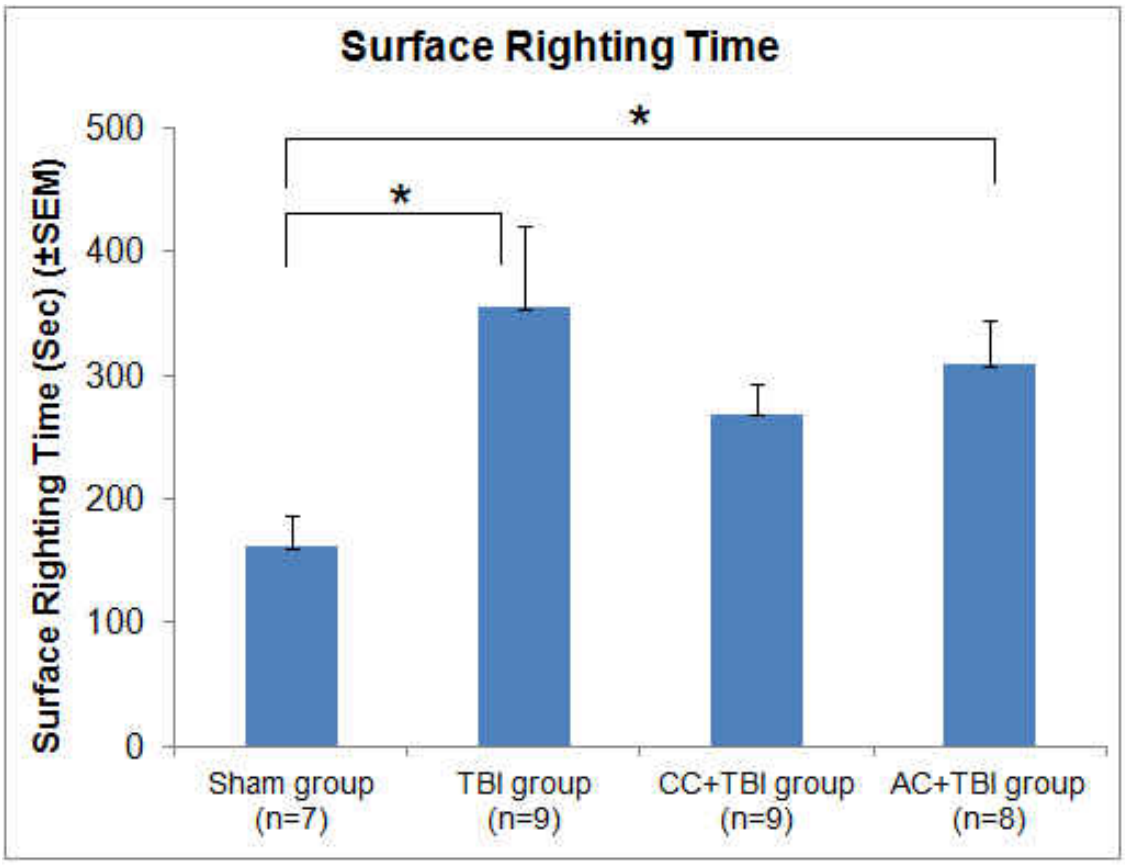


Figure 5-1: Plot showing mean SR duration of rats in different groups: sham, TBI-only, CC+TBI and AC+TBI groups. * indicates significant difference (p<0.05).

5.4.2 CBF Changes following TBI in chronic caffeine pretreated rats

CBF changes in CC+TBI rats were measured at several time points: pre-Rx (pre-caffeine treatment, 21 days before TBI), pre-scan (1 day before TBI), 4 hrs, 24 hrs, 3 days and 7 days post-TBI.

Parietal cortex CBF changes following TBI in chronic caffeine+TBI group rats

CBF in the parietal cortex (PCx) showed an insignificant increase at pre-scan (1 day before TBI), compared to pre-Rx (pre-caffeine treatment) levels (Figure 5-2). A slight drop in PCx CBF was observed at 4 hrs post-TBI with a trend of recovery at 24 hrs post-TBI which again showed a trend of reduction by 3 days post-TBI. All these alterations

were not statistically significant compared to their pre-Rx or pre-TBI levels. By 7 days post-TBI, the CBF levels reached pre-scan levels. However, no significant differences were found in right, left and Avg PCx between any time points.

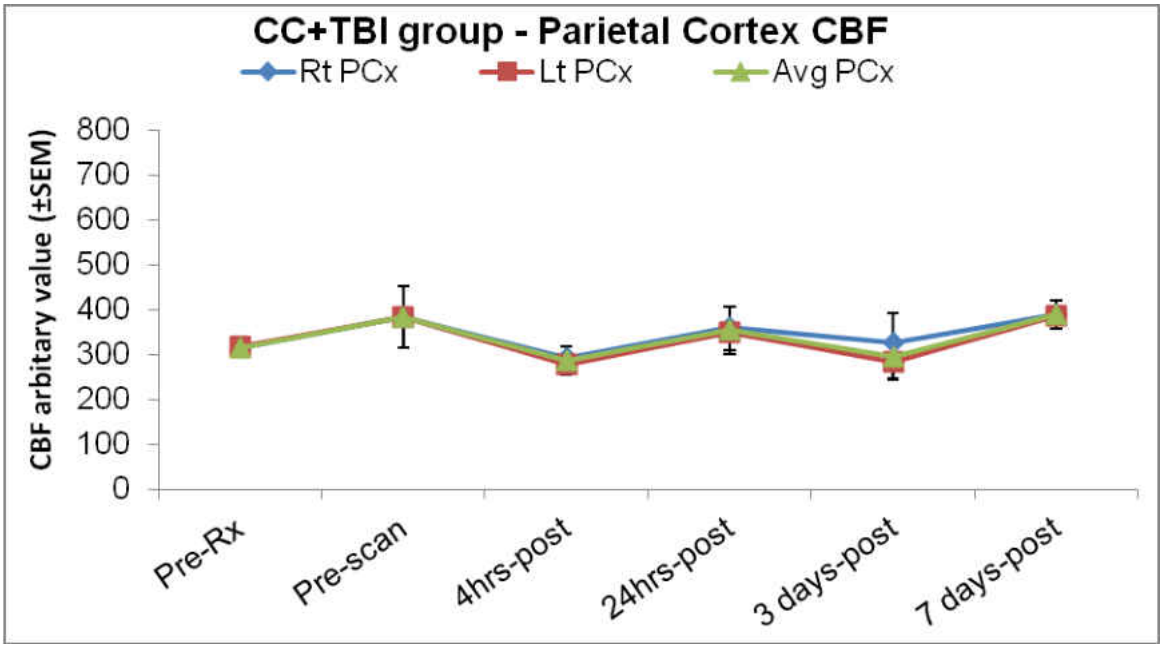


Figure 5-2: Plot showing CBF changes in the right parietal cortex (Rt PCx), left parietal cortex (Lt PCx) and average of right and left parietal cortex (Avg PCx) following TBI in chronic caffeine pretreated rats at various time points: pre-Rx=pre-caffeine treatment 21 days before TBI, pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI.

Striatum CBF changes following TBI in chronic caffeine+TBI group rats

Similar to PCx, striatum also showed an insignificant increase in CBF at pre-scan compared to their pre-caffeine treatment levels. CBF of right, left and Avg St continued to increase at 4 hrs and 24 hrs post-TBI albeit without any significance (Figure 5-3). But, by 3 days post-TBI, CBF in Lt St was significantly decreased compared to 24 hrs post-TBI level (p=0.026); and by 7 days post-TBI, the CBF significantly recovered compared to its 3 days post-TBI level (p=0.040). Avg St also showed a trend similar to Lt St. No

significant CBF changes were observed in right striatum. Left, right and Avg St did not show any significant changes in CBF compared to pre-Rx or pre-scan levels.

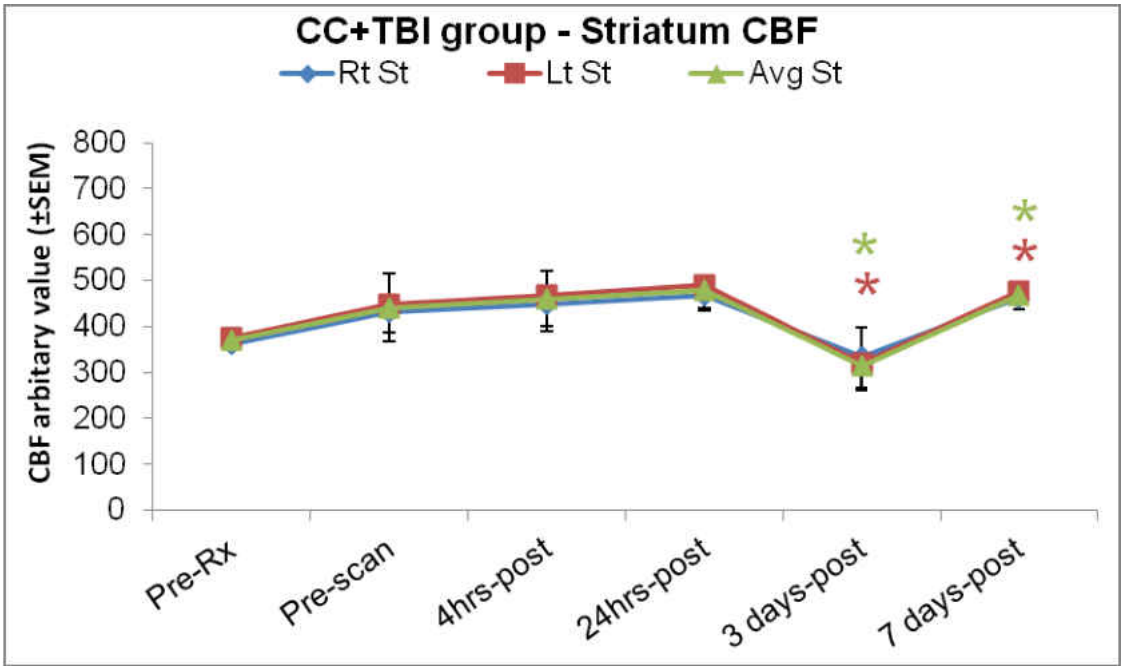


Figure 5-3: Plot showing CBF changes in the right striatum (Rt St), left striatum (Lt St) and average of right and left striatum (Avg St) following TBI in chronic caffeine pretreated rats at various time points: pre-Rx=pre-caffeine treatment 21 days before TBI, pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

Hippocampus CBF changes following TBI in chronic caffeine+TBI group rats

Rt Hp, Lt Hp and Avg Hp showed a similar trend of decreased CBF at 3 days post-TBI, compared to their pre-scan levels; but only Rt Hp and Avg Hp showed significant differences (p=0.017 and 0.036, respectively) compared to their pre-scan levels (Figure 5-4). At 7 days post-TBI, Rt Hp and Avg Hp have showed significant increases (p=0.022 and 0.048, respectively) in CBF compared to their 3 days post-TBI levels. No significant differences were observed in Lt Hp.

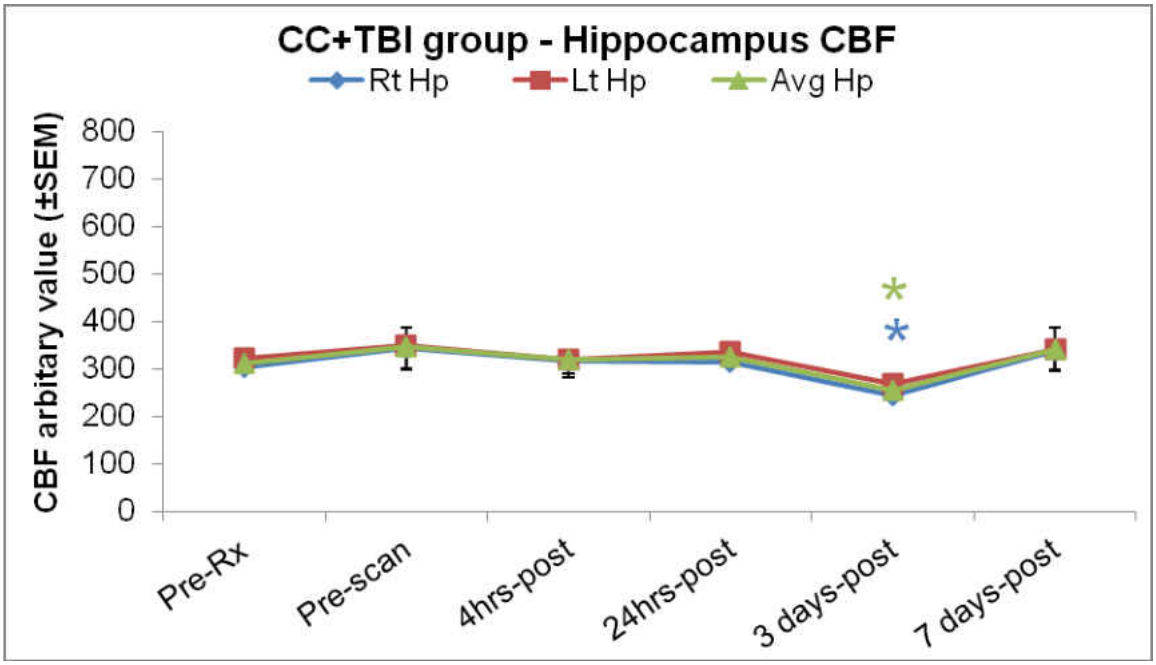


Figure 5-4: Plot showing CBF changes in the right hippocampus (Rt Hp), left hippocampus (Lt Hp) and average of right and left hippocampus (Avg Hp) following TBI in chronic caffeine pretreated rats at various time points: pre-Rx=pre-caffeine treatment 21 days before TBI, pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

Bilateral thalamus and brainstem CBF changes following TBI in chronic caffeine+TBI group rats

CBF of both bilateral thalamus and brainstem at pre-scan (21 days after chronic caffeine treatment) remained the same as their pre-caffeine treatment levels (Figure 5-5). Brainstem showed a slight reduction in CBF at 24 hrs post-TBI and a temporal pattern of recovery at 3 days and 7 days post-TBI. However, these were not found to be significantly different (p<0.05). Similar to sham group rats or TBI-only group rats, both bilateral thalamus and brainstem of chronic caffeine pretreated did not demonstrate any apparent changes in CBF between any time points.

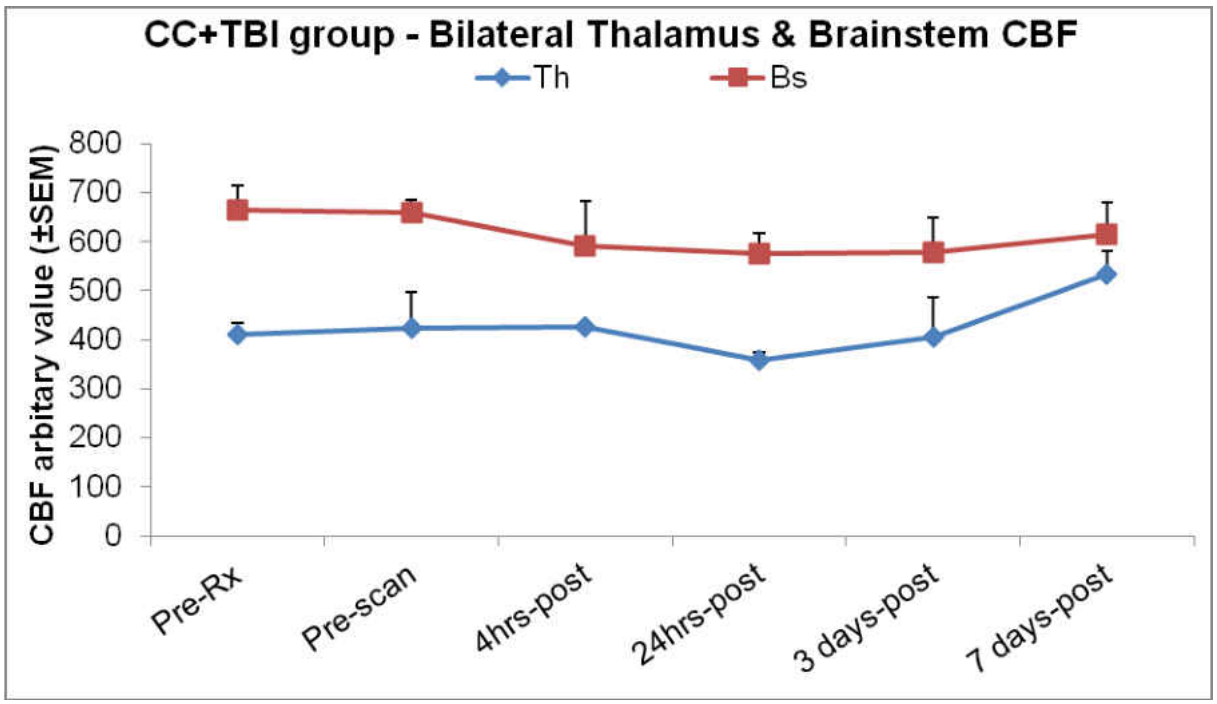


Figure 5-5: Plot showing CBF changes in the bilateral thalamus (Th) and brainstem (Bs) following TBI in chronic caffeine pretreated rats at various time points: pre-Rx=pre-caffeine treatment 21 days before TBI, pre-scan=1 day before TBI, pre-scan (1 day before TBI), 4 hrs, 24 hrs, 3 days and 7 days post-TBI.

Combined regional CBF and global CBF changes following TBI in chronic caffeine+TBI group rats

Both the combined regional CBF (rCBF) and the global CBF followed a similar trend. They did not show any significant differences in CBF compared to their pre-Rx or pre-scan levels (Figure 5-6). But at 3 days post-TBI, combined rCBF was significantly lower (p=0.017) compared to 7 day post-TBI levels, and global CBF showed almost significant reduction (p=0.062) compared to its 7 day post-TBI level. No other significant changes in combined rCBF and global CBF were observed in rats pretreated with chronic

caffeine.

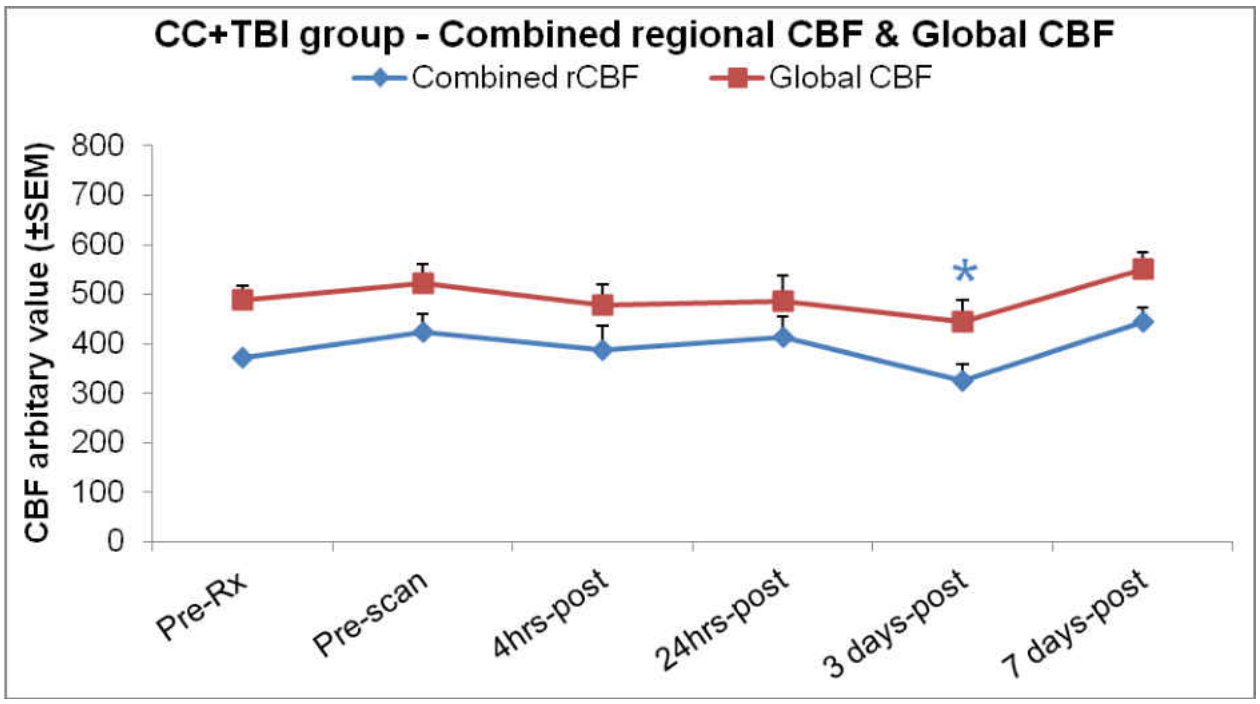


Figure 5-6: Plot showing changes in combined regional CBF (rCBF) and global CBF in chronic caffeine pretreated rats at various time points: pre-Rx=pre-caffeine treatment 21 days before TBI, pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05) in combined rCBF at 3 days post-TBI compared to 7 days post-TBI.

5.4.3 CBF Changes following TBI in acute caffeine pretreated rats

CBF changes following TBI in acute caffeine pretreated rats **(**AC+TBI rats) were measured at several time points: 1 day before TBI (pre-scan), 4 hrs, 24 hrs, 3 days and 7 days post-TBI.

Parietal cortex CBF changes following TBI in acute caffeine+TBI group rats

Lt, Rt and Avg PCx of acute caffeine pretreated rats following TBI showed an insignificant reduction in CBF by 4 hrs post-TBI compared to their pre-scan levels (Figure 5-7). However, a significant reduction (p=0.050) in CBF was observed at 4 hrs post-TBI in Rt PCx (p=0.050) compared to its 7 day post-TBI levels. Avg PCx showed nearly significant (p=0.066) reduction in CBF at 4 hrs post-TBI compared to its 7 day post-TBI level. After 4 hrs post-TBI, CBF in the PCx was found to show a temporal pattern of recovery with an insignificant increase by 7 days post-TBI compared to their pre-TBI levels. No significant differences in CBF were observed in Lt PCx and Avg PCx following TBI between any time points.

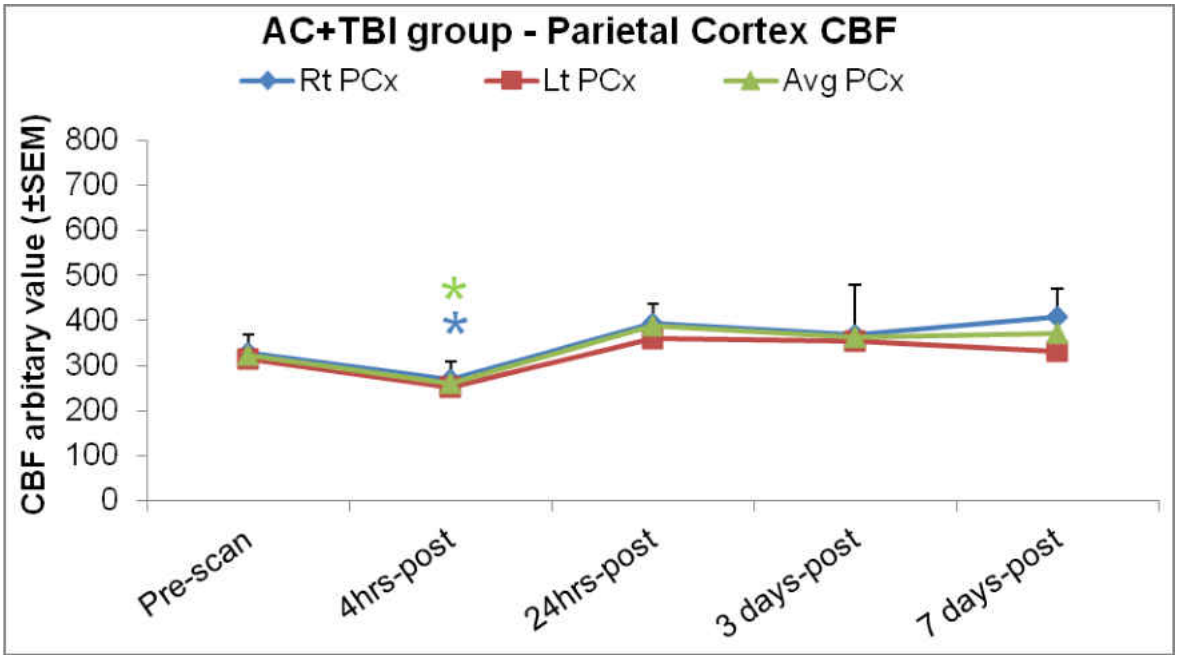


Figure 5-7: Plot showing CBF changes in the right parietal cortex (Rt PCx), left parietal cortex (Lt PCx) and average of right and left parietal cortex (Avg PCx) following TBI in acute caffeine pretreated rats at various time points: pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

Striatum CBF changes following TBI in acute caffeine+TBI group rats

CBF changes following TBI in striatum of the acute caffeine pretreated rats also showed a trend similar to that in parietal cortex. In acute caffeine pretreated rats following TBI, CBF in the Lt, Rt and Avg St showed an insignificant reduction by 4 hrs post-TBI compared to its pre-scan levels (Figure 5-8). But, a significant reduction in CBF was observed at 4 hrs post-TBI in Rt St and Avg St (p=0.033 and 0.039 respectively) compared to their 7 day post-TBI levels. No significant differences were observed in Lt St CBF following TBI between any time points. After 4 hrs post-TBI, a temporal pattern of recovery in CBF was observed at 24 hrs, 3 days and 7 days post-TBI.

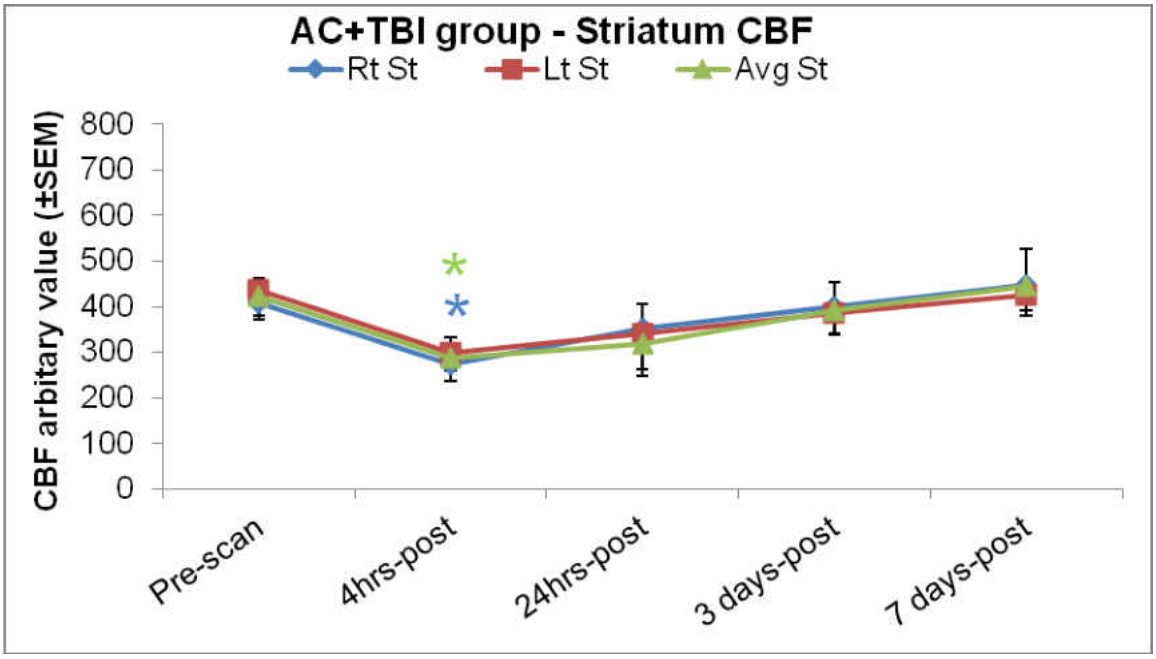


Figure 5-8: Plot showing CBF changes in the right striatum (Rt St), left striatum (Lt St) and average of right and left striatum (Avg St) following TBI in acute caffeine pretreated rats at various time points: pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

Hippocampus CBF changes following TBI in acute caffeine+TBI group rats

In acute caffeine pretreated rats subjected to TBI, significant reduction (p=0.042) in CBF of the left hippocampus was observed at 4 hrs post-TBI compared to its pre-scan (pre-TBI) level (Figure 5-9). Furthermore, Lt Hp CBF at 4 hrs post-TBI showed a significant reduction compared to its 3 days (p=0.012) and 7 days post-TBI (p=0.019). Also, CBF of the Avg Hp showed a significant decrease by 4 hrs post-TBI compared to its 3 days (p=0.027) and 7 days post-TBI (p=0.037). CBF in the Rt, Lt and Avg Hp of acute caffeine pretreated rats showed a complete recovery by 3 days and 7 days post-TBI. In Rt Hp, no significant differences in CBF following were observed between any time points.

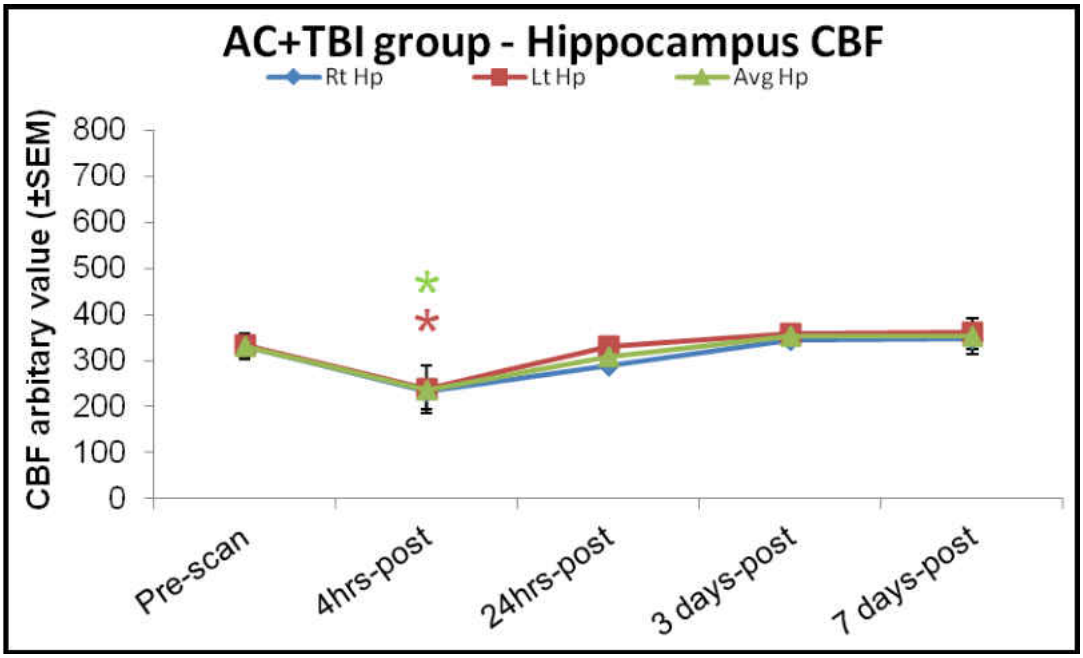


Figure 5-9: Plot showing CBF changes in the right hippocampus (Rt Hp), left hippocampus (Lt Hp) and average of right and left hippocampus (Avg Hp) following TBI in acute caffeine pretreated rats at various time points: pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

Bilateral thalamus and brainstem CBF changes following TBI in acute caffeine+TBI group rats

Unlike sham, TBI-only and CC+TBI groups, significant differences (p<0.05) in CBF of the bilateral thalamus (Th) and brainstem (Bs) were observed (Figure 5-10). The trends in CBF of Th and Bs were similar to TBI-only rats. Although Th did not show any significant changes in CBF following TBI compared to its pre-scan (pre-TBI) level, CBF at 3 days post-TBI was significantly higher from its 4 hrs post-TBI level. CBF in the Bs was observed to be significantly increased at 7 days post-TBI compared to its pre-TBI (p=0.018), 4 hrs (p=0.001) and 3 days post-TBI (p=0.019) levels.

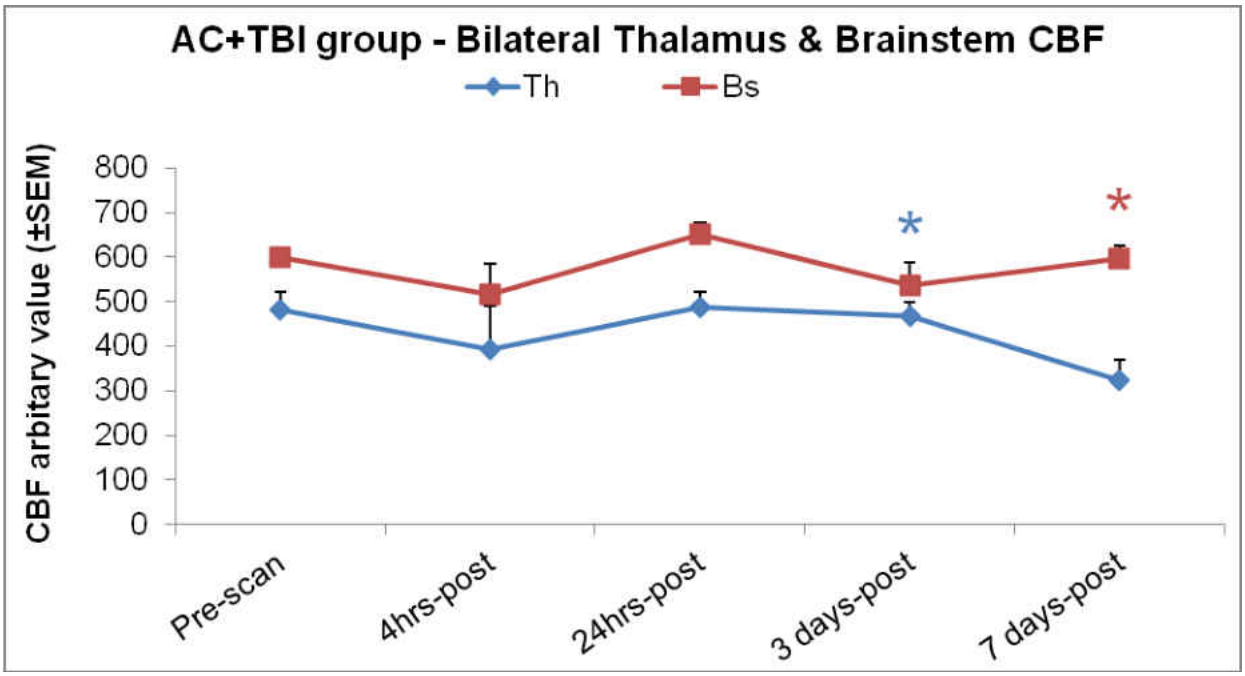


Figure 5-10: Plot showing CBF changes in the bilateral thalamus (Th) and brainstem (Bs) following TBI in acute caffeine pretreated rats at various time points: pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

Combined regional CBF and global CBF changes following TBI in AC+TBI group rats

Both the combined regional CBF (rCBF) and the global CBF followed a similar trend of reduction by 4 hrs post-TBI and temporal pattern of recovery at 24 hrs, 3 days and 7 days post-TBI (Figure 5-11). However, significant reduction (p=0.010) at 4hrs post-TBI was observed only in global CBF. Additionally, this reduction at 4 hrs post-TBI was also found to be significant compared to rest of the post-TBI time points (24 hrs, p= 0.020; 3 days, p= 0.014; and 7 days, p=0.031). Although the reduction in combined rCBF at 4 hrs post-TBI was not significant different from its pre-TBI levels, it was found to be significantly reduced compared to 3 days (p=0.033) and 7 days post-TBI (p=0.020).

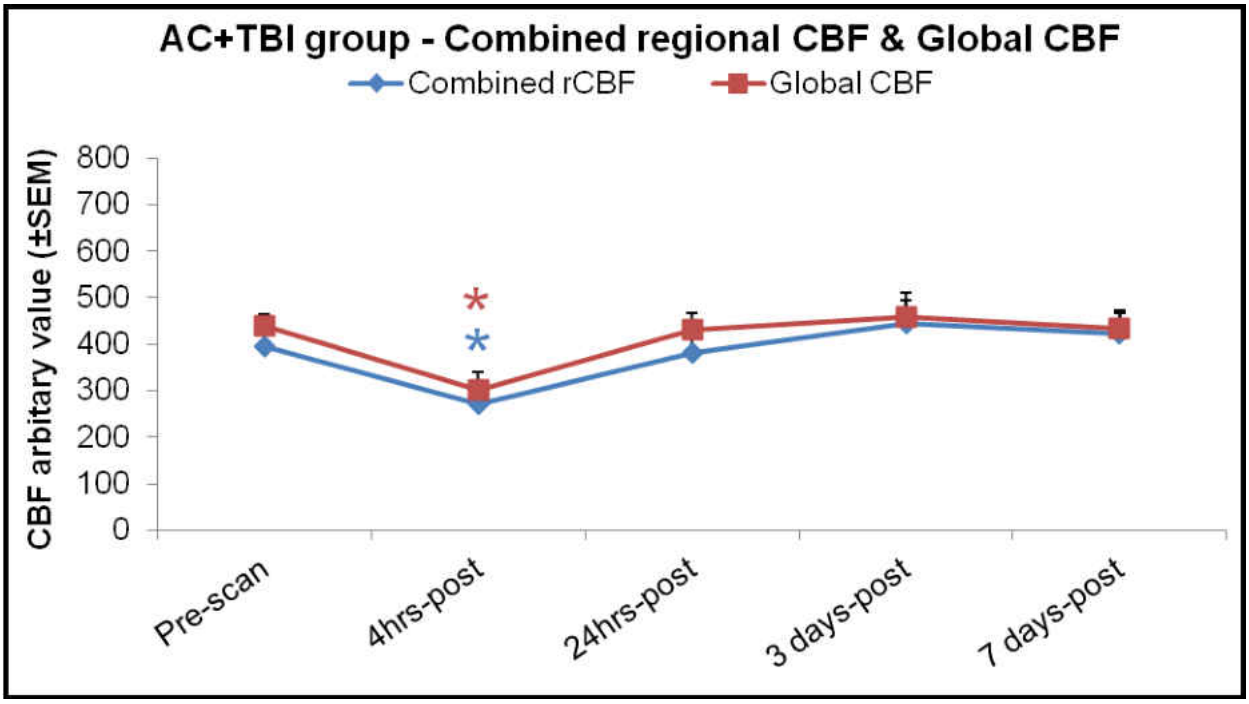


Figure 5-11: Plot showing changes in combined regional CBF (rCBF) and global CBF in acute caffeine pretreated rats at various time points: pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisks indicate significant difference (p<0.05).

5.5 Discussion (Specific Aim 2)

5.5.1 Surface righting (SR) duration: Chronic caffeine pretreated rats exhibited the least SR duration among the rats that underwent TBI

As mentioned before (section 4.2.3), loss of consciousness for a brief duration is commonly observed immediately following TBI, which is often indirectly measured by monitoring SR duration. Although rats in all four groups were exposed to a similar anesthesia levels during surgical procedures, the rats in three groups that underwent TBI (TBI-only, CC+TBI and AC+TBI) had prolonged SR duration compared to rats that underwent sham surgery; this can be attributed to TBI insult. However, only TBI and AC+TBI rats exhibited significantly higher ($p<0.05$) SR duration compared to sham rats. Although not significant, AC+TBI rats had slightly decreased SR duration compared to TBI rats. This can be attributed to acute caffeine pretreatment 30 mins before TBI. Among the rats that underwent TBI, the least SR duration was observed in chronic caffeine pretreated rats. Additionally, this group did not show a significant increase in SR duration (like TBI rats) when compared to sham rats; this can be attributed to chronic caffeine pretreatment before TBI. Since loss of consciousness following TBI is often used as a measure in grading injury severity, it may proposed that chronic caffeine offers potential neuroprotection (as evidenced by decreased duration of loss of consciousness). To conclude, chronic caffeine pretreatment was found to be more beneficial compared to acute caffeine pretreatment in decreasing the duration of loss of consciousness immediately after TBI.

5.5.2 CBF changes following TBI in chronic caffeine pretreated rats

Tolerance to vasoconstrictive effects of caffeine following chronic consumption in rats

Caffeine is well known for its vasoconstrictive effects (decreased CBF) following acute intake. However, it was reported that chronic caffeine consumption results in an adaptation of the adenosine (Ade) receptors, including vascular Ade receptors and creates a tolerance to its vasoconstrictive effect (Echeverri et al., 2010; Johansson et al., 1997; Li et al., 2008). Results of our study also support potential tolerance to caffeine mediated cerebral vasoconstriction in rats following chronic ingestion of caffeinated drinking (1.5 g/L) water for 3 weeks. Both regional (PCx, St, Hp, Th and Bs) and global CBF levels 21 days after chronic caffeine treatment (pre-scan time point) did not show any significant reduction (p<0.05) compared to their pre-caffeine treatment levels (pre-Rx time point). Instead, there was an insignificant increase in both cortical PCx) subcortical (St, Hp, Th) regional CBF and also global CBF. To summarize, no significant changes in regional or global CBF were observed 21 days after chronic caffeine treatment in rats, supporting the development of tolerance to vasoconstrictive effects of caffeine.

Restoration of regional and global CBF following TBI in chronic caffeine pretreated rats

Previous studies have reported the neuroprotective effects of caffeine against TBI through diverse mechanisms (Section 2.6.1). But none of the previous studies have reported caffeine mediated CBF changes following TBI. Our study demonstrated that

chronic caffeine (1.5 g/L) can potentially interfere with decreased CBF (secondary brain damage event) following TBI and restore CBF. Unlike TBI-only rats, CBF in the PCx and St of CC+TBI rats did not show any significant changes following TBI compared to their pre-Rx and pre-scan levels, except Hp, which showed a significant reduction (18%) by 3 days post-TBI compared to its pre-scan level. This clearly indicates that the significant reduction in CBF of PCx, St, Th observed in TBI-only rats is not seen in chronic caffeine pretreated rats, indicating the potential role of chronic caffeine in restoring CBF following TBI.

Similar to sham and TBI-only rats, CBF in the bilateral thalamus and brainstem of chronic caffeine pretreated rats also did not show any significant changes in CBF following TBI. It implies that chronic caffeine only restored CBF of the regions that were affected following TBI, but did not worsen the CBF of the regions which were not affected by TBI-only. Similar to sham rats, combined rCBF and global CBF did not show any significant changes in CBF following TBI compared to their pre-Rx and pre-scan levels; whereas TBI-only rats showed an insignificant reduction by 4 hrs post-TBI (~18%) and a significant reduction at 7 days post-TBI (~37%). This restoration of CBF in the chronic caffeine pretreated rats following TBI may be attributed to adaptation of adenosine receptors on vascular smooth muscle cells following chronic caffeine pretreatment or caffeine withdrawal effect (increased CBF) after TBI.

Caffeine withdrawal from chronic ingestion corresponds with increased CBF after 20 - 24 hrs of caffeine abstention (Couturier et al., 1997; Jones et al., 2000) and caffeine withdrawal effects lasts from 1 to 7 days (Fredholm et al., 1999; JHU, 2003) (Discussed

in Chapter 2). It was also reported that in the absence of caffeine, Ade binds to an increased number of Ade receptors on vascular smooth muscle which are normally antagonized by chronic caffeine levels in the body (Addicott et al., 2009), resulting in increased CBF. However, it is not known when the CC+TBI rats drank caffeinated water for the last time before TBI and whether this CBF restoration following TBI is due to withdrawal effects from chronic caffeine. To the best of our knowledge, no research has been done previously to study withdrawal effects (increased CBF) of chronic caffeine following TBI. To conclude, our data demonstrating the CBF restoration following TBI in chronic caffeine pretreated rats supports the hypothesis that withdrawal effects (increased CBF) of chronic caffeine may be observed even after TBI.

Furthermore, as mentioned previously in Chapter 2 (section 2.4.1), TBI often causes brain swelling (inflammation) resulting in the compression of ventricles and also constriction of cerebral blood vessels (decreased CBF), as the volume within the intact skull is fixed (Lunt et al., 2004). However, chronic (and also acute to some extent) caffeine pretreatment was reported to offer neuroprotection mediated by a decrease in inflammation following TBI (Li et al., 2008). Using CD45 antibody (marker of inflammatory cells), Li et al., showed significant reduction in CD45-positive cells 24 hrs after injury in chronic caffeine pretreated mice. This decreased cerebral inflammation following TBI in chronic caffeine treated rats may be one of the reasons in restoring the CBF that once reduced due to constriction of cerebral vessels due to inflammation. However, we have not yet studied cerebral inflammatory response following TBI in our study.

5.5.3 Acute reduction in CBF following TBI in rats pretreated with acute caffeine

Although previous studies using different pathological mechanisms have reported that acute caffeine pretreatment is not neuroprotective against TBI (Al Moutaery et al., 2003; Li et al., 2008) as discussed under section 2.6.1, we studied the effects of a single dose of acute caffeine (150 mg/kg) on CBF changes following TBI. Acute caffeine is well known for its vasoconstrictive effects mediated by antagonizing Ade receptors on vascular cells (Pelligrino et al., 2010). Unlike CC+TBI rats, acute caffeine pretreatment 30 mins before TBI showed substantial reduction in both regional and global CBF at 4 hrs post-TBI, but these were not significantly different. CBF in the cortical (PCx) and subcortical (St, Hp and Th) regions of both TBI-only rats and AC+TBI rats showed an insignificant reduction up to 19% and 27% respectively, by 4 hrs post-TBI. But, in contrast to TBI rats, global CBF in acute caffeine pretreated rats was observed to be significantly reduced at 4hrs post-TBI compared to its pre-scan levels. This marked reduction in both regional and global CBF by 4 hrs post-TBI in acute caffeine treated rats compared to TBI-only rats can be attributed to the combined effect of TBI and vasoconstrictive effects of acute caffeine. Acute reduction in CBF following TBI may reach ischemic levels and worsen the outcome (Ngai et al., 2001). As mentioned earlier under section 2.4, a study even suggested optimization of CBF in head injury treatment (Ribeiro and Sebastiao, 2010). Therefore, this acute reduction (~27%) in CBF following TBI may worsen the outcome. Unlike sham, TBI-only and CC+TBI rats, CBF in the brainstem region also reduced significantly at 4hrs (22%) and 7 days post-TBI (~23%) compared to its pre-TBI levels. The thalamus did not show any significant changes in CBF following TBI in AC+TBI rats.

In acute caffeine pretreated rats following TBI, CBF in the cortical and subcortical regions (PCx, St, Hp and Th) and global CBF showed a trend towards normalization following reduction at 4 hrs post-TBI. TBI rats have showed significant reduction in regional and global CBF at 7 days post-TBI. In contrast, acute caffeine pretreated rats showed a complete recovery in CBF by 3 days and 7 days post-TBI. This trend of CBF restoration at 3 days and 7 days post-TBI may be attributed to significant decrease in inflammation mediated by acute caffeine treatment 24 hrs after injury, as mentioned before (Li et al., 2008). However, it is not known whether single high dose of caffeine (150 mg/kg) can mediate adaptation of adenosine receptors on cerebral vascular smooth muscle resulting in favorable CBF changes following TBI, as observed in case of chronic caffeine. To conclude, although acute caffeine treated rats reached a normalization in CBF by 3 days and 7 days post-TBI, acute reduction (~27%) in CBF by 4 hrs post-TBI may have worse effects on other secondary brain damage events. This supports the notion that chronic caffeine pretreatment is more beneficial in optimizing CBF changes following TBI, compared to acute caffeine pretreatment.

CHAPTER 6 TO ASSESS GROUP-WISE DIFFERENCES IN CEREBRAL BLOOD FLOW OF RATS FOLLOWING TBI WITH AND WITHOUT CAFFEINE (CHRONIC AND ACUTE) PRETREATMENT (Specific Aim 3)

6.1 Introduction

Although we have analyzed regional and global CBF changes following TBI across various time points within individual groups (specific aim 1 and 2), it is imperative to analyze group-wise differences in CBF following TBI at each time point for better understanding of the effects of caffeine (chronic and acute) pretreatment. Repeated measures of analysis of variance (ANOVA) is a commonly used statistical method for analyzing changes in mean values over three or more time points/treatment conditions within same subjects in each group, often termed as "mixed design". It is also referred to as ANOVA for correlated samples. Our analysis of repeated measures was based on the assumption that all the subjects (SD rats, male) used in different groups (sham, TBI only, CC+TBI, AC+TBI) of this study had similar levels of regional and global CBF before sham surgery or TBI (pre-scan). We have analyzed the group-wise differences in CBF under different treatment conditions (sham surgery, TBI only, CC+TBI, AC+TBI) at each time point using repeated measures ANOVA. A schematic design of the time-course repeated measures of CBF used for this study is shown below (Figure 6-1). Regional (Rt PCx, Lt PCx, Avg PCx, Rt St, Lt St, Avg St, Rt Hp, Lt Hp, Avg Hp, Th and Bs), combined regional and global CBF values obtained from ASL data as a part of specific aim 1 and specific aim 2 were utilized here to perform repeated measures of ANOVA (specific aim 3). Group-wise differences in CBF were investigated across various time points (pre-scan=1 day before sham surgery or TBI, 4hrs, 24 hrs, 3 days

and 7 days post-TBI) in different treatment groups (sham, TBI only, chronic and acute caffeine pretreated rats subjected to TBI).

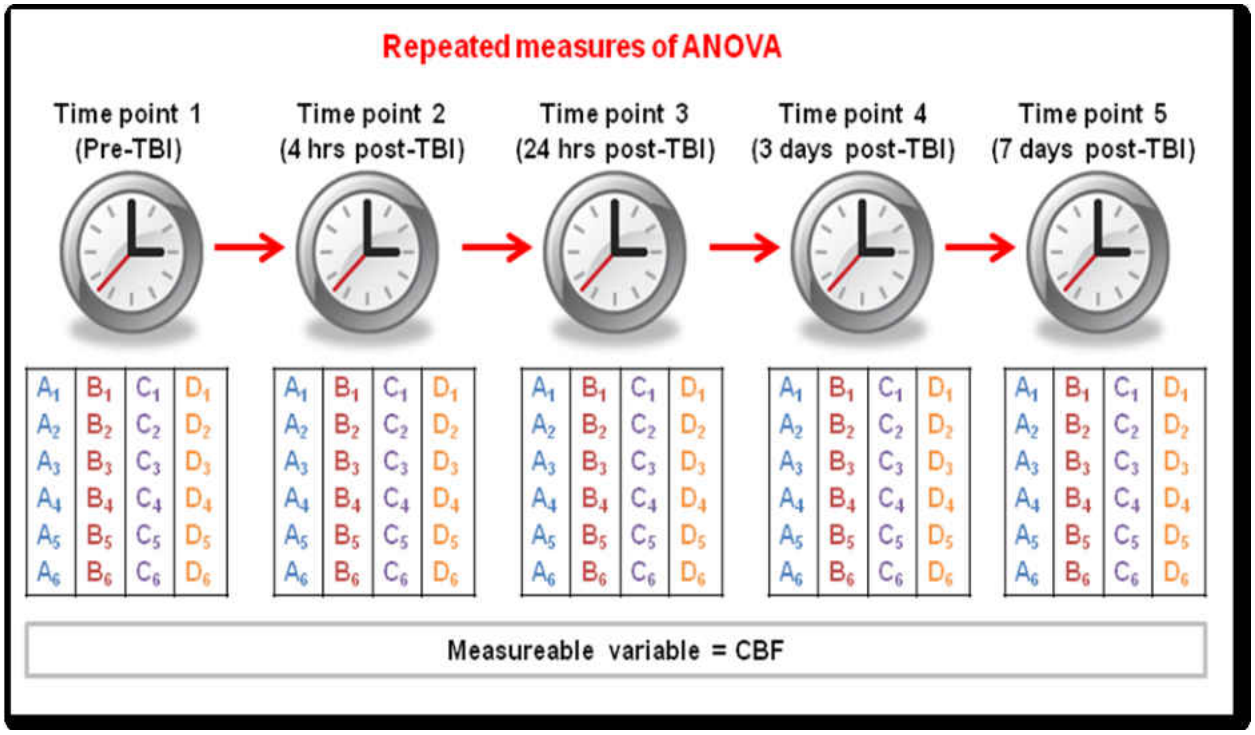


Figure 6-1: Schematic design of the repeated measures of ANOVA used in this study for analyzing group-wise differences in CBF at each time point. A_1 to A_6 represent CBF value of six sham rats, B_1 to B_6 represent CBF value of six TBI rats, C_1 to C_6 represent CBF value of six CC+TBI rats and D_1 to D_6 represent CBF value of six AC+TBI rats. However, the actual samples size of each group used in this study was different from what is shown here.

6.2 Statistical Analysis

All data were analyzed for statistical significance using SPSS (version 20, Chicago, IL). The data were first tested for reliability by Cronbach's alpha test (Table 10). The CBF value obtained from ASL images was expressed as the mean value ± standard error of the mean (SEM). Group-wise differences in CBF at each time point were analyzed by repeated measures of analysis of variance (ANOVA). A p value of <0.05 was considered as significant.

6.3 Results (Group-wise Differences in CBF)

Group-wise differences in the CBF of right parietal cortex (Rt PCx) across time

In the Rt PCx of different groups, no apparent changes in CBF were observed until 7 days post-TBI (Figure 41). At 4 hrs post-TBI rats from all four groups showed an insignificant reduction in the CBF of Rt PCx, compared to their pre-TBI levels. However, by 7 days post-TBI nearly significant reduction (p=0.07) in CBF was observed in TBI rats compared to sham rats. However, CBF in TBI rats at 7 days post-TBI decreased significantly when compared to its pre-scan levels.

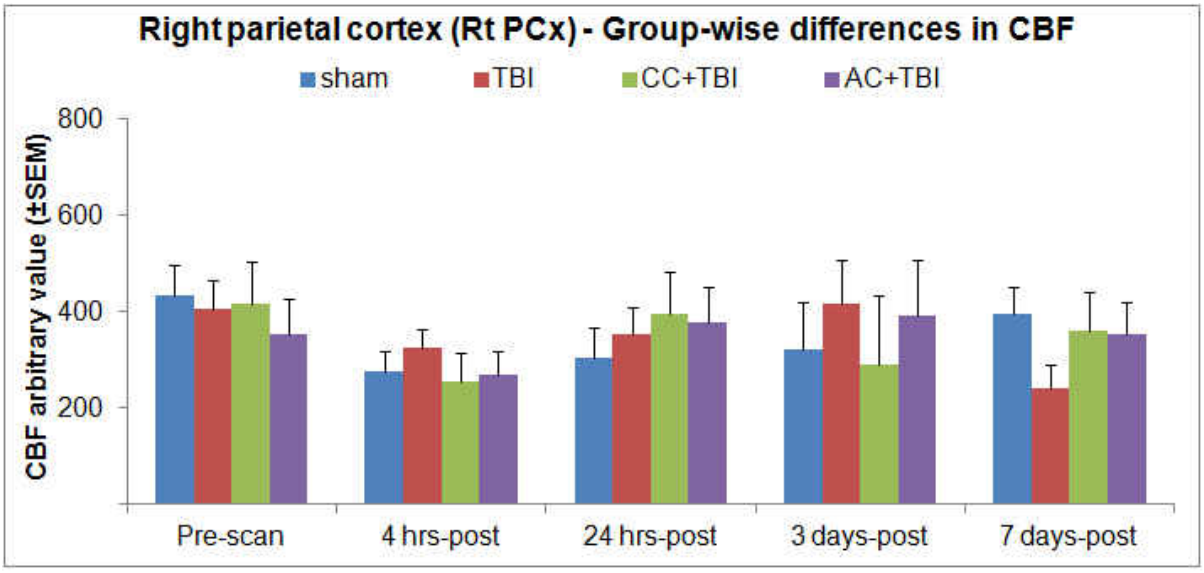


Figure 6-2: Plot showing group-wise differences in CBF of the right parietal cortex (Rt PCx) at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. No significant group-wise differences were observed.

Group-wise differences in the CBF of left parietal cortex (Lt PCx) across time

Similar to Rt PCx, CBF of the Lt PCx also did not show any significant changes between groups until 7 days post-TBI. Lt PCx from all four groups showed an insignificant reduction in the CBF at 4 hrs post-TBI, compared to their pre-scan levels (Figure 6-3). By 7 days post-TBI, the reduction in Lt PCx CBF of TBI rats was close to significance in comparison to sham and chronic caffeine pretreated rats.

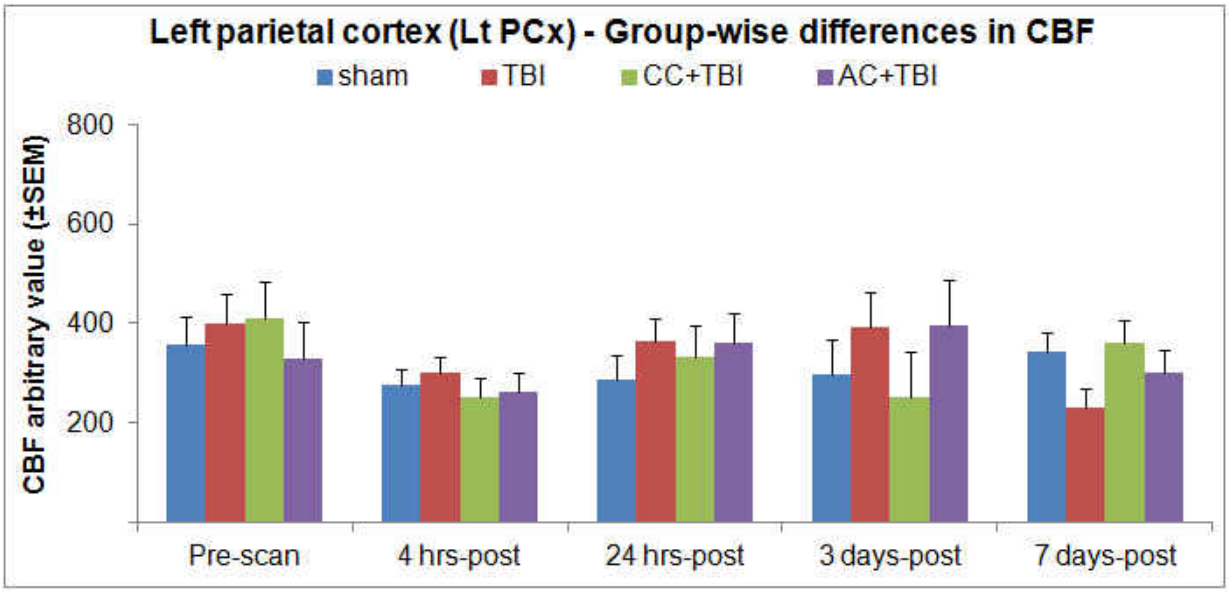


Figure 6-3: Plot showing group-wise differences in CBF of the left parietal cortex (Lt PCx) at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI.

Group-wise differences in CBF of the average of right and left parietal cortex (Avg PCx) across time

Similar to Rt and Lt PCx, CBF in the average of Rt and Lt PCx (Avg PCx) showed no apparent changes in CBF until 3 days post-TBI (Figure 6-4). But, by 7 days post-TBI, CBF in the Avg PCx of TBI rats showed significant reduction compared to sham rats

(p=0.049) and with an insignificant reduction compared to chronic caffeine treated rats (p=0.09). No other significant changes in CBF between the groups were observed.

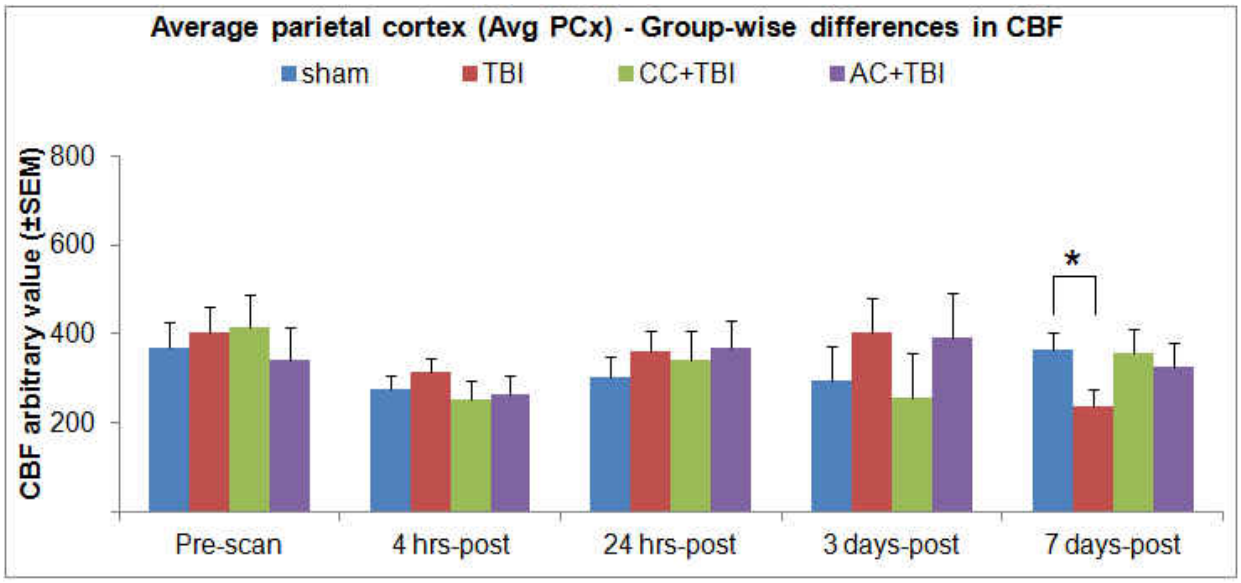


Figure 6-4: Plot showing group-wise differences in CBF of average of the right and left parietal cortex (Avg PCx) at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. Asterisk indicates significant difference (p<0.05).

Group-wise differences in the CBF of right striatum (Rt St) across time

CBF in the Rt St of rats from all four groups did not exhibit any significant changes at pre-TBI, 24 hrs, 3 days and 7 days post-TBI (Figure 6-5). But, a significant reduction (p=0.030) in the CBF of Rt St of acute caffeine treated was observed at 4hrs post-TBI, when compared to TBI rats. However, 7 days after trauma, a nearly significant reduction (p=0.055) in CBF of Rt St from TBI rats was observed when compared to sham rats.

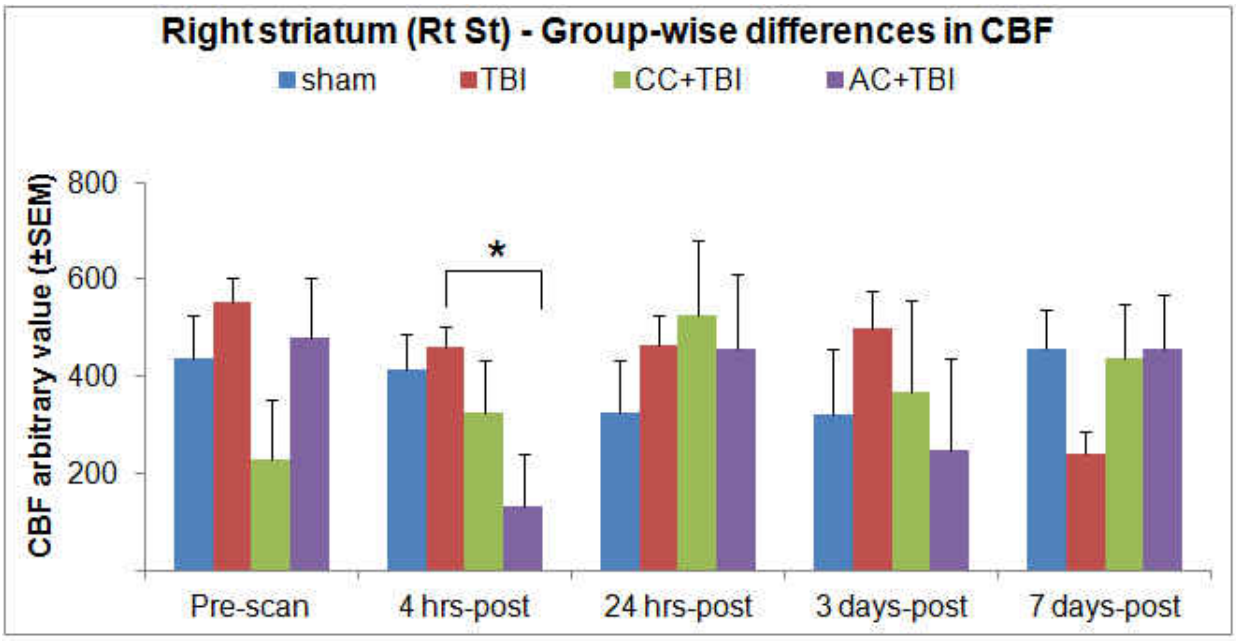


Figure 6-5: Plot showing group-wise differences in CBF of the right striatum (Rt St) at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. Asterisk indicates significant difference (p<0.05).

Group-wise differences in the CBF of left striatum (Lt St) across time

Similar to Rt St, CBF in Lt St also did not show any significant differences between groups before TBI, 24 hrs and 3 days following TBI (Figure 6-6). But, significant changes were observed at 4 hrs and 7 days post-TBI. In acute caffeine pretreated rats, CBF in the Lt St at 4 hrs post-TBI was found to be significantly reduced compared to sham, TBI only, and chronic caffeine pretreated rats (p=0.026, 0.002 *and* 0.012 respectively). Additionally, at 7 days post-TBI CBF in the Lt St of TBI rats was significantly decreased compared to sham, chronic and acute caffeine pretreated rats (p=0.007, 0.016 and 0.004 respectively). However, by 7 days-post TBI, this decrease appears to be normalized. On the other hand, by 7 days post-TBI in the case of rats

subjected to TBI, CBF in the Lt St significantly reduced in comparison to sham, CC+TBI and AC+TBI.

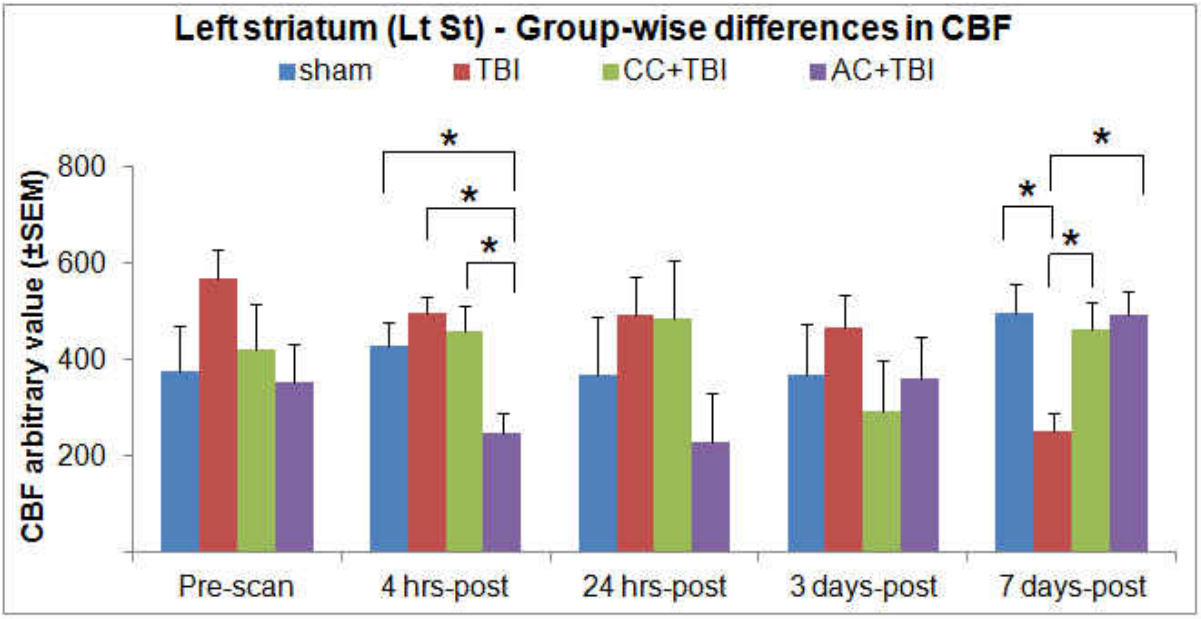


Figure 6-6: Plot showing group-wise differences in CBF of the left striatum (Lt St) at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. Asterisks indicate significant difference (p<0.05).

Group-wise differences in CBF of the average of right and left striatum (Avg St) across time

Unlike CBF in other regions (PCx, St, Th, Bs), Avg St alone showed significant differences in CBF between groups following TBI, as well as before TBI CBF (Figure 6-7). Pre-TBI CBF level in the Avg St of acute caffeine treated rats showed significant reduction (p=0.037) compared to TBI only group. At 4 hrs post-TBI, CBF of Avg St in the acute caffeine treated rats (AC+TBI) was significantly reduced compared to sham (p=0.037), TBI (p=0.006) and CC+TBI rats (p=0.031). In addition, CBF of Avg St in AC+TBI rats decreased significantly (p=0.046) by 24 hrs post-TBI, compared to TBI rats

that showed a trend of recovery by 3 and 7 days post-TBI. After 7 days following TBI, the CBF of the Avg St from TBI rats showed a significant reduction when compared to sham, chronic and acute (p=0.009, 0.018 and 0.001 respectively) caffeine pretreated rats.

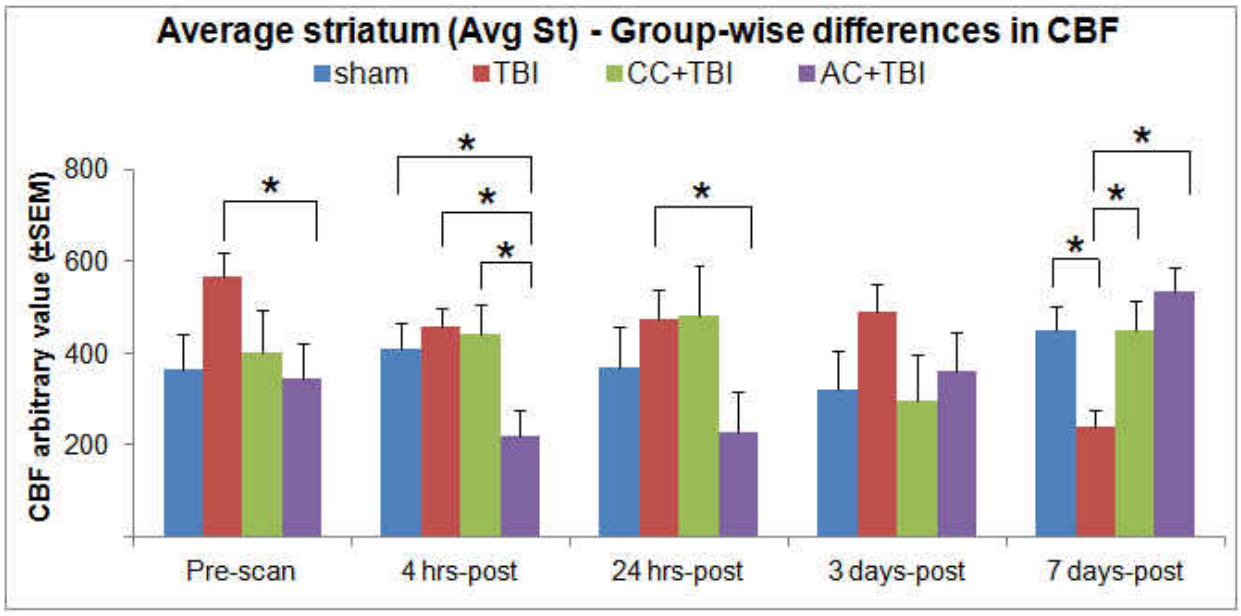


Figure 6-7: Plot showing group-wise differences in CBF of the average of right and left striatum (Avg St) at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. Asterisks indicate significant difference (p<0.05).

Group-wise differences in the CBF of right hippocampus (Rt Hp) across time

Similar to Rt PCx, CBF in the Rt Hp in all four different groups did not show any significant changes at any time points (Figure 6-8).

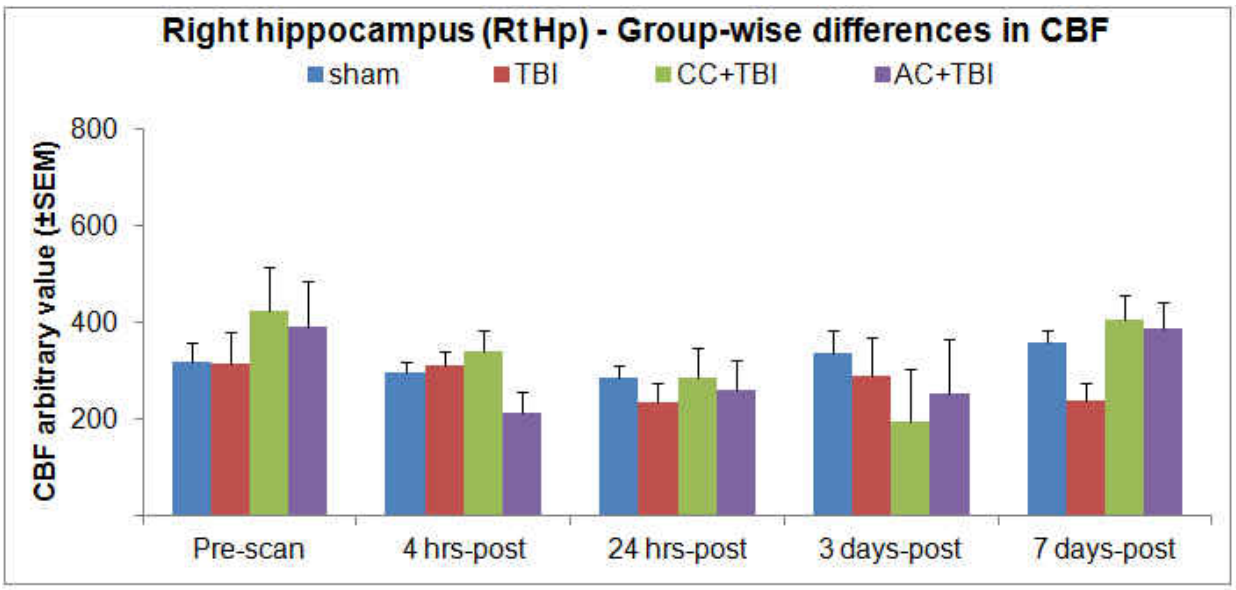


Figure 6-8: Plot showing group-wise differences in CBF of the right hippocampus (Rt Hp) at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. No significant group-wise differences were observed.

Group-wise differences in the CBF of left hippocampus (Lt Hp) across time

No apparent changes in CBF of Lt Hp were observed before-TBI (pre-scan), 24 hrs and 3 days post-TBI (Figure 6-9). However, significant changes (p<0.05) in the CBF of Lt Hp were observed 4 hrs and 7 days post-TBI. By 4 hrs post-TBI, CBF in the Lt Hp of acute caffeine pretreated rats was significantly reduced when compared to sham (p=0.037), chronic caffeine pretreated rats (p=0.023) and nearly significant reduction (p=0.059) compared to TBI rats. Additionally, by 7 days post-TBI, CBF of the Lt Hp in TBI rats was significantly reduced when compared to sham, CC+TBI and AC+TBI rats (p=0.019, 0.039 and 0.042 respectively).

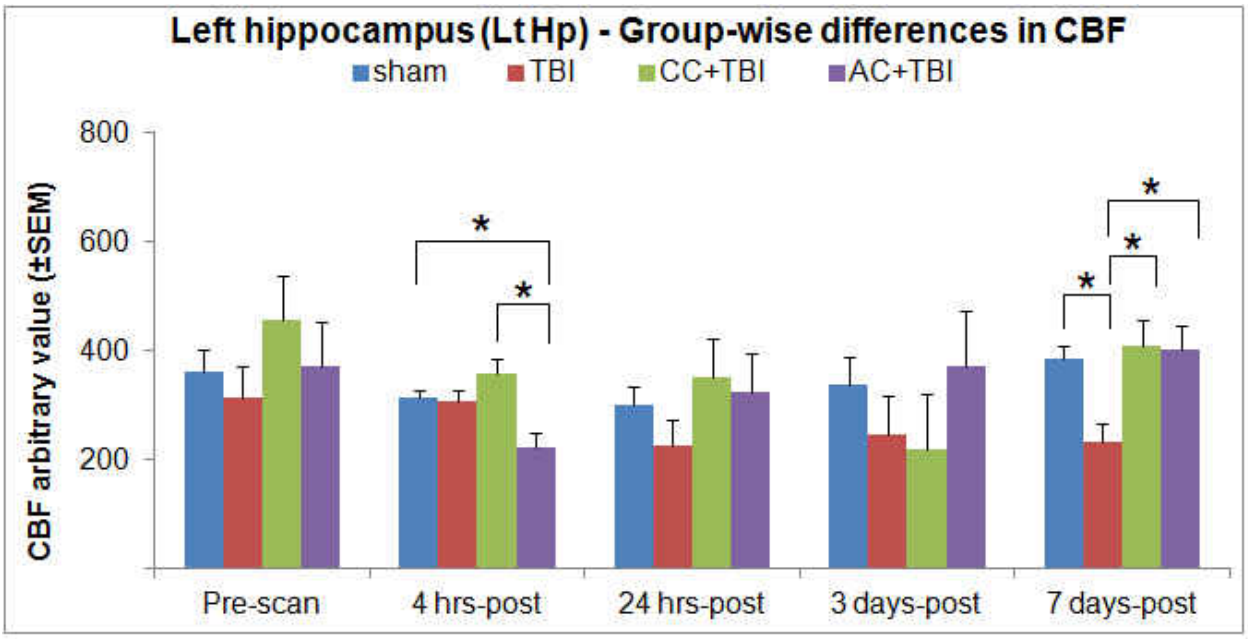


Figure 6-9: Plot showing group-wise differences in CBF of the left hippocampus (Lt Hp) at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. Asterisks indicate significant difference (p<0.05).

Group-wise differences in CBF of the average of right and left hippocampus (Avg Hp) across time

Similar to CBF in the Rt Hp and Lt Hp, the average of Rt and Lt Hp (Avg Hp) did not show any significant changes before TBI, and 24 hrs and 3 days following TBI (Figure 6-10). But, CBF of the Avg Hp in acute caffeine pretreated rats at 4 hrs post-TBI was significantly reduced when compared to sham (p=0.047), CC+TBI rats (p=0.027) and nearly significant reduction (p=0.057) was observed when compared with TBI rats. Furthermore, by 7 days post-TBI, the CBF of Avg Hp in TBI rats was significantly reduced compared to sham (p=0.028), TBI rats (p=0.039) and almost significantly reduced compared to AC+TBI rats (p=0.050).

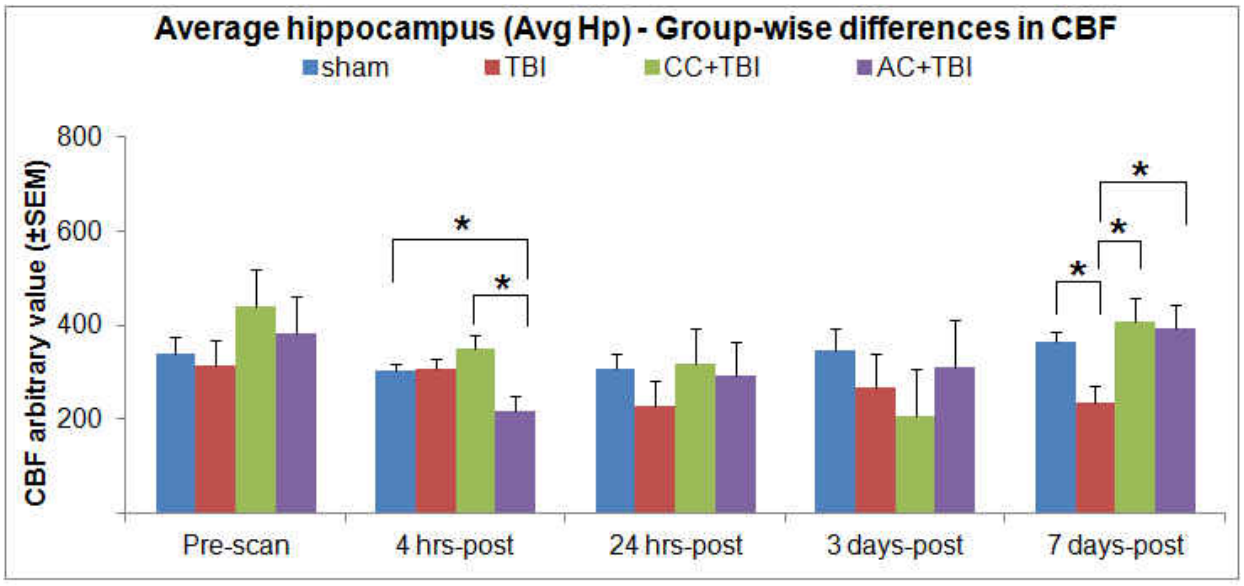


Figure 6-10: Plot showing group-wise differences in CBF of the average of right and left hippocampus (Avg Hp) at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. Asterisks indicate significant difference (p<0.05).

Group-wise differences in the CBF of thalamus (Th) across time

Unlike other PCx, St, Hp, CBF the thalamus did not show any significant group-wise differences at any time point pre and post-trauma (Figure 6-11).

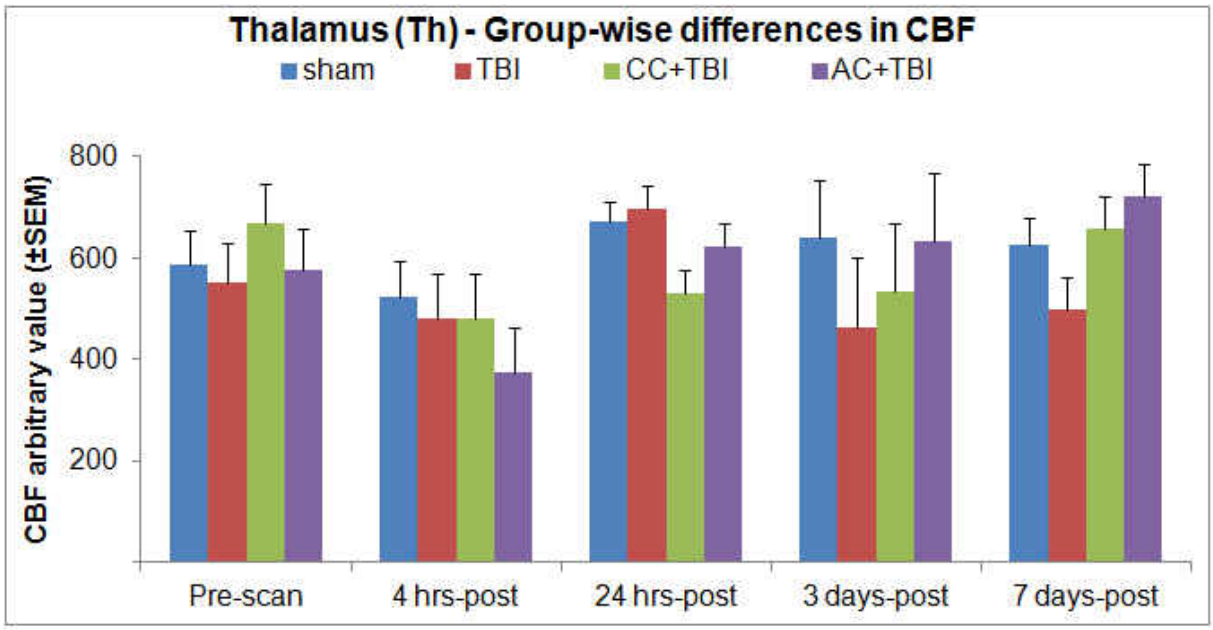


Figure 6-11: Plot showing group-wise differences in the CBF of the thalamus (Th) at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. No significant group-wise differences were observed.

Group-wise differences in the CBF of brainstem (Bs) across time

Similar to thalamus, CBF in the brainstem did not show any significant group-wise differences at any time point before and after TBI (Figure 6-12).

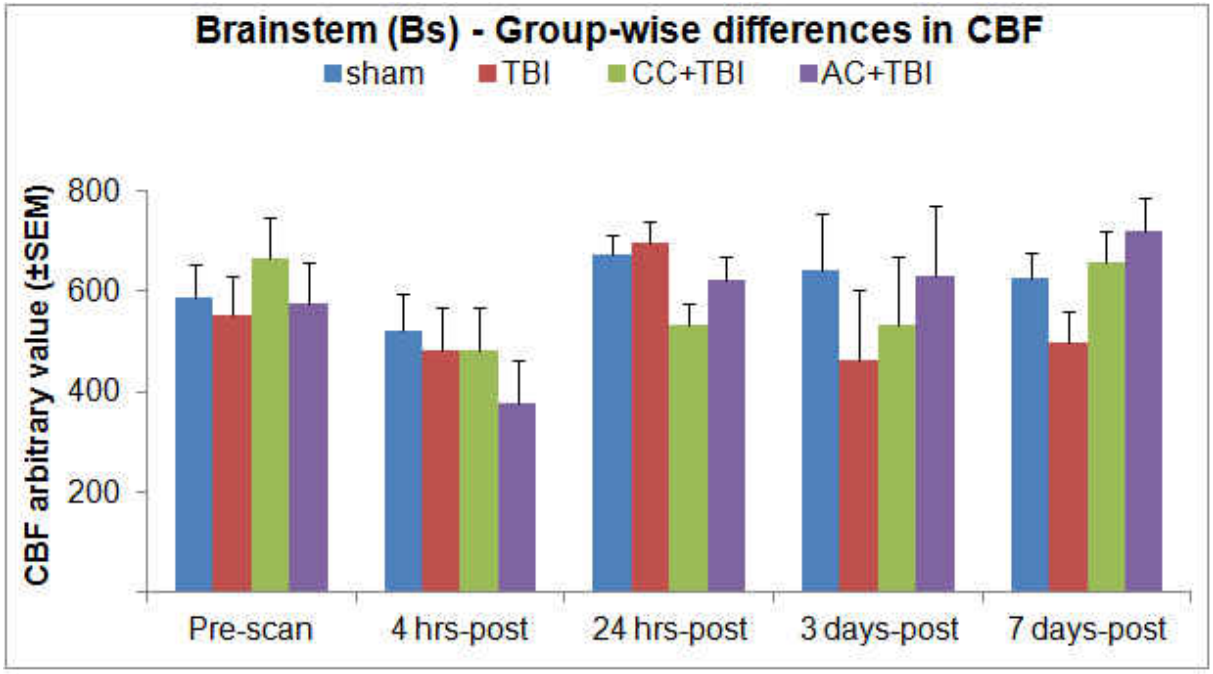


Figure 6-12: Plot showing group-wise differences in the CBF of the brainstem (Bs) at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. No significant group-wise differences were observed.

Group-wise differences in the combined regional CBF (rCBF) across time

Combined regional CBF (average of the CBF in Rt PCx, Lt PCx, Rt St, Lt St, Rt Hp, Lt Hp, Th and Bs) showed no significant group-wise differences before TBI and 24 hrs after TBI (Figure 6-13). But, at 4 hrs post-TBI, the combined rCBF of the acute caffeine pretreated rats was significantly reduced compared to sham (p=0.021) and TBI rats (p=0.006). Also, by 3 days post-TBI, the combined rCBF of the chronic caffeine pretreated rats was significantly reduced (p=0.040) compared to TBI rats. Furthermore, it was observed that the combined rCBF of TBI rats at 7 days post-TBI was significantly reduced when compared to sham (p=0.002), CC+TBI (p=0.005) and AC+TBI rats (p=0.001).

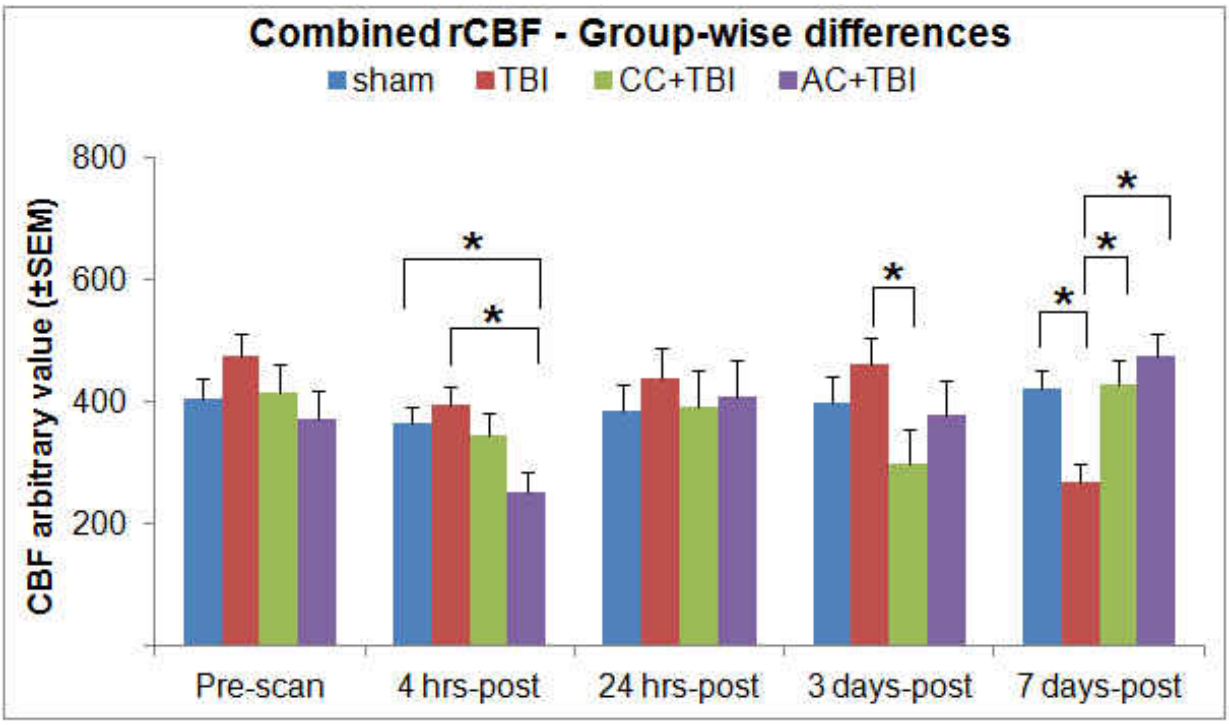


Figure 6-13: Plot showing group-wise differences in the combined regional CBF at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. Asterisks indicate significant difference (p<0.05).

Group-wise differences in the global CBF across time

Global CBF did not show any apparent group wise differences before TBI, 24 hrs and 3 days following TBI (Figure 6-14). Similar to combined rCBF, group-wise differences in global CBF also showed significant differences (p<0.05) by 4 hrs and 7 days post-TBI. At 4 hrs post-TBI, the global CBF in the acute caffeine pretreated rats was significantly reduced compared to sham, TBI, and chronic caffeine pretreated rats (p=0.001, 0.013 and 0.002). But AC+TBI showed a temporal pattern of recovery by 24 hrs and 3 days post-TBI. However, global CBF in the TBI rats at 7 days post-TBI showed significant

reduction in comparisons with sham (p=0.007) and chronic caffeine pretreated rats (p=0.002). No other significant group-wise differences were found.

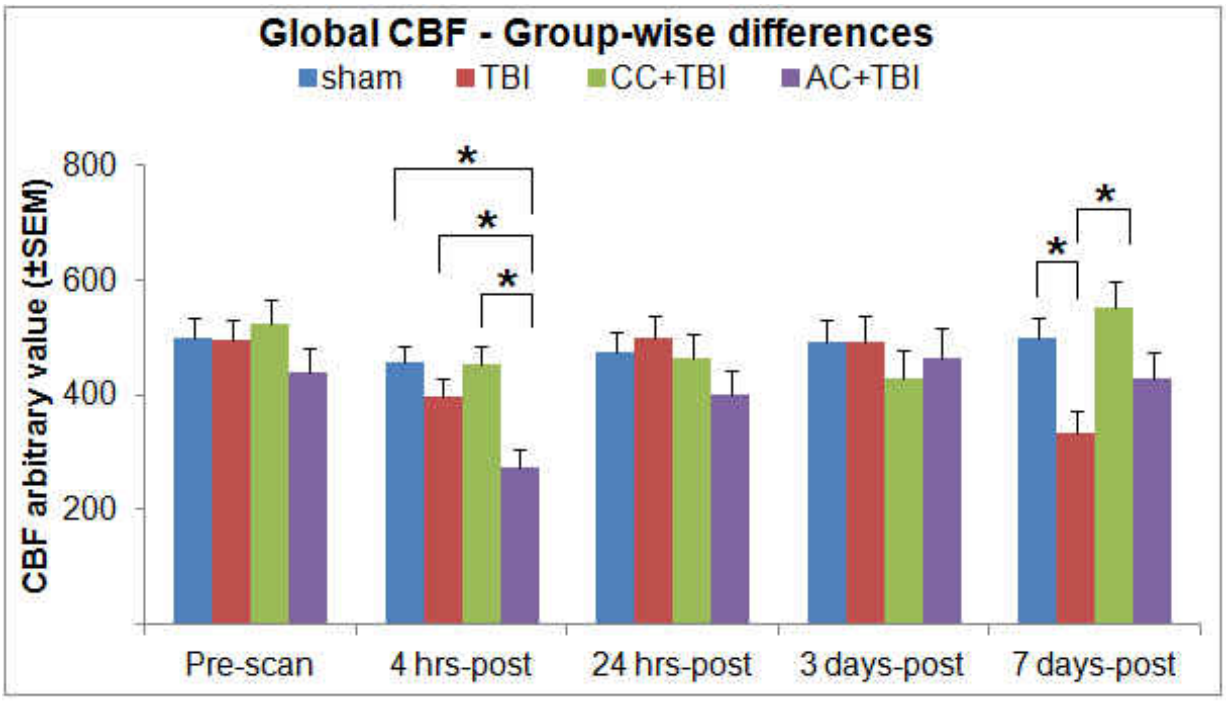


Figure 6-14: Plot showing group-wise differences in the global CBF at various time points (pre-scan=1 day before sham surgery/TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI) in sham rats, TBI only and chronic and acute caffeine pretreated rats subjected to TBI. Asterisks indicate significant difference (p<0.05).

6.4 Discussion (Specific Aim 3)

Using repeated measures of ANOVA, at each MRI scanning time point, our study has statistically analyzed group-wise differences in the CBF between the four groups of rats: sham, TBI only, CC+TBI (chronic caffeine pretreated rats subjected to TBI) and AC+TBI (acute caffeine pretreated rats subjected to TBI).

Pre-scan time point: No significant group-wise differences in regional and global CBF before TBI

Rats from all four groups underwent MRI scanning 1 day before sham surgery or TBI. CBF measured in various brain regions (Rt PCx, Lt PCx, Rt St, Lt St, Rt Hp, Lt Hp, Th and Bs) did not exhibit any apparent changes. However, pre-TBI CBF level of the average in Lt and Rt St in AC+TBI rats was found to be significantly different (p=0.037) compared to TBI group rats. CBF in none of the other regions and also global CBF, did not show any significant differences when compared between different groups (sham, TBI, CC+TBI and AC+TBI) at pre-scan level. This clearly indicates that, both regional and global CBF levels in rats from different groups were similar before TBI. Any respective changes in CBF following TBI, can be attributed to resulting from trauma (TBI rats) and the treatment (CC+TBI and AC+TBI) they have received.

4 hrs post-TBI: Acute caffeine pretreated rats subjected to TBI exhibited significant reductions in regional and global CBF

In general, CBF changes following TBI were most affected at 4 hrs post-TBI and 7 days post-TBI as observed in one-way ANOVA analysis of CBF changes in TBI only rats. Similarly, analysis of group-wise differences using repeated measures ANOVA also showed significant changes in regional and global CBF between different groups at 4 hrs post-TBI. Lt PCx, Rt PCx and average of Lt and Rt PCx (Avg PCx) did not show any significant changes in CBF between groups at 4 hrs post-TBI. But AC+TBI rats showed significant reduction in CBF of Rt St compared to TBI rats. Similarly Lt St and Avg St of AC+TBI rats showed significant reductions in CBF compared to sham, TBI and CC+TBI

rats. This clearly indicates that CBF of the striatum in the AC+TBI rats worsened at 4 hrs following TBI. Similarly, CBF in the Lt HP and Avg Hp of AC+TBI was significantly lower compared to sham and TBI rats. But thalamus and brainstem did not show any significant differences in CBF between groups at 4 hrs post-TBI. Both combined rCBF and global CBF reflected the changes observed in CBF of the striatum and hippocampus. At 4 hrs post-TBI, combined regional and global CBF in the AC+TBI was significantly reduced compared to sham and TBI rats. Furthermore, global CBF in AC+TBI rats was significantly reduced compared to CC+TBI rats. To summarize, acute caffeine pretreated rats subjected to TBI showed significantly reduced regional (Rt St, Lt St, Avg St, Lt Hp and Avg Hp) and global CBF compared to the rest of the groups. Sham, TBI and CC+TBI rats did not show any significant changes in CBF at 4 hrs post-TBI. This clearly indicates the role of vasoconstriction (decreased CBF) mediated by acute caffeine at 4 hrs post-TBI. Implications of this acute reduction in CBF by 4 hrs following TBI was already discussed in Chapter 5 (section 5.5.3).

24 hrs post-TBI: No apparent group-wise differences in regional and global CBF

From Chapters 4 and 5, it is observed that both regional and global CBF in the rats that underwent TBI (TBI, CC+TBI and AC+TBI rats) showed a trend of recovery in CBF by 24 hrs post-TBI. Although, AC+TBI rats showed significant reductions in CBF compared to other groups at 4 hrs post-TBI, this group showed recovery by 24 hrs post-TBI. Similarly, repeated measures of ANOVA also did not show any group-wise differences in CBF of the brain regions (Rt PCx, Lt PCx, Rt St, Lt St, Rt Hp, Lt Hp, Th and Bs). However, CBF of the Avg St alone in the AC+TBI rats was significantly reduced

compared to TBI rats by 24 hrs post-TBI. No other group-wise differences in regional or global CBF were observed at 24 hrs post-TBI. This indicates that cerebral hypoperfusion observed at 4 hrs post-TBI in AC+TBI did not last until 24 hrs post-TBI and that vasoconstrictive effects mediated by acute caffeine were not exhibited 24 hrs post-TBI. Furthermore, both combined rCBF and global CBF at 24 hrs post-TBI did not show any significant group-wise differences, indicating the existence of similar levels of CBF in all four groups.

3 days post-TBI: No significant group-wise differences in regional and global CBF

Similar to 24 hrs post-TBI, group-wise differences in regional and global CBF by 3 days post-TBI did not show any significant changes. The CBF in different regions of the brain (Rt PCx, Lt PCx, Rt St, Lt St, Rt Hp, Lt Hp, Th and Bs) and global CBF at 3 days post-TBI did not any show significant changes when compared between the four groups. Although CBF in various cortical (Rt PCx, Lt PCx) and sub-cortical regions (Rt St, Lt St, Rt Hp, Lt Hp) of the CC+TBI rats showed insignificant reduction when compared to TBI rats, only combined rCBF showed a significant reduction when compared to TBI rats. This indicates that no apparent reductions in CBF were observed between different groups at 3 days post-TBI.

7 days post-TBI: Rats subjected to TBI without chronic or acute caffeine pretreatment exhibited significant reductions in both regional and global CBF

As discussed earlier in Chapter 4 (section 4.5.3), both regional and global CBF in TBI rats was found to be significantly reduced at 7 days post-TBI when compared to their

pre-TBI levels within the group by one-way ANOVA. Similarly, group-wise differences analyzed at 7 days post-TBI using repeated measures of ANOVA also revealed that both regional and global CBF of the TBI rats was significantly reduced when compared to sham rats. An apparent decrease in CBF of cortical (Lt, Rt and Avg PCx) and sub-cortical regions (Lt St, Avg St, Lt Hp and Avg Hp) at 7 days post-TBI was observed, when compared to sham and CC+TBI rats. However, thalamus and brainstem regions did not show any group-wise differences in CBF at 7 days following TBI and also at other time points. Both combined rCBF and global CBF of TBI rats at 7 days post-TBI also showed significant reductions compared to sham and chronic caffeine pretreated rats. In general, regional and global CBF of chronic and acute caffeine pretreated rats at 7 days post-TBI was similar to sham rat CBF levels. This clearly indicates that only TBI group rats have shown decreased CBF levels at 7 days post-TBI when compared to rats from the other three groups (sham, CC+TBI and AC+TBI). This reduction in CBF in TBI rats at 7 days post-TBI may be attributed to post-traumatic events (impaired cerebral autoregulation, changes in cerebral vasculature, etc) leading to secondary brain damage. From this we can infer that chronic and acute caffeine pretreatment can potentially restore the CBF at 7 days following trauma.

To summarize, no apparent group-wise changes in CBF were observed before TBI, 24 hrs and 3 days following TBI. Although TBI rats showed slightly decreased global CBF at 4 hrs post-TBI compared to sham rats, only acute caffeine pretreated rats showed a significant reduction in regional and global CBF compared to sham, TBI and CC+TBI groups at 4hrs post-TBI. This significant decrease in CBF of AC+TBI rats at 4 hrs post-TBI may be attributed to vasoconstrictive effects of acute caffeine pretreatment 30

minutes before TBI. Similar to AC+TBI rats at 4 hrs post-TBI, TBI rats at 7 days post-TBI showed significant reduction in regional and global CBF when compared to sham, CC+TBI and AC+TBI. Although, AC+TBI rats could show recovery in CBF by 7 days post-TBI, its acute reduction in CBF at 4 hrs post-TBI may worsen the effects on the secondary brain damage as discussed earlier in Chapter 4 (section 4.5.3). Among the rats that underwent TBI (TBI, CC+TBI and AC+TBI rats), only chronic caffeine pretreated rats maintained CBF throughout without any apparent changes when compared with TBI and AC+TBI rats. Therefore, it supports our hypothesis that chronic caffeine pretreatment may be beneficial in optimizing CBF changes following TBI, compared to acute caffeine pretreatment.

CHAPTER 7 CONCLUSIONS AND FUTURE WORK

There have only been limited studies on cerebral blood flow (CBF) changes following a closed head injury in an experimental animal model. The few available experimental studies have reported only acute CBF changes (up to 48 hrs post-TBI) following TBI with CBF measurements from only one or two cerebral regions. None of the previous experimental studies have reported sub-acute (up to 7 days post-TBI) changes in CBF in an animal model of closed head injury. Work performed as part of this thesis offers evidence related to CBF changes during acute and sub-acute periods from various brain regions following a traumatic insult. These findings are listed below under section 7.1.

7.1 Conclusions

Surface righting (SR) duration following TBI

- Rats subjected to TBI using the Marmarou impact acceleration device (2 m height, 450 g weight) demonstrated significantly prolonged SR duration (an indirect measure of duration of loss consciousness) compared to rats that underwent sham surgery. This prolonged SR duration can be attributed to the traumatic insult.
- Acute caffeine (150 mg/kg) pretreated rats subjected to TBI (AC+TBI) also demonstrated significantly increased SR duration compared to sham rats. Therefore, it is reasoned that acute caffeine treatment 30 minutes before TBI was not able to mitigate the prolonged SR duration that was demonstrated by TBI-only rats.
- Unlike TBI and AC+TBI rats, on the other hand, chronic caffeine (1.5 g/L) pretreated rats subjected to TBI (CC+TBI) did not show any marked increase in SR duration

compared to sham rats. This reduced SR duration following TBI is attributed to the effects of chronic caffeine pretreatment. Considering that duration of loss of consciousness following TBI is used as a measure in grading the severity of injury, our study suggests that chronic caffeine can potentially alleviate the SR duration (i.e. reduce the SR duration) compared to TBI-only rats and in turn decrease the injury severity.

- To the best of our knowledge, this is the first study to report differences in SR duration immediately after TBI in rats that underwent caffeine (acute and chronic) pretreatment.

Cerebral blood flow changes in sham rats

- In sham rats, using ASL perfusion imaging, we demonstrated no significant changes in regional (Rt PCx, Lt PCx, Avg PCx, Rt St, Lt St, Avg St, Rt Hp, Lt Hp, Avg Hp, Th and Bs) CBF following 21 days of pure-water treatment.
- Furthermore, no apparent changes in regional and global CBF were observed at 4 hrs, 24 hrs, 3 days and 7 days post-sham surgery.
- It can be postulated that a close relationship exists between regional and global CBF, as both of them showed similar trends across various time points.
- Therefore, sham rats served as an ideal control to compare the effects of chronic caffeine treatment on CBF before TBI and also for comparing post-traumatic CBF changes in all rats that underwent TBI.

Cerebral blood flow changes in rats subjected to TBI without caffeine treatment

- Our study showed significant changes in global and regional CBF following TBI induced by the Marmarou impact acceleration device (2 m height, 450 g weight).
- In thalamus and brainstem, no apparent changes in CBF following TBI were observed throughout the study period
- CBF in cortical (Rt PCx, Lt PCx and Avg PCx) and sub-cortical (Rt St, Lt St, Avg St, Rt Hp, Lt Hp and Avg Hp) regions was affected the most following TBI. They showed up to 19% reduction in CBF at 4 hrs post-TBI and ~49% reduction at 7 days post-TBI compared to their pre-TBI levels. Similarly, combined rCBF showed a 16% reduction at 4hrs post-TBI and ~44% reduction by 7 days post-TBI. Furthermore, global CBF also showed slight reduction (20%) at 4 hrs post-TBI and significant reduction (~32%) by 7 days post-TBI. No apparent changes in CBF were observed at 24 hrs and 3 days following TBI. These findings indicate CBF changes as early as 4 hours after TBI and may continue to evolve until 7 days despite signs of normalization at 24 hrs and 3 days post TBI.
- Decreased CBF changes during the acute and sub-acute periods following TBI may even reach ischemic or sub-ischemic levels and may potentially trigger secondary injury events. This underscores the need for optimal clinical interventions well beyond the traumatic insult.
- Therefore, it is strongly recommended to routinely measure CBF changes in the critical care management of TBI patients to alleviate possible secondary brain damage.

- These findings indicate a potential need to develop potential therapeutic agents which can offer neuroprotection by modulating CBF following TBI.
- To the best of our knowledge, our study is the first to extensively measure both regional (Rt PCx, Lt PCx, Avg PCx, Rt St, Lt St, Avg St, Rt Hp, Lt Hp, Avg Hp, Th and Bs) and global CBF changes up to 7 days in an animal model of closed head injury induced by the Marmarou impact acceleration device.

Cerebral blood flow changes in chronic caffeine pretreated rats subjected to TBI

- Data from our study suggest the development of tolerance to vasoconstrictive effects of caffeine (1.5g/L) following 21 days of caffeinated water treatment in rats as evidenced by regional and global CBF levels before and after chronic caffeine treatment.
- This study also supports our hypothesis that chronic caffeine treatment for 21 days before TBI can potentially help in restoration of the TBI-induced CBF reductions.
- Similar to sham and TBI rats, CBF in thalamus and brainstem of CC+TBI rats also did not show any significant changes.
- Unlike TBI rats, CBF in the cortical (Rt PCx, Lt PCx and Avg PCx) and sub-cortical (Rt St, Lt St, Avg St, Rt Hp, Lt Hp and Avg Hp) regions of CC+TBI rats did not show any reduction by 7 days post-TBI.
- In addition, both combined rCBF and global CBF did not show any apparent changes in CBF following TBI in CC+TBI rats.
- Therefore, it is suggested that chronic caffeine pretreatment can potentially optimize CBF following TBI. This optimization has been postulated to be related to adenosine

receptor mediated changes on cerebrovascular smooth muscle, various brain regions or due to withdrawal effects (increased CBF) of chronic caffeine.

- To the best of our knowledge, ours is the first study to demonstrate chronic caffeine mediated CBF changes in rats subjected to a closed head injury in an experimental model of TBI in rats.

Cerebral blood flow changes in acute caffeine pretreated rats subjected to TBI

- In accordance with previous studies, our study demonstrated putative vasoconstrictive effects of caffeine (150 mg/kg) upon acute intake.
- Unlike sham, TBI and CC+TBI rats, significant reductions in CBF of the thalamus and brainstem were observed, compared to pre-TBI levels. This may suggest the negative effects of acute caffeine on CBF following TBI.
- In the case of TBI rats, acute reduction (19%) in CBF in the cortical (PCx) and sub-cortical (St, Hp and Th) regions by 4 hrs post-TBI was observed. But in the case of AC+TBI rats, this CBF reduction was exacerbated (decreased by 27%) by 4 hrs post-TBI. Furthermore, unlike TBI rats, global CBF in AC+TBI rats was significantly reduced at 4hrs post-TBI compared to its pre-TBI levels. This marked reduction in both regional and global CBF by 4 hrs post-TBI in acute caffeine pretreated rats can be attributed to the combined effect of TBI and vasoconstrictive effects of acute caffeine.
- Acute reduction (4 hrs post-TBI) in CBF following TBI plus acute caffeine pretreatment (150 mg/kg) leading to vasoconstriction may exacerbate reduction in

CBF following TBI and may even reach ischemic or sub-ischemic levels potentially worsening the outcome. This is an area requiring further study.

7.2 Future Work

Our study has demonstrated CBF changes following closed head injury up to 7 days post-TBI. But, ischemic cell death or ischemia mediated cell changes following TBI were not studied. Association between cerebral hypoperfusion and traumatic axonal injury (TAI) as well as neuronal cell injury needs to be studied, TAI is commonly observed sequel of TBI. Our study also demonstrated effects of repeated doses of chronic caffeine (1.5 g/L) and also a single dose of acute caffeine (150 mg/kg) on CBF changes following TBI. However, further studies are required to determine if these CBF changes are neuroprotective and helped reduced secondary injury changes. These can include behavioral, histological and biomarker studies. CSF samples from the rats in this study as well as fixed brains from these rats have been saved for such studies in the near future, as discussed below. Furthermore, the effects of other doses of chronic and acute caffeine on CBF following TBI need to be tested.

In this study, several other MRI sequences (T1 and T2 imaging, Susceptibility Weighted Imaging-SWI, Diffusion Tensor Imaging-DTI, Magnetic Resonance Spectroscopy-MRS) data were collected along with ASL data for future analysis. These data sets can be used to better understand the secondary axotomy of TBI and also effects of caffeine in offering neuroprotection against TBI. T1 and T2 images can be used to study structural or inflammatory changes following TBI. SWI data can provide information on oxygen saturation levels and micro hemorrhages. Changes in DTI parameters (FA - fractional

anisotropy, AD - axial diffusivity, RD – radial diffusivity, ADC - apparent diffusion coefficient) provide crucial information on axonal injury progression following TBI. Furthermore, MRS imaging data can be used to do quantitative analysis of changes in neurometabolites (N-acetylaspartate (NAA), creatine/phosphocreatine (Cr), choline containing compounds (Cho), lactate, etc) in vivo and to explore metabolic and biochemical changes in TBI; thus helping in prognosis of the outcome. Correlative studies between ASL and these MRI data sets can help in attaining a better understanding of TBI pathology and effects of caffeine treatment.

In addition to MRI data, biological samples (CSF, serum and brain tissue) have been collected from all the rats used in this study. It is planned to perform immunohistochemical and biomarker studies on these samples to better establish the effects of caffeine mediated CBF changes following TBI. Neuroprotective effects of caffeine (chronic & acute) can be tested by performing biomarker studies (e.g. ELISA or Western blotting) on CSF and serum samples. Effects of caffeine on inflammation following TBI can be studied using CSF and serum samples with antibodies, including IL-1, TNF-α and others. By determining caffeine concentration in CSF and serum following TBI using high pressure liquid chromatography (HPLC), its correlation to TBI outcome can be studied. Furthermore, histological assessment of inflammatory cell infiltration in brain (using standard immunofluorescence immunohistochemistry) with CD45 antibody (marker of inflammatory cells) can provide vital information regarding occurrence of inflammation following TBI and effects of caffeine pretreatment on inflammation following TBI can be better understood. Also, histological assessment of impaired axonal transport using β-APP and RMO-14 immunostaining and detection of

apoptotic cells by TUNEL staining can be done. These studies, independently and collectively, can provide crucial information in understanding and establishing the potential neuroprotective effects of caffeine in relation to TBI.

APPENDIX A

Glossary of Acronyms and Abbreviations

ABP - Arterial blood pressure

AC+TBI - Acute caffeine pretreated rats subjected to TBI

Ade - Adenosine

ADP - Adenosine diphosphate

AIS - Abbreviated injury severity

ANOVA - Analysis of variance

ASL - Arterial spin labeling

ATP - Adenosine triphosphate

Avg Hp - Average of left and right hippocampus

Avg PCx - Average of left and right parietal cortex

Avg St - Average of left and right striatum

BBB - Blood-brain-barrier

Bs - Brainstem

CASL - Continuous arterial spin labeling

cAMP - Cyclic adenosine monophosphate

CBF - Cerebral blood flow

CBV - Cerebral blood volume

CC+TBI - Chronic caffeine pretreated rats subjected to TBI

CDC - Centers for Disease Control and Prevention

CHI - Closed head injury

CNS - Central nervous system

CPA - Cerebral pressure autoregulation

CPP - Cerebral perfusion pressure

CVP - Cerebral venous pressure

CVSM - Cerebrovascular smooth muscle

DAI - Diffuse axonal injury

DTI - Diffusion tensor Imaging

EC50 - Half maximal effective concentration value

FoV - Field of view

g - grams

gCBF - Global cerebral blood flow

GPCR - G protein coupled receptor

GCS - Glasgow coma scale

ICP - Intracranial pressure

Lt Hp - Left hippocampus

Lt PCx - Left parietal cortex

Lt St - Left striatum

LDF - Laser-Doppler flowmetry

LD_{50} - Median lethal dose

LOC - Loss of consciousness

LSD - Least significant difference

m - meters

MABP - Mean arterial blood pressure

min - Minutes

MRI - Magnetic resonance imaging

MRS - Magnetic resonance spectroscopy

NO - Nitric oxide

OHI - Open head injury

PASL - Pulsed arterial spin labeling

PET - Positron emission tomography

PTA - Posttraumatic amnesia

rCBF - Regional cerebral blood flow

RF - Radio frequency

ROI - Region of interest

RT - Repetition time

Rt Hp - Right hippocampus

Rt PCx - Right parietal cortex

Rt St - Right striatum

sec - Seconds

SEM - Standard error of the mean

SMC - Smooth muscle cell

SNR - Signal-to-noise ratio

SPECT - Single photon emission computed tomography

SR - Surface righting

STDEV - Standard deviation

SWI - Susceptibility weighted imaging

TA - Time of acquisition

TAI - Traumatic axonal injury

TBI - Traumatic brain injury

TE - Echo time

Th - Bilateral thalamus

VSM - Vascular smooth muscle

XeCT - Xenon-enhanced computed tomography

APPENDIX B

Analysis of combined regional CBF (rCBF) by taking size of the cerebral regions into account.

Previously (Chapters 4, 5 & 6), combined rCBF of each rat at each time point was calculated by taking the simple average of the CBF values from all the regions where CBF was measured (Rt PCx, Lt PCx, Rt St, Lt St, Rt Hp, Lt Hp, Th and Bs). The size (number of pixels in ASL image) of the each region was not taken into account. Here, the combined rCBF was calculated by taking the number of pixels in each region into account. The number of pixels in the ASL image for each region represents the size of the corresponding region. The formula below demonstrates the combined rCBF calculation used here.

$$\frac{\{(\text{CBF of A} \times \text{No. of pixels in A}) + (\text{CBF of B} \times \text{No. of pixels in B}) + (\text{CBF of C} \times \text{No. of pixels in C}) + ...\}}{\text{No. of pixels in A} + \text{No. of pixels in B} + \text{No. of pixels in C} + ...}$$

Where, A, B, C,.... represent the different cerebral regions from where CBF was measured.

Combined regional CBF changes in sham group rats

Combined rCBF levels of the sham group rats calculated by taking size of cerebral regions into account remained at the same pre and post pure-water treatment (Figure B1). However, a significant decrease (23%) was observed at 24 hrs post-sham surgery and with a trend towards recovery by 3 days post-sham surgery. But, no significant changes in combined rCBF of sham group rats were found when the size of the cerebral regions (number of pixels in ASL image) was not included in combined rCBF calculation (Figure 4-14).

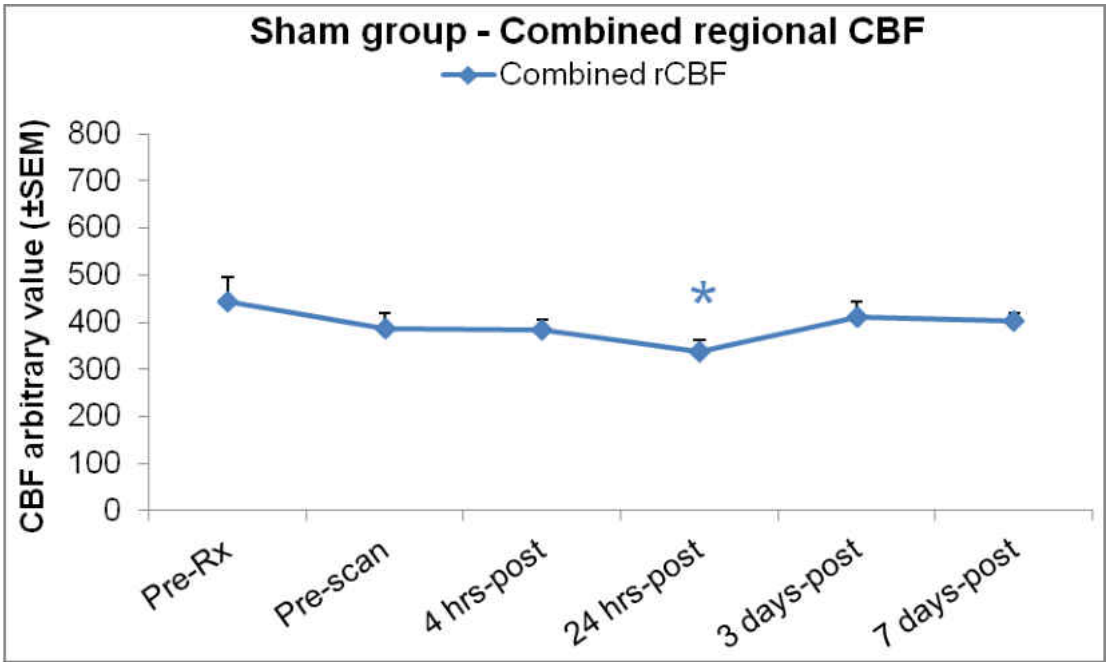


Figure B1: Plot showing changes in combined regional CBF (rCBF) of sham rats at various time points (pre-Rx=pre-water treatment, pre-scan=1 day before sham surgery, 4 hrs, 24 hrs, 3 days and 7 days post-sham surgery). Asterisk indicates significant difference (p<0.05) in combined rCBF at 24 hrs post-TBI compared to its pre-water treatment (pre-Rx) level.

Combined regional CBF changes in TBI-only group rats

In rats subjected to TBI, combined rCBF calculated by taking the size of cerebral regions into account showed a significant reduction (36%) by 7 days post-TBI compared to their pre-TBI level, as well as to their 24 hrs post-TBI and 3 days post-TBI levels (Figure B2). Although a slight reduction in combined rCBF was observed at 4 hrs post-TBI, it was not found to be significant. Combined rCBF of TBI rats calculated without taking the size of cerebral regions into account also showed a similar trend with a significant reduction by 7 days post-TBI compared to their pre-TBI level, as well as to their 24 hrs post-TBI and 3 days post-TBI levels (Figure 4-19). Also, global CBF in TBI rats showed a similar trend.

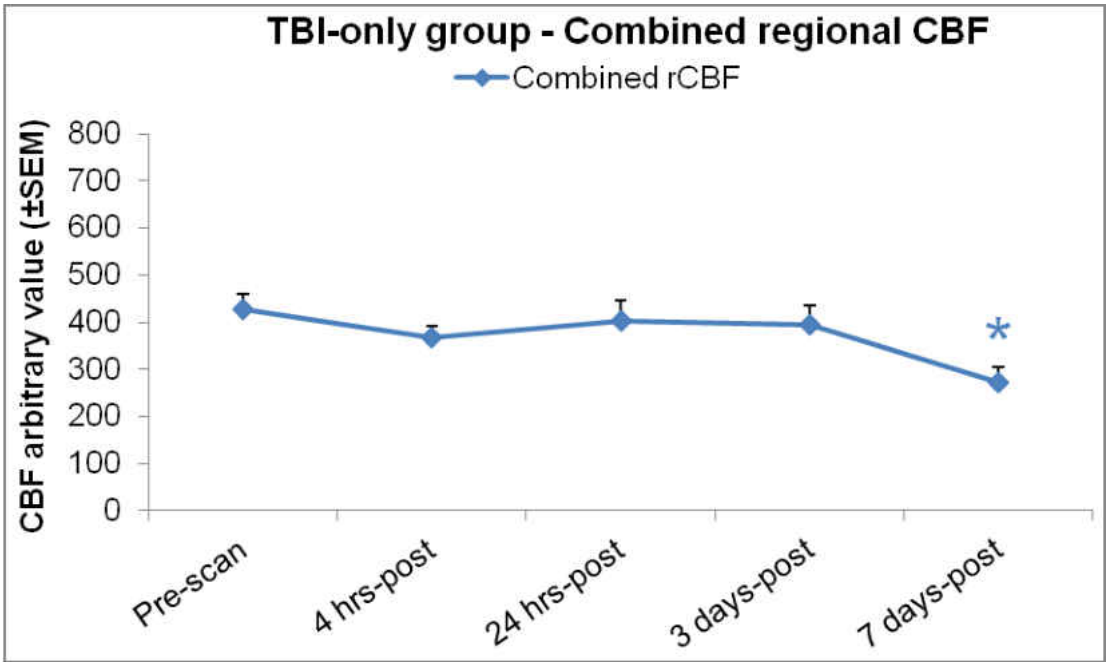


Figure B2: Plot showing changes in combined regional CBF (rCBF) in rats subjected to TBI. CBF was measured at various time points: pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisk indicates significant difference (p<0.05) in combined rCBF at 7 days post-TBI compared to its pre-TBI, 24 hrs and 3 days post-TBI.

Combined regional CBF changes in CC+TBI group rats

Combined rCBF of CC+TBI rats calculated by taking the size of cerebral regions into account showed a significant reduction (25%) by 4 hrs post-TBI compared to its pre-caffeine treatment levels and then a trend of recovery by 24 hrs and 3 days post-TBI (Figure B3). But, combined rCBF calculated without taking size of cerebral regions into account did not show any significant changes across the time points, when compared to its pre-caffeine treatment levels (Figure 5-6).

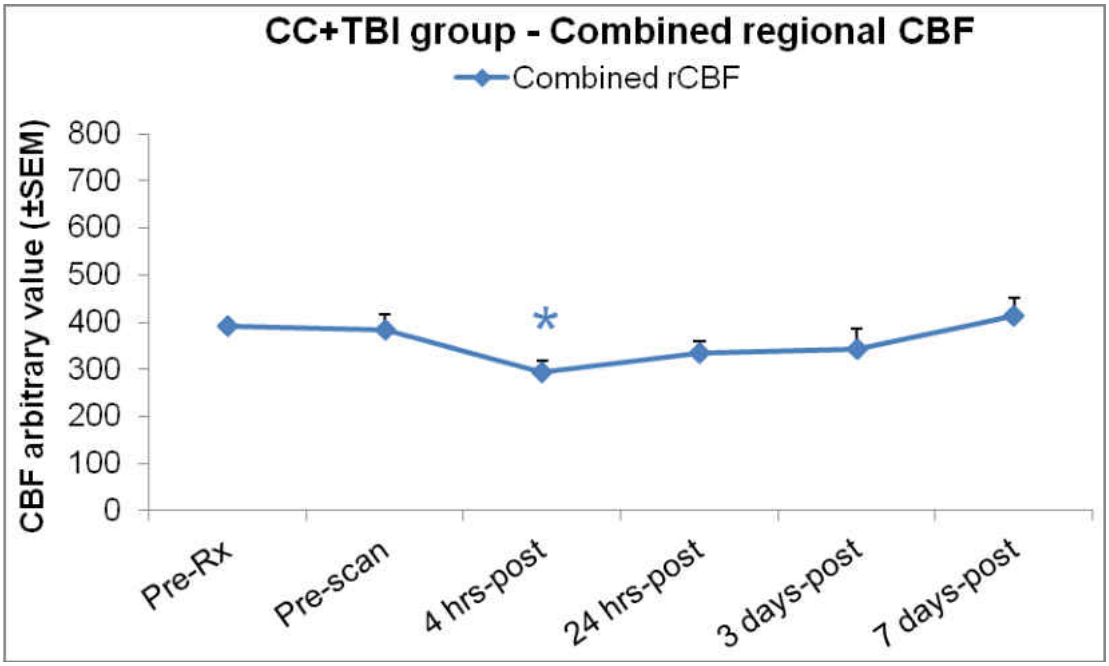


Figure B3: Plot showing changes in combined regional CBF (rCBF) in chronic caffeine pretreated rats at various time points: pre-Rx=pre-caffeine treatment 21 days before TBI, pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisk indicates significant difference (p<0.05) in combined rCBF at 4 hrs post-TBI compared to pre-caffeine treatment and 7 days post-TBI levels.

Combined regional CBF changes in AC+TBI group rats

Combined rCBF of AC+TBI rats calculated by taking size of cerebral regions into account showed a significant reduction (32%) by 4 hrs post-TBI compared to its pre-TBI and 7 days post-TBI levels (Figure B4). Combined rCBF calculated without taking size of cerebral regions into account also showed a significant decrease by 4 hrs post-TBI compared to its 7 days post-TBI level (Figure 5-11).

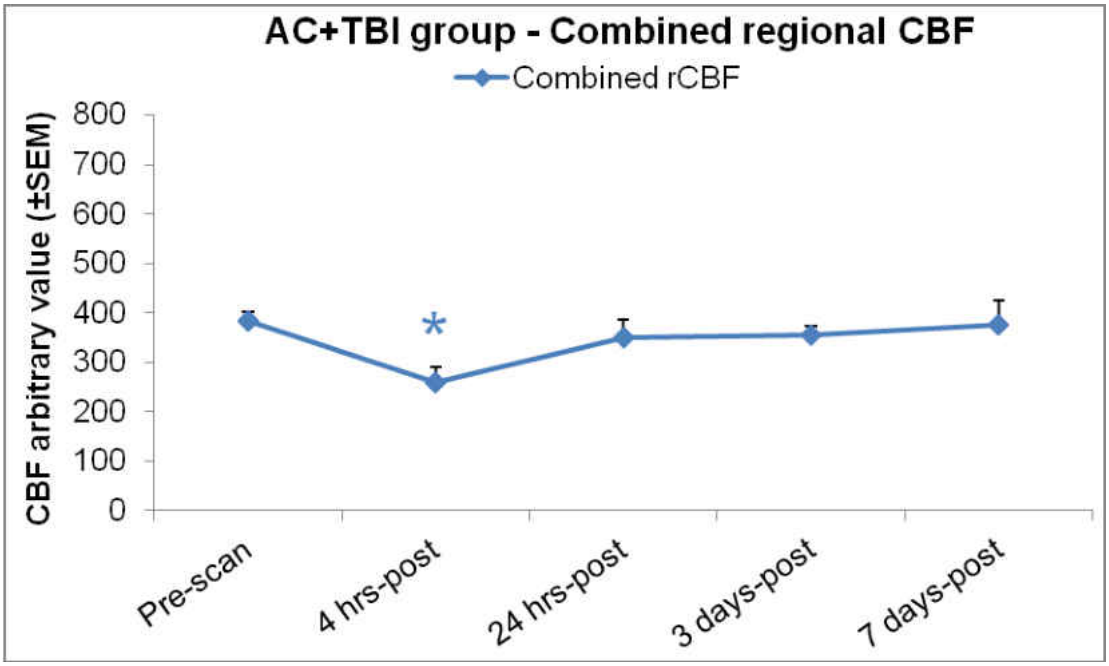


Figure B4: Plot showing changes in combined regional CBF (rCBF) in acute caffeine pretreated rats at various time points: pre-scan=1 day before TBI, 4 hrs, 24 hrs, 3 days and 7 days post-TBI. Asterisk indicates significant difference (p<0.05) in combined rCBF at 4 hrs post-TBI compared to its pre-TBI and 7 days post-TBI levels.

In general, combined rCBF calculated by taking the size of cerebral regions into account showed trends similar to combined rCBF calculated without considering the size of cerebral regions. The differences which resulted between the two combined rCBF calculation methods are: 1) Sham group rats showed significant decrease by 24 hrs post-sham surgery compared to pretreatment levels. 2) CC+TBI rats showed significant decrease by 4 hrs post-TBI compared to its pre-caffeine treatment levels.

APPENDIX C

Tables representing mean, standard deviation and percentage (%) change in CBF compared to the pre-Rx or pre-scan levels of rats in different groups.

CBF measurements from sham group rats:

Sham group - Right Parietal Cortex CBF						
Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	229	157		359	177	329
Rat 2	535	286	282	439	290	357
Rat 3	559	448	220	322	153	347
Rat 4	364	343			393	282
Rat 5	283	308		160	594	437
Rat 6	333	574	252	202	395	464
Rat 7	434	431	353	254	443	413
Average	391	364	277	289	349	376
STDEV	124	134	57	104	155	65
SEM	47	51	28	42	59	24
% Change	0	-6.9	-29.2	-26.0	-10.7	-3.9

Sham group - Left Parietal Cortex CBF						
Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	189	267	273	366	266	309
Rat 2	467	262	261	367	229	286
Rat 3	479	353	221	292	219	313
Rat 4	345	354	214		403	315
Rat 5	239	300		163		392
Rat 6	328	563	286	183	400	463
Rat 7	389	340	338	233	370	350
Average	348	348	266	267	315	347
STDEV	108	102	46	89	86	62
SEM	41	39	19	36	35	23
% Change	0	0.1	-23.7	-23.2	-9.6	-0.3

Sham group - Average of right and left parietal Cortex CBF

Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	209	212	273	363	221	319
Rat 2	501	274	271	403	260	322
Rat 3	519	400	220	307	186	330
Rat 4	354	348	214		398	298
Rat 5	261	304		161	594	415
Rat 6	331	569	269	192	398	464
Rat 7	411	386	345	243	406	381
Average	369	356	265	278	352	361
STDEV	116	114	47	96	141	61
SEM	44	43	19	39	53	23
% Change	0	-3.6	-28.2	-24.7	-4.8	-2.2

Sham group - Right striatum CBF						
Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	294		352	436	233	429
Rat 2	561	365	424	415	167	328
Rat 3	661		333	447	151	341
Rat 4	354	412			448	393
Rat 5	345	361		252	521	426
Rat 6	384	541		203	485	577
Rat 7	493	509	402	236	475	588
Average	442	438	378	332	354	440
STDEV	134	83	42	112	163	105
SEM	51	37	21	46	62	40
% Change	0	-0.9	-14.5	-25.0	-19.8	-0.3

Sham group - Left striatum CBF						
Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	331	227	445	495	260	442
Rat 2	474	343		383	318	349
Rat 3	646		377	466	213	374
Rat 4	403	424			462	420
Rat 5	364	337		239	535	420
Rat 6	398	560			487	550
Rat 7	481	522	409	237	471	551
Average	442	402	410	364	392	444
STDEV	105	125	34	122	126	79
SEM	40	51	20	55	48	30
% Change	0	-9.1	-7.3	-17.7	-11.3	0.3

Sham group - Average of right and left striatum CBF

Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	313	227	399	466	247	436
Rat 2	518	354	424	399	243	339
Rat 3	654		355	457	182	358
Rat 4	379	418			455	407
Rat 5	355	349		246	528	423
Rat 6	391	551		203	486	564
Rat 7	487	516	406	237	473	570
Average	442	403	396	335	373	442
STDEV	118	119	29	119	143	92
SEM	45	49	15	49	54	35
% Change	0	-9	-10.5	-24.4	-15.6	0

Sham group - Right hippocampus CBF						
Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1		173	297	327	170	292
Rat 2	384	271	356	360	360	318
Rat 3	342	371	228	293	365	383
Rat 4	367	383			373	313
Rat 5	365	325				388
Rat 6		440	279	226	420	395
Rat 7	299	331	321	211	361	400
Average	351	328	296	283	342	356
STDEV	33	86	48	64	87	46
SEM	15	33	21	29	36	17
% Change	0	-6.7	-15.7	-19.4	-2.8	1.2

Sham group - Left hippocampus CBF						
Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	230	249	315	386	248	338
Rat 2	433	380	303	450	444	
Rat 3	346	347	280	346	290	411
Rat 4	379	343			393	320
Rat 5	320	298				410
Rat 6		469	307	233	383	398
Rat 7	322	377	354	235	426	395
Average	338	352	312	330	364	379
STDEV	68	69	27	95	78	39
SEM	28	26	12	43	32	16
% Change	0	4	-7.8	-2.5	7.6	11.9

Sham group - Average of right and left hippocampus CBF

Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	230	211	306	357	209	315
Rat 2	409	326	330	405	402	318
Rat 3	344	359	254	320	328	397
Rat 4	373	363			383	317
Rat 5	343	312				399
Rat 6		455	293	230	402	397
Rat 7	311	354	338	223	394	398
Average	335	340	304	307	353	363
STDEV	61	73	33	79	76	43
SEM	25	28	15	36	31	16
% Change	0	1.5	-9.2	-8.4	5.4	8.4

Sham group - Thalamus CBF						
Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1		351	578	541	368	515
Rat 2	691	416	571	543	494	503
Rat 3	604	571	328	452	388	498
Rat 4	440	577	345		569	448
Rat 5	281	464	270	323		483
Rat 6	508	749		310	580	682
Rat 7	474	466	496	322	464	492
Average	500	513	431	415	477	517
STDEV	141	131	134	111	89	76
SEM	58	50	55	45	36	29
% Change	0	2.8	-13.7	-16.9	-4.5	3.5

Sham group - Brainstem CBF						
Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	564	600	545	626	537	580
Rat 2	737	431	493	748	444	602
Rat 3	718	524		842	567	547
Rat 4	717	671	689	590		658
Rat 5	645	735	532	646	944	696
Rat 6	741	788	792	654		680
Rat 7	708	748	617	601		693
Average	690	642	611	672	623	637
STDEV	64	131	113	91	220	60
SEM	24	49	46	34	110	23
% Change	0	-6.9	-11.4	-2.5	-9.7	-7.7
Sham group - Combined regional CBF						

Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	288	267	378	429	267	391
Rat 2	519	337	372	447	332	372
Rat 3	534	422	282	413	277	391
Rat 4	407	421	366	590	428	379
Rat 5	346	372	401	274	619	444
Rat 6	427	569	354	264	444	512
Rat 7	437	453	398	276	428	476
Average	422	406	364	385	399	424
STDEV	88	95	40	121	122	54
SEM	33	36	15	46	46	21
% Change	0	-3.9	-13.8	-9	-5.5	0.3

Sham group - Global CBF						
Rat ID	Pre-Rx	Pre-scan	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	390	410	423	494	369	392
Rat 2	559	381	435	580	388	481
Rat 3	517	490		535	375	439
Rat 4	476	539	452	409	561	502
Rat 5	442	495	449	543	582	508
Rat 6	510	602	505	384	577	490
Rat 7	586	574	477	425	463	612
Average	497	499	457	481	474	489
STDEV	67	81	30	76	98	68
SEM	25	31	12	29	37	26
% Change	0	0.3	-8.1	-3.2	-4.7	1.6

CBF measurements from TBI-only group rats:

TBI-only group - Right parietal cortex CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	254	304	175	596	157
Rat 2	450	307	239	326	419
Rat 3	298	363	452	189	256
Rat 4	529	221	347	484	169
Rat 5	502	434	557	490	201
Rat 6	539	379	562		202
Average	429	335	389	417	234
STDEV	123	74	163	160	97
SEM	50	30	66	71	40
% Change	0	-21.9	-9.3	-2.7	-45.4
TBI-only group - Left parietal cortex CBF					

Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	276	290	245	438	191
Rat 2	402	272	330	336	387
Rat 3	270	345	379	154	222
Rat 4	541	191	301	471	134
Rat 5	515	411	560	567	226
Rat 6	486		585		207
Average	415	302	400	393	228
STDEV	120	82	141	157	85
SEM	49	37	57	70	35
% Change	0	-27.3	-3.6	-5.3	-45.1

TBI-only group - Average of right and left parietal cortex CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	265	297	210	517	174
Rat 2	426	290	285	331	403
Rat 3	284	354	416	171	239
Rat 4	535	206	324	478	152
Rat 5	508	423	559	528	213
Rat 6	512	379	573		205
Average	422	325	395	405	231
STDEV	120	77	149	153	90
SEM	49	31	61	68	37
% Change	0	-23	-6.4	-4	-45.2

TBI-only group - Right striatum CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	386	494	231	605	216
Rat 2	451	555	353	430	416
Rat 3	488	462	466	164	250
Rat 4	624	238	457	577	182
Rat 5	695	555	601	539	215
Rat 6	670	462	670	679	159
Average	552	461	463	499	240
STDEV	128	117	160	183	92
SEM	52	48	65	75	38
% Change	0	-16.5	19.3	-9.7	-56.6

TBI-only group - Left striatum CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	426	450	268	463	172
Rat 2	546	496	423	434	416
Rat 3	484	415	435	190	231
Rat 4	650	248	467	549	
Rat 5	724	617	674	533	239
Rat 6	661	501	662	706	191
Average	582	455	488	479	250
STDEV	115	122	155	170	97
SEM	47	50	63	70	43
% Change	0	-21.9	-16.1	-17.6	-57.1

TBI-only group - Average of right and left striatum CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	406	472	250	534	194
Rat 2	499	526	388	432	416
Rat 3	486	439	451	177	241
Rat 4	637	243	462	563	182
Rat 5	710	586	638	536	227
Rat 6	666	482	666	693	175
Average	567	458	476	489	239
STDEV	120	117	156	174	90
SEM	49	48	64	71	37
% Change	0	-19.3	-16.1	-13.8	-57.8

TBI-only group - Right hippocampus CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	286	300	202	398	192
Rat 2	423		402	363	306
Rat 3	340	317	262	180	284
Rat 4	426		283		133
Rat 5	403			394	144
Rat 6			418		182
Average	376	309	313	334	207
STDEV	61	12	93	104	72
SEM	27	9	42	52	29
% Change	0	-17.9	-16.6	-11.1	-44.9

TBI-only group - Left hippocampus CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	279	306	195	348	181
Rat 2	368		403	353	324
Rat 3	345	310	253	141	285
Rat 4	440		313		123
Rat 5	403			466	188
Rat 6	436		420		174
Average	379	308	317	327	213
STDEV	61	3	96	135	76
SEM	25	2	43	68	31
% Change	0	-18.6	-16.3	-13.6	-43.9

TBI-only group - Average of left and right hippocampus CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	283	303	199	373	187
Rat 2	396		403	358	315
Rat 3	343	314	258	161	285
Rat 4	433		298		128
Rat 5	403			430	166
Rat 6	436		419		178
Average	382	309	315	331	210
STDEV	59	8	94	117	73
SEM	24	6	42	59	30
% Change	0	-19.3	-17.5	-13.6	-45.1

TBI-only group - Thalamus CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	384	352	283	537	253
Rat 2	454	410	524	425	585
Rat 3	425	417	514	251	283
Rat 4	663		522		242
Rat 5				652	
Rat 6			588		258
Average	482	393	486	466	324
STDEV	124	36	117	171	147
SEM	62	21	53	85	66
% Change	0	-18.4	1.0	-3.2	-32.7

TBI-only group - Brainstem CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	638	553	721		546
Rat 2	554		491	768	673
Rat 3	509	435	718	446	521
Rat 4	596	526	673	483	476
Rat 5	624	546		496	695
Rat 6	685			483	676
Average	601	515	651	535	598
STDEV	63	55	109	131	94
SEM	26	27	54	59	39
% Change	0	-14.3	8.3	-10.9	-0.5

TBI-only group - Combined regional CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	353	375	271	481	224
Rat 2	452	408	386	414	424
Rat 3	388	379	419	202	282
Rat 4	552	268	404	515	192
Rat 5	549	510	598	512	251
Rat 6	566	441	556	640	237
Average	477	397	439	461	268
STDEV	92	81	120	146	82
SEM	38	33	49	60	33
% Change	0	-16.8	-7.9	-3.3	-43.7

TBI-only group - Global CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	429	411	402	636	283
Rat 2	436	461	458	559	437
Rat 3	396	359	513	271	331
Rat 4	578	288	442	472	276
Rat 5	629	469	675	512	341
Rat 6	592	454	669		392
Average	510	407	527	490	343
STDEV	101	71	118	137	62
SEM	41	29	48	61	25
% Change	0	-20.2	3.2	-3.9	-32.7

CBF measurements in chronic caffeine pretreated rats subjected to TBI:

CC+TBI group - Right parietal cortex CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	314	283	328		258	444
Rat 2	366			502	562	397
Rat 3	290	269	249	388	375	255
Rat 4	298	561	260	402	205	461
Rat 5	282		375	240	233	432
Rat 6	335	426	256	268		348
Average	314	385	294	360	327	390
STDEV	32	137	56	107	147	77
SEM	13	69	25	48	66	31
% Change	0	22.5	-6.5	14.6	4.0	24

CC+TBI group - Left parietal cortex CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	326	310	276		287	476
Rat 2	343			490	453	355
Rat 3	265	278	219	311	338	308
Rat 4	280	582	285	439	204	465
Rat 5	317		368	263	209	411
Rat 6	370	366	249	249	212	307
Average	317	384	279	350	284	387
STDEV	39	137	56	108	99	75
SEM	16	68	25	48	40	31
% Change	0	21.2	-11.8	10.6	-10.4	22.1

CC+TBI group - Average of right and left parietal cortex CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	320	296	302		273	460
Rat 2	355			496	507	376
Rat 3	278	273	234	350	356	282
Rat 4	289	571	272	420	205	463
Rat 5	299		371	252	221	422
Rat 6	353	396	253	258	212	327
Average	316	384	286	355	296	388
STDEV	33	136	54	105	118	74
SEM	13	68	24	47	48	30
% Change	0	21.2	-9.6	12.1	-6.7	22.6

CC+TBI group - Right striatum CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	303	357	297	451		545
Rat 2	362			638	424	506
Rat 3		230	325	528	369	436
Rat 4	393	535	518	438		447
Rat 5	389		687	397	213	474
Rat 6	364	603	420	354		365
Average	362	431	449	468	335	462
STDEV	36	170	159	102	109	62
SEM	16	85	71	42	63	25
% Change	0	36.1	41.8	47.6	5.8	45.9

CC+TBI group - Left striatum CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	317	366	293	539		607
Rat 2	393			688	459	499
Rat 3		325	372	501	352	410
Rat 4	378	512	546	466	229	511
Rat 5	422		684	385	241	485
Rat 6	373	588	452	358		338
Average	377	448	469	490	320	475
STDEV	38	123	152	119	108	92
SEM	17	62	68	49	54	38
% Change	0	18.9	24.6	30.0	-15.0	26.1

CC+TBI group - Average of right and left striatum CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	310	362	295	495		576
Rat 2	378			663	442	503
Rat 3		278	349	515	361	423
Rat 4	386	524	532	452	229	479
Rat 5	406		686	391	227	480
Rat 6	369	596	436	356		352
Average	370	440	460	479	315	469
STDEV	36	146	155	109	105	76
SEM	16	73	69	44	53	31
% Change	0	19.0	24.3	29.4	-14.9	26.8

CC+TBI group - Right hippocampus CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	272	226	281	323	237	
Rat 2	314	364			300	413
Rat 3			282	343	223	203
Rat 4	361	423	339	286	194	404
Rat 5	269				256	
Rat 6	292	363	363	297		337
Average	302	344	316	312	242	339
STDEV	38	84	41	26	40	97
SEM	17	42	21	13	18	48
% Change	0	14.1	4.9	3.5	-19.8	12.5

CC+TBI group - Left hippocampus CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	287	233	236	331	262	
Rat 2	356	414			332	412
Rat 3			282	365	248	229
Rat 4	360	455	358	351	217	409
Rat 5	312				277	
Rat 6	296	290	404	290		314
Average	322	348	320	334	267	341
STDEV	34	104	75	33	42	87
SEM	15	52	38	16	19	44
% Change	0	8.0	-0.7	3.7	-17.1	5.8

CC+TBI group - Average of right and left hippocampus CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	280	230	259	327	250	
Rat 2	335	389			316	413
Rat 3			282	354	236	216
Rat 4	361	439	349	319	206	407
Rat 5	291				267	
Rat 6	294	327	384	294		326
Average	312	346	319	324	255	341
STDEV	34	90	58	25	41	92
SEM	15	45	29	12	18	46
% Change	0	10.9	2.0	3.6	-18.3	9.1

CC+TBI group - Thalamus CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	343	314				598
Rat 2	437				664	612
Rat 3		395			521	414
Rat 4	450	563	423	373	281	
Rat 5					322	
Rat 6	409		431	342	237	516
Average	410	424	427	358	405	535
STDEV	48	127	6	22	181	91
SEM	24	73	4	16	81	46
% Change	0	3.5	4.2	-12.8	-1.2	30.6

CC+TBI group - Brainstem CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	812	613		569	678	449
Rat 2	781	647		673	847	446
Rat 3	705	731	321	469	438	538
Rat 4	631	604	643	594	628	778
Rat 5	537		690		476	796
Rat 6	526	705	709		404	685
Average	665	660	591	576	579	615
STDEV	121	56	182	84	170	159
SEM	50	25	91	42	70	65
% Change	0	-0.8	-11.2	-13.4	-13.1	-7.5

CC+TBI group - Combined regional CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	353	326	285	434	321	519
Rat 2	402	454		593	482	448
Rat 3	385	347	292	412	347	338
Rat 4	381	524	411	413	260	482
Rat 5	352		552	321	267	500
Rat 6	362	466	396	307	266	383
Average	372	424	387	413	324	445
STDEV	20	84	109	102	85	71
SEM	8	38	49	42	35	29
% Change	0	13.7	4.0	11.0	-13.0	19.5

CC+TBI group - Global CBF						
Rat ID	Pre-Rx	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	612	424	462	422	468	577
Rat 2	534			697	624	508
Rat 3	463	506	339	534	477	410
Rat 4	445	562	488	538	338	630
Rat 5	433		583	364	328	592
Rat 6	437	598	523	358	425	594
Average	487	523	479	486	443	552
STDEV	72	76	90	130	109	80
SEM	29	38	40	53	44	33
% Change	0	7.2	-1.7	-0.4	-9.0	13.2

CBF measurements in acute caffeine pretreated rats subjected to TBI:

AC+TBI group - Right parietal cortex CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1		291	442		595
Rat 2	450	417	447		393
Rat 3	277	117	244	284	524
Rat 4	202	185		345	341
Rat 5	263	200		328	663
Rat 6	341	313	419	113	338
Rat 7	439	377	470	776	197
Average	329	268	395	369	409
STDEV	100	109	91	245	164
SEM	41	41	41	110	62
% Change	0	-18.4	20.2	12.3	24.5

AC+TBI group - Left parietal cortex CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1		282	429		576
Rat 2	418	306			327
Rat 3	249	158	211	294	371
Rat 4	219	235		326	308
Rat 5	262	183		259	460
Rat 6	296	264	406	175	308
Rat 7	442	361	460	717	221
Average	314	251	359	354	333
STDEV	93	70	113	210	117
SEM	38	27	56	94	44
% Change	0	-20.1	14.2	12.7	5.8

AC+TBI group - Average of left and right parietal cortex CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1		287	435		586
Rat 2	434	362	447		360
Rat 3	263	138	228	289	448
Rat 4	211	210		335	324
Rat 5	262	192		293	562
Rat 6	319	289	412	144	323
Rat 7	440	369	465	747	209
Average	322	260	388	362	371
STDEV	96	87	97	227	137
SEM	39	33	43	102	52
% Change	0	-19.1	20.7	12.5	15.4

AC+TBI group - Right striatum CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	446	462	393		677
Rat 2	458	431	371		389
Rat 3	266	182	162	405	
Rat 4		136		508	466
Rat 5	266	278		436	726
Rat 6	480	133	455	246	457
Rat 7	579	485	426		207
Average	410	274	354	399	449
STDEV	125	156	116	111	191
SEM	51	59	52	55	78
% Change	0	-33.1	-13.7	-2.7	9.6

AC+TBI group - Left striatum CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	423	444	369		606
Rat 2	484	386	514		410
Rat 3	310	272	149	420	544
Rat 4		222		467	431
Rat 5	271	265	71	405	501
Rat 6	477	201	465	252	432
Rat 7	637	451	505		229
Average	436	300	341	386	425
STDEV	132	105	191	93	120
SEM	54	40	78	47	45
% Change	0	-31.3	-21.8	-11.4	-2.6

AC+TBI group - Average of right and left striatum CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	435	453	381		642
Rat 2	471	409	443		400
Rat 3	288	227	156	413	544
Rat 4		179		488	449
Rat 5	269	272	71	421	614
Rat 6	479	167	460	249	445
Rat 7	608	468	466		218
Average	423	287	319	393	445
STDEV	128	130	172	102	144
SEM	52	49	70	51	55
% Change	0	-32.2	-24.5	-7.2	5.2

AC+TBI group - Right hippocampus CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	387			396	
Rat 2	367	338	365		356
Rat 3	274			318	351
Rat 4				431	243
Rat 5	224	154		381	530
Rat 6	391	212	259	253	388
Rat 7	392		243		209
Average	330	235	289	346	346
STDEV	72	94	66	71	114
SEM	29	54	38	32	47
% Change	0	-28.8	-12.3	4.9	5.0

AC+TBI group - Left hippocampus CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	393			442	
Rat 2	368	326	368		386
Rat 3	344			370	384
Rat 4				351	351
Rat 5	214	170		347	449
Rat 6	371	223	323	371	400
Rat 7	367		298		197
Average	333	240	330	360	361
STDEV	65	79	35	38	87
SEM	27	46	20	17	35
% Change	0	-28	-0.9	8.1	8.5

AC+TBI group - Average of right and left hippocampus CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	390			419	
Rat 2	368	332	367		371
Rat 3	309			344	368
Rat 4				391	297
Rat 5	219	162		364	490
Rat 6	381	218	291	312	394
Rat 7	380		271		203
Average	331	237	310	353	354
STDEV	67	87	51	41	97
SEM	27	50	29	18	39
% Change	0	-28.4	-6.6	6.4	6.8

AC+TBI group - Thalamus CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	496		386		
Rat 2	434		381		423
Rat 3	458			592	491
Rat 4				564	395
Rat 5	304			482	660
Rat 6	581	226	536	453	474
Rat 7	561	422	411		331
Average	468	324	443	523	462
STDEV	100	139	73	66	113
SEM	41	98	36	33	46
% Change	0	-30.7	-5.3	11.8	-1.1

AC+TBI group - Brainstem CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	607	581	671		
Rat 2	577	556	718		717
Rat 3	518			511	582
Rat 4	562		574	580	733
Rat 5	547		576	428	643
Rat 6	534	276	668	513	761
Rat 7	621	476	579	751	683
Average	560	436	623	557	687
STDEV	38	138	62	121	66
SEM	14	69	26	54	27
% Change	0	-22.1	11.3	-0.6	22.6

AC+TBI group - Combined regional CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	447	400	438	419	614
Rat 2	439	386	442		412
Rat 3	323	182	192	385	461
Rat 4	299	195	574	435	394
Rat 5		208	239	377	573
Rat 6	423	229	427	280	429
Rat 7	497	426	418	748	264
Average	396	271	382	445	422
STDEV	77	109	131	160	117
SEM	31	41	50	65	44
% Change	0	-31.5	-3.6	12.4	6.6

AC+TBI group - Global CBF					
Rat ID	Pre-TBI	4 hrs-post	24 hrs-post	3 days-post	7 days-post
Rat 1	550	456	508		
Rat 2	489	447	550		425
Rat 3	385	262		434	464
Rat 4	440	236	323	455	408
Rat 5	351	225	377	387	579
Rat 6	472	249	481	421	445
Rat 7	492	388	422	595	284
Average	438	301	431	458	434
STDEV	68	103	85	80	95
SEM	26	39	35	36	39
% Change	0	-31.3	-1.7	4.6	0.9

APPENDIX D

Tables showing the sample size (n) for CBF measurements in each region at their corresponding time point for rats in all four different groups:

Sham Group: Sample size (n) for CBF measurements in each region at each time point						
Cerebral Regions	**Pre-Rx**	**Pre-scan**	**4 hrs-post**	**24 hrs-post**	**3 days-post**	**7 days-post**
Rt Pcx	7	7	4	6	7	7
Lt Pcx	7	7	6	6	6	7
Avg PCx	7	7	6	6	7	7
Rt St	7	5	4	6	7	7
Lt St	7	6	3	5	7	7
Avg St	7	6	4	6	7	7
Rt Hp	5	7	5	5	6	7
Lt Hp	6	7	5	5	6	6
Avg Hp	6	7	5	5	6	7
Th	6	7	6	6	6	7
Bs	7	7	6	7	4	7
Combined rCBF	7	7	7	7	7	7
Global CBF	7	7	6	7	7	7

TBI-only Group: Sample size (n) for CBF measurements in each region at each time point					
Cerebral Regions	**Pre-scan**	**4 hrs-post**	**24 hrs-post**	**3 days-post**	**7 days-post**
Rt Pcx	6	6	6	5	6
Lt Pcx	6	5	6	5	6
Avg PCx	6	6	6	5	6
Rt St	6	6	6	6	6
Lt St	6	6	6	6	5
Avg St	6	6	6	6	6
Rt Hp	5	2	5	4	6
Lt Hp	6	2	5	4	6
Avg Hp	6	2	5	4	6
Th	4	3	5	4	5
Bs	6	4	4	5	6
Combined rCBF	6	6	6	6	6
Global CBF	6	6	6	5	6

CC+TBI Group: Sample size (n) for CBF measurements in each region at each time point						
Cerebral Regions	**Pre-Rx**	**Pre-scan**	**4 hrs-post**	**24 hrs-post**	**3 days-post**	**7 days-post**
Rt Pcx	6	4	5	5	5	6
Lt Pcx	6	4	5	5	6	6
Avg PCx	6	4	5	5	6	6
Rt St	5	4	5	6	3	6
Lt St	5	4	5	6	4	6
Avg St	5	4	5	6	4	6
Rt Hp	5	4	4	4	5	4
Lt Hp	5	4	4	4	5	4
Avg Hp	5	4	4	4	5	4
Th	4	3	2	2	5	4
Bs	6	5	4	4	6	6
Combined rCBF	6	5	5	6	6	6
Global CBF	6	4	5	6	6	6

AC+TBI Group: Sample size (n) for CBF measurements in each region at each time point					
Cerebral Regions	**Pre-scan**	**4 hrs-post**	**24 hrs-post**	**3 days-post**	**7 days-post**
Rt Pcx	6	7	5	5	7
Lt Pcx	6	7	4	5	7
Avg PCx	6	7	5	5	7
Rt St	6	7	5	4	6
Lt St	6	7	6	4	7
Avg St	6	7	6	4	7
Rt Hp	6	3	3	5	6
Lt Hp	6	3	3	5	6
Avg Hp	6	3	3	5	6
Th	6	2	4	4	6
Bs	7	4	6	5	6
Combined rCBF	6	7	7	6	7
Global CBF	7	7	6	5	6

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ABSTRACT

TRAUMATIC BRAIN INJURY INDUCED CEREBRAL BLOOD FLOW CHANGES – A POTENTIAL ROLE FOR CAFFEINE

by

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Traumatic brain injury (TBI) is a global health problem with significant socio-economic costs. Closed head TBIs are one of the major causes of physical disability and cognitive disorder in young adults and a leading cause of death in children.

Alteration in cerebral blood flow due to an impaired autoregulation is one of the most common consequences of TBI. However, studies related to understanding the temporal changes in cerebral blood flow (CBF) following TBI in experimental models are limited. The few available studies report acute reduction in CBF following TBI; knowledge related to CBF changes at sub-acute periods extending to 7 days after TBI is still not known. Furthermore, reduction in CBF has been associated with unfavorable neurological outcome and can render the brain vulnerable to secondary damage. However, interventions that can restore neuronal function after TBI are lacking. A few available studies have demonstrated that caffeine acts as a neuroprotectant in several neurological disorders acting through diverse mechanisms. It has been postulated that caffeine may offer neuroprotection by restoring or maintaining adequate CBF following

TBI. Thus, studying the CBF changes following TBI and its potential modulation by caffeine pretreatment forms the central theme of this research.

We investigated the CBF changes in male Sprague Dawley rats at 4hrs, 24 hrs, 3 days and 7 days following closed head injury, with and without caffeine (chronic and acute) pretreatment. TBI was induced using the Marmarou impact acceleration device (2 m height, 450 g weight). Rats subjected to TBI showed reduced regional and global CBF at 4hrs and 7 days following TBI. In contrast, rats that underwent chronic caffeine pretreatment (1.5 g/L) for 3 weeks did not show apparent changes in regional and global CBF following TBI, indicating a potential benefit after TBI. Acute caffeine treatment (150 mg/kg, i.p. injection 30 minutes before TBI) showed significant reductions in CBF at 4 hrs post-TBI, further deteriorating the cerebral perfusion.

In addition, chronic caffeine pretreated rats demonstrated significantly reduced surface righting duration following TBI, compared to acute caffeine treated rats subjected to TBI and rats subjected to TBI without caffeine treatment. Therefore, chronic caffeine treatment may be beneficial in offering a degree of neuroprotection against TBI. This study was able to support the hypothesis that chronic caffeine can restore or optimize CBF following TBI and this optimization may be related to the adaptation of adenosine receptors on vascular smooth muscle cells. This may form the stepping stone for further studies on beneficial effects of caffeine in TBI.

AUTOBIOGRAPHICAL STATEMENT

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Master of Science: Biomedical Engineering, Wayne State University, Detroit, MI, USA (2010 - 2012).

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- Graduate Research Scholarship for the year 2012 from the Office of the Vice President for Research (OVPR) **Program for Traumatic Brain Injury Research (PTBIR)**, Wayne State University.
- Best poster award at the 3rd Annual Traumatic Brain Injury Workshop, Wayne State University.

PUBLICATIONS

- Nisrine Zakaria, Srinivas Kallakuri, **Sharath Bandaru** and John Cavanaugh (2012). Temporal assessment of traumatic axonal injury in the rat corpus callosum and optic chiasm. Brain Research 1467, 81-90.

- Srinivas Kallakuri, Yan Li, Runzhou Zhou, **Sharath Bandaru**, Nisrine Zakaria, Liying Zhang and John Cavanaugh (2012). Impaired axoplasmic transport is the dominant injury induced by an impact acceleration injury device: an analysis of traumatic axonal injury in pyramidal tract and corpus callosum of rats. Brain Research 1452, 29-38.