University of Alberta

Assessing Outcome after Hyperthermia in a Rat Model of Intracerebral Hemorrhage

by

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A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Psychology

Fall 2010

Edmonton, Alberta

Examining Committee

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Abstract

Hyperthermia worsens outcome after ischemia. While it seems reasonable that hyperthermia would also worsen outcome after intracerebral hemorrhage (ICH), clinical studies attempting to find a causative relationship between hyperthermia and outcome have been inconclusive. We induced ICH with an injection of autologous whole blood (100 µl) immediately followed by 3 hours of hyperthermia (HYPER; 39°C) or normothermia (NORMO; 37°C). Surprisingly, hyperthermia reduced edema at 72 hours, and improved outcome on day 3 post-ICH. There were no behavioural differences at later time points (day 11 and 32 post-ICH) and no difference in lesion volume (NORMO 14.0 mm^3, HYPER: 14.5 mm^3). Overall, this study does not support the hypothesis that mild, transient hyperthermia worsens outcome after ICH. Further research is needed to determine if more severe or prolonged hyperthermia worsens outcome, or if the cause of hyperthermia (e.g. infection) is important.

Acknowledgements

This research was supported by a grant from the Canadian Institutes of Health Research (CIHR) to Fred Colbourne, who is supported by an Alberta Heritage Foundation for Medical Research senior medical scholar award. I was supported by a Natural Sciences and Engineering Research Council Canada Graduate Scholarship (NSERC) as well as through the department of psychology.

I would like to extend a thank you to my supervisor Dr. Frederick Colbourne for mentoring me over the past four years. Thank you for your understanding and patience, I know it has not been easy and you have many more grey hairs as a result.

In addition, I would like to thank all of the lab members that I have worked with during my degree. Thank you for your knowledge and expertise, but more importantly thank you for providing me with a much needed distraction. I should especially thank two graduate students that have been by my side throughout this journey. Angela, you are really funny and have provided me with many comedic moments! And Greg, well, you try!

I also need to thank my family for all of the encouragement over the many years of my education. Thanks for all of the support, both emotional and financial, especially the financial. Financial support is always encouraged. I especially need to thank my dog Jack, who always plays with me when I go home for Christmas. Jack, you are always so happy to see me. That is such a rare thing to find in a person or in this case a dog. Thank you so much!

I also need to thank podcasting. You have always given me a much needed distraction. I owe you a debt of gratitude. Speaking of podcasts, I need to thank my fellow podcaster and office mate Michele Moscicki. Listening and to podcasts and your great stories all day gives me a reason to come in to work. You're the greatest!!!

Thank you all! You have all given me the motivation I needed to get this degree.

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Figure 5. The amount of tissue lost (mm^3) is presented. The volume of tissue lost did not differ between groups at day 32 post-ICH.

List of Abbreviations

ANOVA	Analysis of Variance
AP	Anterior-Posterior
BBB	Blood-Brain Barrier
BP	Blood Pressure
BWC	Brain-Water Content
c3	complement component 3
CBF	Cerebral Blood Flow
CSF	Cerebrospinal Fluid
DV	Dorsal-Ventral
DWI	Diffusion Weighted MRI
GCS	Glasgow Coma Scale
g/dL	grams/decilitre
HYPER	Hyperthermia group
ICH	Intracerebral Hemorrhage
ICP	Intracranial Pressure
LPS	Lipopolysaccharide

MABP	Mean Arterial Blood Pressure
MAC	Membrane Attack Complex
MCA	Middle Cerebral Artery
ML	Medial-Lateral
mmHg	millimetres of mercury
MMPs	Matrix Metalloproteinases
N_2O	Nitrous Oxide
NDS	Neurological Deficit Scale
NORMO	Normothermia group
O_2	Oxygen
pCO_2	Partial Pressure of Carbon Dioxide
PET	Positron Emission Tomography
PGE_2	Prostaglandin E_2
pO_2	Partial Pressure of Oxygen
PWI	Perfusion Weighted MRI
RBCs	Red Blood Cells
RCTs	Randomized Clinical Trials

ROS	Reactive Oxygen Species
SEM	Standard Error of the Mean
TBI	Traumatic Brain Injury
TNF-α	Tumor Necrosis Factor-Alpha
t-PA	tissue-Plasminogen Activator
Tukey HSD	Tukey Honestly Significant Difference

1. Introduction

1.1 Stroke Background

Stroke is a leading cause of death and disability (Donnan et al., 2008; Murray & Lopez, 1997). Ischemic stroke accounts for the majority of strokes, with estimates ranging from 80 to 90 % of all strokes. Ischemia results from blockage of blood flow to the brain. The two forms of ischemia are global and focal. Global ischemia results from blockage of blood flow to a large area of the brain, often due to cardiac arrest. Focal ischemia results from blockage to a specific area, often through blockage of the Middle Cerebral Artery (MCA). Since ischemia is the most common form of stroke, the majority of the research and hospital resources are directed towards treatment of ischemic stroke.

1.2 Intracerebral Hemorrhage

Hemorrhage accounts for the remainder of the strokes (10 to 20%). There are many types of hemorrhage including intracerebral (parenchymal or intraventricular), subarachnoid, epidural, and subdural. Intracerebral hemorrhage (ICH) accounts for 10 to 20 % of strokes in North America and ~ 30 % worldwide (Woo & Broderick, 2002). Intracerebral hemorrhage causes a significant financial burden in Canada (Goeree et al., 2005), the United States (Holloway et al., 1996), and worldwide (Christensen et al., 2009).

Intracerebral hemorrhage has a higher mortality rate than any other type of stroke (Broderick et al., 1999), and has not improved over the past two decades

(Aronowski & Hall, 2005). The primary cause of ICH is spontaneous rupture of one or more blood vessels (primary ICH) in the brain. This type of ICH accounts for 70 to 80 % of all intracerebral hemorrhages. Secondary ICH results from malformations that predispose patients to an ICH such as arteriovenous malformation, moyamoya disease, aneurysyms, tumours, coagulopathy, and vasculitis (Feldmann, 1991; Qureshi et al., 2001; Skidmore & Andrefsky, 2002). Outcome depends on many factors including size and location (putaminal, thalamic, lobar, cerebellar, & pontine). Pontine hemorrhages, for instance, result in higher mortality rate than other types (Xi, Keep, & Hoff, 2006).

1.3 Risk Factors

There are a number of risk factors for ICH, with age being the primary risk factor. The risk of ICH increases with each decade, with a sharp increase in the incidence of ICH past the age of 65 (Ariesen et al., 2003; Sturgeon et al., 2007). Race and sex are also important factors for ICH. Both African-Americans (Sturgeon et al., 2007) and Asians have higher incidence of ICH than caucasians (Qureshi et al., 2001). Males have a higher risk for ICH than females (Ariesen et al., 2003; Qureshi et al., 2001).

The most important modifiable risk factor for ICH is hypertension. The incidence of hypertension significantly increases the risk of ICH (Calandre et al., 1986). Song and colleagues (2004b) studied the relationship between hypertension and risk of ICH. They found that risk of ICH increased with each level of hypertension (systolic/diastolic; normal: < 140/< 90 mmHg; stage 1:

blood pressure (BP) = 140-159/90-99 mmHg; stage 2: BP = 160-179/100-109 mmHg; stage 3: BP > 179/> 109 mmHg). Alcohol consumption (Juvela, 1995; Thrift, Donnan, & McNeil, 1999), and body weight (Song et al., 2004a) are also risk factors for ICH. Drugs can cause ICH either through hypertension or coagulopathy (Diringer, 1993). These drugs include cocaine, amphetamine, and methylphenidate among others (Diringer, 1993).

1.4 Pathophysiology of Intracerebral Hemorrhage

When a blood vessel ruptures, it causes formation of a pocket of blood within the tissue, termed a hematoma. A number of pathophysiogical events contribute to the hematoma. There are three major pathophysiological phases for ICH: hematoma formation, hematoma enlargement, and cerebral edema (Ferro, 2006; Rincon & Mayer, 2004). Cerebral edema refers to the accumulation of extracellular fluids in the brain. The first phase is vascular rupture, which results in hematoma formation. The second phase (hematoma expansion) is mediated by a number of factors including continuous bleeding from the ruptured vessel, re-bleeding from the same place, bleeding from surrounding compressed vessels, and local clotting defects (Ferro, 2006). Cellular toxicity and blood degradation products (Rincon & Mayer, 2004) mediate the final phase (edema formation). Rupture of the blood vessel causes immediate destruction of the tissue (Brott et al., 1997) due to shearing forces of the expanding hematoma. This causes immediate cell death that is unlikely to be salvageable. Hematoma formation is mediated by blood pressure and coagulation abnormalities. In ischemia, there is

some immediate destruction of tissue, but tissue surrounding the main infarct is also compromised. In this area, blood flow is reduced to the extent that it causes dysfunction, but normal function can return if blood flow can be restored quickly. If blood flow is not restored, the tissue will continue to degrade over the first few days after the stroke, and much of the tissue will eventually die. This territory has been termed the penumbra, and treatment has been focused on salvaging this tissue.

There has been debate about whether or not there is an ischemic penumbra surrounding the ICH. Studies have shown that there is a reduction in blood flow in the peri-hematoma surrounding the ICH (Butcher et al., 2004; Zazulia et al., 2001). Butcher and colleagues (2004) studied blood flow in the peri-hematoma using perfusion-weighted MRI (PWI) and diffusion-weighted MRI (DWI). They found that blood flow was reduced in the peri-hematoma, but it recovered within 3 to 5 days and was not associated with MRI markers of ischemia. It has been reported that reduction in blood flow is more likely to due to reduced metabolic demand than ischemia. Zazulia and colleagues (2001) used positron emission tomography (PET) and found that there was a lower oxygen extraction fraction in the peri-hematoma rather than an increased oxygen extraction fraction that results from ischemia.

Initially, it was believed that there was no hematoma expansion due to the tamponade effect. Tamponade refers to the stoppage of blood flow due to compression by outside forces. Enlargement would stop due to equilibrium

between bleeding pressure and intracranial pressure and surrounding brain areas would "tamponade" the injury and promote clotting (Ferro, 2006). However, recent studies have shown that there is an enlargement of the hematoma in both rat (Nguyen et al., 2008) and human studies (Brott et al., 1997; Davis et al., 2006).

A number of factors contribute to the expanding hematoma. Immediately following the bursting of the blood vessels, blood products start to degrade the tissue surrounding the rupture. The coagulation cascade is initiated once the blood vessels rupture. A major component of the coagulation cascade is thrombin, which is a serine protease generated by the cleavage of prothrombin. Thrombin is a major factor in the production of early edema following ICH and neuronal injury (Hua et al., 2007; Kitaoka et al., 2002). Thrombin activates reactive oxygen species (ROS) and matrix metallproteinases (MMPs), and increases pro-inflammatory factors (Hua et al., 2007). Thrombin can produce damage through glutamate-mediated pathways and blood-brain barrier (BBB) disruption (Gingrich et al., 2000). Injection of thrombin directly into the striatum of rats causes cerebral edema (Kawai et al., 2001), and inhibiting thrombin reduces edema in animal models of ICH (Kitaoka et al., 2002; Lee et al., 1996).

A significant contributor to ICH injury is the complement cascade. The complement cascade consists of the activation of 30 serum and membrane-bound proteins. Activation of the cascade occurs via one of three pathways (classical, mannose-binding lectin, or alternative) each of which act on complement component 3 (c3). This activation results in the initiation of multiple injury

pathways. When an ICH occurs, thrombin is released at the site of the hematoma leading to c3 cleavage (Ducruet et al., 2009). This leads to a number of pathological events including Anaphylatoxin-mediated inflammation, membrane attack complex (MAC)-lysis of erythrocytes, hemoglobin release, blood-brain barrier (BBB) breakdown, cerebral edema, cytokine release, and iron toxicity. Complement component 3 expression peaks at 48 to 72 hours post-ICH, and markers indicative of BBB breakdown and edema peak at 72 hours post-ICH.

Erythrocyte lysis occurs within the first 24 hours of the ICH (Wu et al., 2003). Lysis of red blood cells (RBCs) can result from either depletion of the intracellular energy store or activation of the complement cascade (Hua et al., 2000). Lysis of RBCs causes release of thrombin and iron (Hua et al., 2007). Injection of lysed RBCs leads to edema (Huang et al., 2002; Xi, Keep, & Hoff, 1998), DNA damage (Wu et al., 2002; Xi, Keep, & Hoff, 1998), and BBB disruption (Xi et al., 2001). Metabolism of heme oxygenase occurs after RBC lysis, and increases iron accumulation. Inhibition of heme oxygenase decreases edema formation (Huang et al., 2002).

Iron accumulation in the brain is increased following ICH, and remains high for up to 1 month post-ICH (Wu et al., 2003) which can lead to increased edema, inflammation, oxidative stress, and cerebral injury. The importance of the role iron has after ICH has been illustrated by success using deferoxamine, an iron chelator, to reduce functional impairment following ICH in rats (Hua et al., 2008).

Free radical production also leads to secondary brain injury after ICH. Oxidative stress from the ICH increases free radical production leading to neuronal damage, edema, and neurological deficits (Nakamura et al., 2005; Wu et al., 2002). The free radical scavenger NXY-059 showed some beneficial effects such as reduced inflammation and neurological deficits in rat studies (Peeling et al., 2001a). Unfortunately, a clinical trial (CHANT trial) failed to improve functional outcome in ICH patients (Lyden et al., 2007).

Inflammation is also an important process following ICH. A number of inflammatory mechanisms have been identified including local inflammation by mediators from extravasation proteins and osmotically active electrolytes (Power et al., 2003), induction of proteases such as thrombin, fibrinogen, and tissue plasminogen activator (Sansing et al., 2003), and clotting factors associated with blood degradation components (Huang et al., 2002). A number of inflammatory mediators have been identified including interleukin-1β (Masada et al., 2001), and MMPs (Abilleira et al., 2003; Lapchak et al., 2000). These substances can lead to edema through opening of the BBB (Rosenberg et al., 1998).

A number of MMPs (MMP-2, 3, 9, and 12) have been studied to identify their effect on ICH. MMP-9 has been found in collagenase-induced ICH brains with edema, necrosis, and BBB disruption (Rosenberg & Navratil, 1997). MMP inhibition has been effective at reducing oxidative stress, edema, cell death, and neurological deficits (Wang & Tsirka, 2005). In addition, edema is reduced in MMP knockout studies (Tejima et al., 2007).

Studies have shown that the presence of microglia around the hematoma increases up to 7 days post-ICH and then subsequently decreases (Gong, Hoff, & Keep, 2000). Microglia is activated to clear the hematoma (Wang & Doré, 2007). However, microglia also release factors that can be harmful to nearby cells including cytokines, nitric oxide, and ROS (Hanisch, 2002; Wang & Doré, 2007). In addition, Wang and colleagues (2003) showed that microglial inhibition with microglia inhibitory factor improved outcome after ICH.

Blood-brain barrier disruption begins to occur shortly after ICH, but is still resistant to large molecules in the first few hours. By 8 to 12 hours post-ICH, large molecules are able to pass through the BBB, leading to infiltration of macrophages and neutrophils (Wang & Tsirka, 2005). The degree of BBB disruption significantly affects functional outcome in humans (Lampl et al., 2005). In addition to necrotic cell death, apoptotic cell death occurs following ICH (Matsushita et al., 2000). Apoptotic cells have been found in the peri-hematoma zone in humans (Qureshi et al., 2003), and are associated with a number of processes that occur following ICH. Apoptosis is associated with increased levels of tumor necrosis factor-alpha (TNF-α), MMPs, interleukins, microglial inhibitory protein, and nuclear factor-κβ (Power et al., 2003). However, Peeling and colleagues (2001b) showed that FK-506 decreased apoptosis at 2 days but did not reduce neuronal loss at 9 weeks.

Edema is a major issue for patients following ICH, and is a significant contributor to morbidity and mortality. Edema following ICH elevates intracranial

pressure (ICP), which can lead to herniation, brain stem compression, and death (Diringer, 1993). Proteins from vasogenic edema can lead to secondary brain injury. Early edema formation results from osmotic proteins from the clot, starting immediately following the bleed and peaking at 3 to 5 days post-ICH in rats (Xi, Keep, & Hoff, 1998; Yang et al., 1994). In humans, edema forms within 3 hours of injury and peaks at 10-20 days post-ICH (Zazulia et al., 1999). Delayed edema is both vasogenic (due to BBB breakdown) and cytotoxic (cellular) in nature and lasts 2 to 4 weeks following ICH, depending on hematoma size (Rincon & Mayer, 2004). There are several phases of edema formation in the first few hours. Phase 1 results from clot retraction and hydrostatic pressure, the second phase (first 2 days) results from the coagulation cascade and thrombin production, and the final phase results from erythrocyte lysis and hemoglobin toxicity (Xi, Keep, & Hoff, 2006).

A number of these processes have dual roles where they can be detrimental following ICH, but also play a role in recovery. Inflammation, for example, can be either detrimental or beneficial. While a number of studies indicate a negative role for inflammation in ICH, inflammation also helps to limit and remove the hematoma (Wang et al., 2003). Therefore, it may be beneficial to manage the inflammatory process, but not to eliminate it.

The complement cascade is also involved in both the process of injury and recovery in ICH. The complement cascade contributes to injury through multiple pathways (Ducruet et al., 2009), but is also involved in opsonization and cell

clearance, which contributes to the recovery process. Current therapies are being aimed at reducing complement activation shortly after ICH, while trying to minimize the effect on complement activation during the repair process (Ducruet et al., 2009).

Microglia contribute to injury (Hanisch, 2002), but are also involved in many adaptive responses including clearing of necrotic cells, and attempting to save cells by releasing brain-derived neurotrophic factor (Imamura et al, 2003). In some cases where microglia activation has been inhibited, there are more necrotic cells, and hematoma volume is not reduced (Wang et al., 2003).

These processes outline the complexity of the response to ICH. Protecting the brain from injury is not as simple as inhibiting the processes that are activated after ICH. In some cases, antagonizing these processes may actually impair recovery. Therefore, it is important to understand under what circumstances it is beneficial to inhibit these processes, and under what circumstances it is harmful to inhibit them.

Hematoma volume is a major determinant of long-term outcome in ICH patients. Each of these pathophysiological factors contributes to increased hematoma expansion and worse functional outcome. Therefore, it is important to take into consideration multiple mechanisms of injury when identifying potential treatments. The three main goals of ICH care are to 1) prevent early death, 2) reduce disability by limiting secondary consequences of ICH, and 3) provide early rehabilitation (Ferro, 2006). Treatment for ICH primarily is used to achieve the

first goal. A number of treatments are used upon admission to reduce the chance of death and disability, but a number of these treatments have not been validated by randomized clinical trials (RCTs).

1.5 Therapeutic Approaches

There are currently no proven early treatment interventions that limit long-term disability following ICH. The primary method of care following ICH involves managing the early consequences of ICH. One of the primary concerns after ICH is elevated intracranial pressure, which is a leading cause of death for ICH patients (Diringer, 1993). There are several methods used to control ICP in ICH patients including head elevation, CSF drainage, osmotic therapy, and hyperventilation (Broderick et al., 2007). Often patients have their head elevated to improve venous outflow and lower ICP. Drainage of cerebrospinal fluid (CSF) is also done to reduce ICP in some cases (Broderick et al., 2007). Mannitol is an osmotic diuretic that will decrease intracranial hypertension by reducing water, CSF, and cerebral blood volume (Ferro, 2006). Unfortunately, there are potential complications from use of mannitol including acute renal failure, hydro-electrolyte imbalances, and pulmonary edema. A clinical trial assessing the efficacy of mannitol for treating ICH found that it did not improve outcome (Misra et al., 2005). Another diuretic agent glycerol also failed to improve outcome in a clinical trial (Yu et al., 1992). Hyperventilation is effective at lowering ICP, but is not often used due to the transient nature of the effect and the simultaneous effect of lowering CBF (Broderick et al., 2007).

Early elevations in BP are common after ICH and are associated with increased risk of death, dependency, hematoma growth, and functional outcome (Broderick et al., 2007). Guidelines for blood pressure management suggest that aggressive management be considered if mean arterial blood pressure (MABP) is higher than 150 mmHg or if it is higher than 130 mmHg and there is an increase in ICP (Morgenstern et al., 2010). There is a concern, however, that blood pressure medication may increase the risk of ischemia. A trial (ICH ADAPT) is currently being conducted to determine if lowering blood pressure affects CBF (Butcher et al., 2010). A recent clinical trial (Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage Trial) demonstrated that aggressive management for patients with high blood pressure (150 to 200 mmHg) is safe and feasible (Anderson et al., 2008). Currently, a follow-up trial is being conducted to determine if aggressive management will improve ICH outcome.

Management of ICH also includes aggressive airway management, fluid management, prevention of seizures, and temperature management (Mayer & Rincon, 2005). Patients with a large hemorrhage, brain stem compression, or hydrocephalus are at risk for airway obstruction (Testai & Aiyagari, 2008). Patients that have a Glasgow Coma Scale (GCS) score of ≥ 8 are usually intubated and mechanically ventilated to ensure proper ventilation and oxygenation (Testai & Aiyagari, 2008). Isotonic solutions such as 0.9% saline are used to replace fluids in ICH patients to keep osmality within normal range. Passero and colleagues (2002) have reported that 8.1 % of ICH patients develop seizures within 30 days of the ICH. They also found that lobar location and small

volume of ICH were independently predictive of seizure onset. Seizures are associated with midline shift and neurological worsening, but are not an independent predictor of worse outcome (Ferro, 2006; Vespa et al., 2003). Temperature management is used to maintain normothermia, largely based on the negative impact that hyperthermia has on ischemia patients.

Surgery to remove the hematoma has been used in severe cases of ICH for many years. A number of clinical trials have been undertaken to assess whether surgery is a viable option for ICH patients, with varying results. The International Surgical Trial in Intracranial Haemorrhage (International STICH) studied the efficacy of early hematoma evacuation combined with medical treatment in comparison to conservative early medical treatment. About 26% had favourable outcome in the surgical group, whereas 24 % had favourable outcome in the control group, thus, failing to provide strong evidence that surgery is a viable treatment option (Mendelow et al., 2005).

A number of other surgical techniques have been used in an attempt to improve outcome including aspiration, craniotomy with hematoma evacuation, decompressive craniectomy, ventricular external drainage for hydrocephalus and intraventricular bleeding, endoscopic evacuation, and stereotactic CT-guided aspiration and thrombolysis of the hematoma but have not been validated by a large RCT (Broderick et al., 2007). Although there is no evidence that surgery is helpful, there is consensus that evacuation be used for large (> 3 cm in diameter) cerebellar hemorrhages (Morgenstern et al. 2010). Patients with large cerebellar

hemorrhages treated with medical management have poor outcomes. Brain stem compression and hydrocephalus are common in this type of hemorrhage and are a main reason why these types of hemorrhages are evacuated (Broderick et al., 2007). In addition, many neurosurgeons perform surgeries on young patients showing neurological deterioration and not responding to treatment.

A number of drugs have been used to limit hematoma growth including recombinant factor VIIa, aminocaproid acid, tranexamic acid, and aprotinin (Mayer, 2002; Mayer, 2003). A phase II trial using recombinant factor VIIa showed very promising results, where the group that received the drug had a smaller hematoma, less mortality, and less functional impairment than the control group (Mayer et al., 2005). This led to a large phase III clinical trial, but unexpectedly it produced negative results (Mayer et al., 2008). The treatment limited hematoma volume but did not improve functional outcome.

Currently, efforts are being made to identify a sub-population of patients that may be helped by this treatment. For instance, there is a current trial underway to identify if it is effective in treating warfarin-related ICH. Warfarin is an anti-coagulation drug that is used to prevent blood clots in susceptible patients. Unfortunately, in rare cases this treatment can lead to the development of intracerebral hemorrhage. Common treatments for this type of ICH include vitamin K and fresh-frozen plasma, which can reverse the coagulation defect but act slowly and do not always prevent the expansion of the hematoma. A study was conducted recently to determine if it would be safe to assess recombinant

factor VIIa in a large RCT (Robinson et al., 2010). They found that it was safe, despite an increase in thromboembolic complications. This study suggests that a clinical trial be undertaken comparing the efficacy of recombinant factor VIIa and fresh-frozen plasma in reversing warfarin-related ICH.

Management of ICH currently involves a normalization approach. When a bodily process is out of normal range, physicians try to normalize the process. However, in many cases this type of care is initiated before there is evidence that a particular process (i.e. temperature) is detrimental to ICH patients. For instance, the primary reason that normothermia is maintained is that there is evidence that hyperthermia is detrimental to ischemia patients.

1.6 Animal Models of ICH

The two major models of intracerebral hemorrhage are the autologous whole-blood model (Bullock et al., 1984) and the bacterial collagenase model of ICH (Rosenberg et al., 1990). The whole blood model consists of injecting blood into the brain of an animal, thus, mimicking a single large bleed. Most studies using the whole blood model use rats, but there have also been studies using cats, dogs, rabbits, pigs, and monkeys (Andaluz, Zuccarello, & Wagner, 2002). The whole blood model used in rat studies involves taking blood from the tail artery and injecting it into the brain (usually the striatum). The collagenase model involves injecting bacterial collagenase into the brain, which dissolves the extracellular matrix and disrupts the basal lamina of the cerebral blood vessels,

causing BBB permeability and bleeding. This model mimics the prolonged bleeding that occurs in some ICH patients (Brott et al., 1997).

There are several differences between these two models (Andaluz, Zuccarello, & Wagner, 2002; MacLellan et al., 2008). Although neither model completely mimics the process that occurs in humans, there are advantages and disadvantages to each model. A disadvantage of the whole-blood model is blood moving up the needle track, reducing the amount of blood remaining in the striatum, which is not common in the collagenase model. A disadvantage of the collagenase model is that it causes a greater inflammatory response than either the whole-blood model or that which occurs in humans (Andaluz, Zuccarello, & Wagner, 2002). In addition, the degrading of the extracellular matrix introduces another element that is not present in the human condition.

These two models also display a different time course of injury and impairment. The collagenase model seems to cause more significant long-lasting injury and impairment than the whole blood model. In a study by MacLellan and colleagues (2008), in which the initial volume of injury was similar, lesion volume and functional impairment was greater in the collagenase-induced ICH group than the whole blood-induced ICH group. Rats in the collagenase group had more tissue loss at six weeks, fewer surviving neurons in the ipsilateral striatum and substantia nigra pars compacta, and a thinner cortex and corpus callosum than rats in the whole blood group. They also had more BBB disruption and more deficits on the neurologic deficit scale (NDS). This study showed that

tissue loss increased in the collagenase model from 1 to 4 weeks, whereas it remained relatively unchanged in the whole blood model. Similarly, Nguyen and colleagues (2008) showed that lesion volume increases from 7 days post-ICH to 60 days post-ICH in the collagenase model.

Differences in the models can sometimes lead to varying results for a particular treatment (Hua et al., 2006; MacLellan et al., in press; Warkentin et al., 2010). For instance, a number of studies have shown that deferoxamine treatment following whole-blood injection in rats improves outcome (Hua et al., 2006; Okauchi et al., 2010). Deferoxamine reduces forelimb placing, forelimb asymmetry, turning preference deficits, and edema formation. However, a recent study by Warkentin and colleagues (2010) indicates that deferoxamine does not improve outcome following injection of bacterial collagenase. They found no difference between deferoxamine and non-deferoxamine treated rats on turning preference, walking ability, skilled reaching, edema formation, and lesion assessment. Due to the response difference between models, it is important to test the potential impact of treatments and other variables in both models.

1.7 Assessment of Injury and Impairment

There are a variety of ways to assess injury and impairment in rat models of ICH. A simple way to assess injury in experimental models of ICH is to quantify the amount of injury that is visible when brain slices are imaged with a high-powered microscope. In our lab, we stain the tissue with cresyl violet, a nissl stain, to allow us to delineate healthy from abnormal tissue. This provides a

quantifiable and repeatable assessment of injury. Several mechanisms of injury can be studied including edema formation, BBB disruption, and inflammation. To study edema formation, for instance, we can measure the weight of specific brain structures at the time of euthanasia and then measure the weight of the brain structure once it has been dehydrated to determine the water content of the brain structure at the time of death.

It is important to reduce tissue loss after ICH, but it is even more important to reduce the impairments that patients have after the hemorrhage. Several behavioural tests have been developed to mimic various functional impairments. In our lab, we use behavioural tests to determine many post-ICH deficits including general neurological impairment, walking ability, skilled reaching ability, forelimb asymmetry, and turning preference. These tests are used to detect initial impairment as well as recovery over time. It is important to use sensitive tests so that even mild improvements can be detected. MacLellan and colleagues (2006a) found that some tests were better at detecting gradations in injury than others. Therefore, when considering which tests are best to use, it is important to consider what type of impairment you want to study as well as how sensitive your test is.

1.8 Hypothermia Following Stroke

Hypothermia has been studied as a potential mechanism for treating stroke for many decades (Colbourne, Sutherland, & Corbett, 1997). Initially, deep hypothermia (30°C) for cardiac arrest patients was used. However, this was

discontinued due to inconsistent results and negative side effects (Michenfelder & Milde, 1977; Mullan, Raimondi, & Suwanela, 1961). Complications from induced hypothermia include coagulation, infection, hypovolemia, and shivering (Polderman, 2004).

In the late eighties and early nineties a number of animal studies were conducted that renewed interest in the potential of using hypothermia treatment for stroke patients (Busto et al., 1987). The early animal studies used intraischemic hypothermia to show that hypothermia could prevent injury. Typically, hypothermia would be initiated just prior to the insult and then brought back to normothermia after the insult. These studies found that hypothermia significantly reduced the amount of tissue damage following ischemia (Baker, Solomon, & Onesti, 1992; Busto et al., 1987; Onesti et al., 1991) using short-term (up to 7 days post-ischemia) outcome measures.

Some studies also showed that very short delays (1 to 2 hours) were effective at providing short-term (≤ 7 days) protection (Caroll & Beek, 1992). Unfortunately, attempts to provide long-term protection with delayed hypothermia were unsuccessful. Dietrich and colleagues (1993), for instance, assessed normothermia, intraischemic hypothermia, and post-ischemic hypothermia (3 minutes into recirculation) for both short and long-term protection after a 10-minute global ischemia insult. Intraischemic hypothermia demonstrated long-term (2 months post-ischemia) protection. In contrast, post-ischemic

hypothermia provided strong protection at 3 days, less protection at 7 days, and no protection at 2 months.

In order for hypothermia to be a clinically relevant treatment, delayed hypothermia needs to provide long-term neuroprotection. It was hypothesized that a more prolonged hypothermia at a moderate temperature (~32°C) would be more beneficial. Colbourne & Corbett (1994) found that 24 hours of hypothermia (32°C) one hour after a five-minute global ischemic insult provided significant protection at 30 days post-ischemia. A number of other studies have since demonstrated that prolonged hypothermia provides neuroprotection in models of both global and focal ischemia. A number of reviews have outlined the many hypothermia studies conducted and mechanisms of neuroprotection for hypothermia (MacLellan et al., 2009; Marion & Bullock, 2009).

Based on the success of hypothermia treatment in rodent studies, clinical trials were undertaken for cardiac arrest patients. Two clinical trials demonstrated that hypothermia was successful at treating cardiac arrest patients (Bernard et al., 2002; The Hypothermia After Cardiac Arrest Study Group, 2002). In addition, a number of feasibility trials have been undertaken to determine if hypothermia treatment would be beneficial for focal ischemia patients (Schwab et al., 1998). Schwab and colleagues (1998) determined that hypothermia was safe to conduct in patients suffering from a large MCA stroke. Since then, a number of trials have been conducted that have demonstrated feasibility for hypothermia alone (DeGeorgia et al., 2004; Georgiadis et al., 2001; Kammersgaard et al., 2000;

Krieger et al., 2001) and in combination with other treatments (Martin-Schild et al., 2009; Meloni et al., 2009). A large randomized trial is likely to be conducted; however, several issues need to be resolved first. These issues include depth, duration, and method of hypothermia (Liu, Yenari, & Ding, 2009; MacLellan et al., 2009; van der Worp, MacLeod, & Kollmar, 2010).

1.9 Hypothermia Following Intracerebral Hemorrhage

Recently, there have been attempts to apply hypothermia treatment to ICH. If hypothermia can reduce edema following ICH, it may prevent death in patients with severe edema. A recent study applying hypothermia to 12 patients with a severe intracerebral hemorrhage showed that hypothermia prevented the increase in perihemorrhagic edema during the first 14 days after ICH that occurred in 25 patients from a local hemorrhage data bank (Kollmar et al., 2010). This finding was predicted by a study by Fingas, Clark, & Colbourne (2007) that found hypothermia that reduced edema after ICH (Fingas, Clark, & Colbourne, 2007). However, hypothermia does not seem to provide substantial functional improvement or reduce hematoma volume in rat models of ICH (Fingas, Clark, & Colbourne, 2007; MacLellan, Girgis, & Colbourne, 2004; MacLellan et al., 2006b). These studies do not suggest that routine use of hypothermia for ICH patients would be beneficial. One of the primary reasons why it is thought that hypothermia mitigates injury in ischemia is that it protects vulnerable cells in the ischemic penumbra. However, there is not very good evidence to suggest that there is a similar penumbra surrounding the hematoma in ICH (Herweh et al.,

2007; Schellinger et al., 2003). Therefore, treatments that are successful in ischemia, such as hypothermia, may not be successful in treating ICH.

1.10 Fever and Hyperthermia

Among the many consequences following stroke is elevation of body temperature, which occurs in ischemia, hemorrhage, and other pathological conditions. A large number of stroke patients develop fever upon hospital admission (Kilpatrick et al., 2000). Kilpatrick and colleagues (2000) studied all febrile episodes (> 38.5°C) following stroke over a six-month period. Approximately, 50% of ICH patients developed a fever and approximately 35% of embolic stroke patients developed a fever. Castillo and colleagues (1998) studied 260 patients with cerebral infarction and found that 60.8 % developed fever, which was defined as temperature > 37.5°C on any measurement.

In the majority of cases, elevation of body temperature is caused by an infection, resulting in a fever (Deogaonkar et al., 2005). Whenever the cause of the body temperature increase is unknown, central hyperthermia is suspected. There are several important differences between hyperthermia and fever. Fever results from a change in the body's set point due to an infection. In this case, the body maintains a higher than normal body temperature to fight against an infection. The body's set point is maintained by the hypothalamus (Axelrod & Diringer, 2007). Two separate pathways for induction of fever have been identified. The classical pathway consists of leukocytes and macrophages producing pyrogenic cytokines once the body is exposed to a large range of

harmful substances (i.e. toxins, microorganisms, proteins, etc.). This causes the synthesis of prostaglandin E_2 (PGE_2) in the hypothalamus, and an elevation of body temperature (Axelrod & Diringer, 2007).

The alternate method that fever is initiated and maintained is through the production of PGE_2 through the cyclooxygenase pathway (Axelrod & Diringer, 2007; Blatteis et al., 2005). This pathway is initiated by the synthesis of PGE_2 from arachidonic acid by Kupffer cells in the liver. These cells are activated by the complement cascade component 5a in response to an invading pathogen. Then, PGE_2 is sent to the hypothalamus and causes an increase in body temperature. This type of pathway seems to lead to a delayed pyretic response. In addition to pyretics, there are also molecules that limit the degree to which temperature is increased so that it is not detrimental. These are referred to as cryogens. Once PGE_2 is cleared from the hypothalamus, the body's set point returns to normal and the body begins to initiate the cooling process (Axelrod & Diringer, 2007).

In contrast to fever, hyperthermia results from a source that overpowers the body's attempt to maintain normal temperature. Hyperthermia can result from four primary sources: an increase in metabolic demand, impairment of heat dissipating mechanisms, a decrease in the heat-absorbing mechanisms under hot conditions, or the influence of a drug (Roth et al., 2006). Types of non-experimental hyperthermia include heat stroke, exertional heat stroke, malignant hyperthermia, neuroleptic malignant syndrome, serotonin syndrome, hemorrhage,

endocrine disturbance, hypothalamic damage, and drug-induced hyperthermia (Axelrod & Diringer, 2007; Sung, Lee, & Chu, 2009).

The body's response to hyperthermia is much different from its response to fever. When hyperthermia occurs, the blood flow to the skin is increased and the individual perspires to increase heat loss. In contrast, blood flow is reduced and individuals shiver to decrease heat loss in the initial phase of the fever (Roth et al., 2006). Essentially, hyperthermia mimics the increased temperature aspect of fever but fails to mimic other aspects (i.e. response to infection).

1.11 Fever and Hyperthermia Following Stroke

Hindfelt (1976) was the first study to suggest that development of a fever after an ischemic insult was detrimental. This study found that patients who developed a fever were more likely to have an unfavourable outcome in comparison to those that did not develop a fever. Since this initial report, a number of clinical studies have correlated fever with worse outcome (Azzimondi et al., 1995; Reith et al., 1996). Hajat, Hajat, & Sharma (2000) did a meta-analysis of nine different studies focusing on fever and functional outcome. They found that fever was correlated with higher mortality and morbidity.

A number of animal studies have shown that increasing temperature (2 to 3°C) will increase brain injury and worsen functional outcome. Experimental studies involving hyperthermia (39-40°C) have demonstrated increased brain damage in both global and focal ischemia. Numerous studies have demonstrated a

worsening of outcome when initiated during global ischemia (Busto et al., 1987; Dietrich et al., 1990; Minamisawa, Smith, & Siesjö, 1990). Dietrich and colleagues (1990) showed that when they maintained brain temperature at 39°C, brain damage was increased in the cortex, hippocampus, thalamus, and striatum.

In addition, several studies have demonstrated worsening of outcome even when hyperthermia is delayed a number of hours after the insult (Baena et al., 1997; Hickey et al., 2003). Baena and colleagues (1997) induced global ischemia for 5 or 7 minutes and initiated hyperthermia (39 to 40°C) 24 hours following the insult. Hyperthermia was initiated for 3 hours and increased injury in rats that received a 7-minute insult. Specifically, hyperthermia more than doubled the number of ischemic neurons in the hippocampus. Hickey and colleagues (2003) studied delayed hyperthermia following asphyxiation (8 minutes) in rats. Hyperthermia was initiated with a ceramic warming device placed under the cage of rats to achieve the desired temperature. Hyperthermia (40°C) was initiated either 24 or 48 hours following asphyxia and compared to normothermia. Hyperthermia delayed by 24 hours significantly increased histological injury, but this effect disappeared when hyperthermia is initiated 48 hours following asphyxia.

Morikawa and colleagues (1992) demonstrated that hyperthermia worsens outcome when it is initiated during focal ischemia. They initiated hyperthermia (39°C) during a 120-minute proximal MCA occlusion and found that it tripled the infarct volume of rats kept at normothermia. Noor, Wang, & Shuaib (2003)

studied the effect of hyperthermia during embolization of a preformed clot by raising brain temperature to 39°C prior to embolizing the clot. Hyperthermia doubled the lesion volume and worsened general neurological impairment.

Hyperthermia also influences functional outcome after focal ischemia when it is delayed by a few hours. Kim and colleagues (1996) studied whether delayed hyperthermia alters outcome following focal ischemia. Twenty-four hours following 60 minutes of transient MCA occlusion, the rat's body temperature was raised to 39 or 40°C or kept normothermic for 3 hours in a heating chamber. Heating rats to 40°C increased histological damage and neurological scores compared to the other two groups.

Hyperthermia can also inhibit the benefit of a drug that improves outcome following focal ischemia such as tissue plasminogen activator (t-PA) or the glutamate antagonist MK-801. Noor, Wang, & Shuaib (2005) embolized a preformed clot at various temperatures (37, 38, and 39°C) and then initiated t-PA treatment (or control). Treatment with t-PA significantly reduced infarct volume in the 37°C and 38°C groups but not the 39°C group. In addition, Memezawa and colleagues (1995) showed that when temperature was controlled in rats subjected to an MCA occlusion, MK-801 reduced infarct size. However, when body temperature was allowed to rise (39 to 39.5°C) spontaneously, MK-801 failed to affect infarct volume.

Based on many studies where hyperthermia was initiated after ischemia, a number of mechanisms have been identified. Hyperthermia worsens outcome

through a variety of mechanisms including enhanced neurotransmitter release, free radical production, BBB opening, and ischemic depolarizations (Axelrod & Diringer, 2007; Ginsberg & Busto, 1998). These are important factors in the pathophysiology in ischemia (Nagel et al., 2008; Rothman & Olney, 1986; Siesjö, 1992) and are increased by hyperthermia (Dietrich et al., 1990; Globus, Prado, & Busto, 1995).

1.12 Hyperthermia Following Traumatic Brain Injury

Experimental studies have indicated that traumatic brain injury (TBI) is increased following hyperthermia (Chatzipanteli et al., 2000; Dietrich et al., 1996; Suh, Frederickson, & Danscher, 2006). Chatzipanteli and colleagues (2000) induced TBI using the fluid-percussion model. Temporal muscle temperature (30, 37, or 39°C) was manipulated for 3 hours following TBI. Hyperthermia increased and hypothermia decreased myeloperoxidase activity at 3 hours and 3 days post-TBI, suggesting that hyperthermia affects the inflammatory response in TBI. Dietrich and colleagues (1996) initiated TBI using the fluid-percussion injury model, and initiated hyperthermia (39°C) 24 hours later. Four days following TBI, hyperthermia increased mortality and contusion volume.

1.13 Fever and Hyperthermia Following ICH

Fever is very common following ICH (Kilpatrick et al., 2000; Schwarz et al., 2000). Schwarz and colleagues studied 251 ICH patients, and only 9 % of patients did not develop a fever (≥ 37.5°C) within the first 3 days. Of the 91% that

did develop a fever, 34% developed a fever for less than 24 hours, 36% developed a fever between 24 and 36 hours, and 21% developed a fever for more than 48 hours. Clinicians treat both ischemic (Adams et al., 2007) and hemorrhagic (Broderick et al., 2007) patients with anti-pyretics. The rationale for using anti-pyretics for ICH patients is that fever is common after ICH and some studies suggest that duration of fever is related to outcome (Morgenstern et al., 2010). Despite the routine use to treat fever with anti-pyretics, some question this practice (Aiyagari & Diringer, 2007; Laupland, 2009). In many disease states, this is not beneficial (Bernard et al., 1997; Doran et al., 1989). In ischemic patients, fever worsens outcome but a recent clinical trial for use of acetaminophen has failed to provide benefit in acute stroke (den Hertog et al., 2009).

Clinical studies have failed to reach a consensus on the impact of fever in hemorrhagic stroke patients. Some studies claim fever is detrimental (Leira et al., 2004; Schwarz et al., 2000), whereas others note that fever is correlated with worse outcome but there is not a causative relationship between the two (Szczudlik et al., 2002; Wang et al., 2000). Leira and colleagues (2004) studied the impact of fever (≥ 37.5°C) in 251 patients. They found that body temperature was an independent factor predictive of early neurologic deterioration. In contrast, Wang and colleagues (2000) studied 72 ICH patients, and found that hyperthermia (≥ 37.5°C) was not a significant contributor to mortality rate. The three variables that did predict in-hospital mortality were impaired consciousness, fecal incontinence, and hyperglycemia. Szczudlik and colleagues (2002) studied the effect that fever has on ICH using 152 ICH patients. They found that body

temperature correlated with mortality and neurological deficit but did not independently predict mortality at 30 days post-ICH.

Although there are no current recommendations specific to temperature management (Morgenstern et al., 2010), patients that develop fever in the first day are often treated with anti-pyretics. This makes it difficult to interpret the results. Perhaps, if temperature were not treated, body temperature would have more of an impact on mortality. Therefore, it is important to conduct controlled experimental studies in a non-clinical setting.

To date, there is only one published study on hyperthermia following experimental ICH. MacLellan and Colbourne (2005) used a bacterial collagenase model of ICH to study the effect of hyperthermia under a variety of conditions. In one group, hyperthermia (38.5 ºC) was initiated immediately following ICH and maintained for 24 hours. In another group, hyperthermia (38.5 ºC) was delayed by 24 hours and maintained for 24 hours. In the third hyperthermia group, hyperthermia (40 ºC) was delayed by 24 hours and maintained for 3 hours. These groups were compared to a normothermic group (36.5 ºC). In order to initiate hyperthermia, rats were kept in their home cage with an overhead heating lamp that was activated whenever the rat's body temperature dropped below 38.5 ºC. Rats were assessed using a variety of behavioural tasks and histological techniques. Hyperthermia did not alter skilled reaching, forepaw inhibition, forelimb asymmetry, walking ability, or general neurological impairment. In addition, it did not alter lesion volume, inflammation, or hematoma volume. This

study would tend to indicate that hyperthermia does not worsen functional outcome or hematoma volume. Unfortunately, no other studies have investigated whether hyperthermia affects ICH outcome.

1.14 Thesis Research

The goal of this research project was to further investigate the impact that hyperthermia has on ICH. Our current study differs from our previous study (MacLellan & Colbourne, 2005) in two major ways. We used a different ICH model (whole blood instead of collagenase) and induction protocol (induction under anesthesia instead of while awake in their cage). There are many key differences between these ICH models, making it imperative to do experiments using both models. We induced hyperthermia under anesthesia primarily for ease of induction. Previously, we found that it was difficult to heat rats above 38.5 ºC. We avoided this problem by heating them under anesthesia. This method of heating is not unprecedented, as several other studies have initiated hyperthermia while the rats were under anesthesia (Chatzipanteli et al., 2000; Noor, Wang, & Shuaib, 2003). This also allowed us to monitor physiological variables (pH, pCO_2, pO_2, glucose, and hemoglobin) and blood pressure during surgery. This allowed us to observe whether hyperthermia altered these variables.

We tested whether 3 hours of post-ICH hyperthermia, as used in previous stroke and TBI experiments (Chatzipanteli et al., 2000; Hickey et al., 2003; Kim et al., 1996), affects outcome in the whole-blood model of ICH. We initiated hyperthermia immediately after all of the blood was injected into the striatum. We

maintained hyperthermia for 3 hours, or maintained normothermia throughout the surgery, and monitored the rats afterwards. In experiment 1, brain-water content (BWC) was measured at 24 hours post-ICH. In experiment 2, we assessed behaviour for 3 days and measured BWC at 72 hours. Previously, we found that edema is highest 3 days following ICH (Fingas, Clark, & Colbourne, 2007). Therefore, we chose 24 and 72-hour time points to assess whether the rate of edema was accelerated (24 hours) or the peak of the edema was elevated (72 hours). In experiment 3, we assessed rats on a variety of behavioural tasks including forelimb asymmetry, walking ability, skilled reaching, turning preference and general neurological impairment. Each of these tasks can detect deficits following ICH (MacLellan et al., 2006a; Schallert, 2006). We tested rats at many different time points to identify if there are any early treatment effects, and whether any effects are maintained or are transient. We also assessed lesion volume at 32 days post-ICH to identify if hyperthermia alters lesion size.

2. Methods

2.1 Animals

All procedures were approved by the University of Alberta Biological Sciences Animal Care and Use Committee and conformed to Canadian Council on Animal Care guidelines. We used 98, male, Sprague Dawley rats, weighing between 200 and 350 g at study onset. They were housed in a humidity and temperature-controlled vivarium and maintained on a 12-hour light-dark cycle (07:00-19:00). Rats were given free access to food and water, except during food restriction occurring 2 days prior to and during single pellet training and testing.

Rats were randomly assigned to either the hyperthermic (HYPER) or the normothermic (NORMO) group for all experiments. In experiment 1, BWC was measured at 24 hours post-ICH (n = 21 per group). In experiment 2, we measured BWC at 72 hours post-ICH (n =15 per group), in addition to assessing functional outcome on several behavioural tasks. In experiment 3, we assessed long-term (32 days post-ICH) behavioural and histological outcome (n = 13 per group). Experimenters blind to group assignment analyzed all data.

2.2 Surgical Procedures

All surgical procedures were done aseptically under isoflurane anesthesia (4% induction; 2 % maintenance in 60% N_2O, balance O_2). We implanted telemetry probes (model TA10TA-F40; Transoma Medical, St. Paul, MN, USA) into the peritoneal cavity of rats three days prior to ICH (Debow & Colbourne,

2003). Body temperature was measured with A.R.T. computer software V 2.2 (Transoma Medical) that sampled every 30 seconds starting one day prior to ICH and continued for three days following ICH (or until euthanasia).

Rats were subjected to an ICH via autologous whole blood injection (Bullock et al., 1984; Fingas et al., 2009). Rats were anesthetized with isoflurane and placed in a stereotactic frame. We monitored rectal temperature and maintained normothermia (37ºC) with an electric heating pad. Two bilateral burr holes were drilled in the skull 3.5 mm lateral to bregma. One was used for the ICH, and the other was to hold a guide cannula. A third burr hole was drilled 5 mm posterior to the guide cannula, and a metal screw (model MX-080-2; Small Parts, Miami Lakes, FL) was inserted for support. The cannula was held in place by applying dental cement around the cannula and screw. Once the cannula was secure, we lowered a thermocouple probe (HYP1-30-1/2-T-G-60-SMPW-M, Omega, Stanford, Conn.) 5 mm below the surface of the skull in the contralateral striatum.

We catheterized the tail artery to extract arterial blood to be injected into the striatum. Once we extracted the blood, we attached the catheter to a blood pressure line to measure mean arterial blood pressure (MABP; BP-1, World Precision Instruments, Sarasota, Fla.) and blood gases during surgery. We slowly lowered a 26 G needle into the ipsilateral striatum (AP: 0, ML: 3.5, DV: 6.5 mm) and injected 100 µL of arterial blood over ten minutes. We induced an ICH on the left side in experiments 1 and 2 and on the side contralateral to paw preference (as

determined by single pellet reaching) in experiment 3. The needle remained in place over ten minutes before being slowly raised to prevent blood from going up the needle tract. Afterwards, the hole was sealed with a metal screw.

2.3 Temperature Manipulation

We increased the brain temperature of rats assigned to the hyperthermia group to 39ºC with a heating pad and overhead infrared lamp (250 W) immediately after blood injection. Their temperature was maintained for three hours, while monitoring BP and blood gas measurements (pH, pCO_2, pO_2, hemoglobin, and glucose; Radiometer ABL 810 blood gas analyzer). We measured brain temperature, rectal temperature, and blood pressure every 15 minutes. In addition, we took an arterial blood sample every 30 minutes to analyze physiological variables. If the pO_2 was out of range, we attempted to normalize it by increasing or decreasing the inspired oxygen concentration (normal range of pO2 = 125 to 135 mmHg). Following three hours of hyperthermia, we stopped heating to allow rats to spontaneously cool for thirty minutes. Normothermic rats remained at 37 ºC throughout the duration of surgery. We then sutured the tail and head wounds closed, and applied a local anesthetic (Marcaine, Sanofi Canada, Markham, Ontario, Canada). We discontinued anesthesia and returned rats to their home cage to monitor body temperature. Each day, we weighed rats and gave them a mixture of rat chow, peanut butter, and honey in a small petri dish. Rats also had access to their normal rat chow.

2.4 Brain Water Content (Experiments 1 and 2)

We assessed BWC at 24 hours post-ICH in experiment 1, and at 72 hours in experiment 2. We anesthetized rats with 4% isoflurane, decapitated them, and then extracted the brain. We cut a coronal section from 2 mm anterior to 2 mm posterior to the site of injection, and dissected out the cerebellum, the ipsilateral cortex and striatum, and the contralateral cortex and striatum. We calculated BWC as follows (MacLellan et al., 2006b): BWC = [(Wet Weight-Dry Weight)/Wet Weight] x 100.

2.5 Experiment 2 (Behaviour)

We tested rats on the neurological deficit scale (NDS) on days 1, 2, and 3 post-ICH. We also used the cylinder test and the horizontal ladder test to assess deficits on day 3 post-ICH. Baseline testing was conducted 2 days prior to ICH.

2.6 Neurological Deficit Scale

We used a modified version of the NDS scale (Del Bigio et al., 1996; MacLellan & Colbourne, 2005). In this task, we measure rats on a variety of motor tasks to give us a general assessment of neurological deficit following ICH. We assessed rats on beam walking, spontaneous circling, bilateral forepaw grasp, hind limb replacement, forelimb flexion, and vibrissae-elicited forelimb placing.

For the beam-walking test, we trained rats to cross a narrow beam. Four baseline crosses were used to assess their walking ability. We also gave them four crosses for each of the testing sessions. For the spontaneous circling task, we placed rats in a cylindrical tube and monitored how much they circled in a five-

minute time period. To assess forepaw grasping, we allowed rats to grasp on to a bar 30 cm above the ground to evaluate whether they could grasp with both forepaws. To assess hind limb replacement, we displaced the rat's hind limb and measured how long it took rats to return their hind limb to its normal position. To assess forelimb flexion, we picked rats up by the base of the tail and assessed their ability to stretch their arms out straight. To assess forelimb placing, we brushed the rat's whiskers against the edge of a table and recorded how many times the rat placed its forepaw on the table. The criteria for each of the tasks are represented in Table 1. A score of 17 indicated maximum impairment.

2.7 Cylinder

We used the cylinder test to assess forelimb asymmetry (Schallert et al., 2000). We placed rats in a clear Plexiglas cylinder (45 cm in height; 20 cm in diameter) for ten minutes, videotaped their behaviour, and analyzed independent wall touches. Wall touches were considered independent when only one paw was touching the cylinder (ipsilateral or contralateral). When rats were touching the wall with both paws it was scored as a touch with both forepaws. We then calculated an asymmetry score, to indicate how much the rats use their impaired paw when exploring the walls of the cylinder. The asymmetry score was calculated as follows:

(contralateral forelimb contact + ½ both) / (ipsilateral forelimb contact + contralateral forelimb contact + both) × 100.

Essentially this formula gives you the frequency that the rat uses its injured paw as a percentage of total usage. Unimpaired rats will have a percentage close to 50 %, while injured rats will have a lower percentage. Rats that did not display 10 independent wall touches were excluded from analysis (Clark et al., 2008). Rats that did not reach this minimum criterion were excluded due to the possibility that including rats that made few touches could cause abnormally high variation in the scores, making it difficult to detect small differences between groups (Clark et al., 2008; Silasi & Colbourne, 2009).

2.8 Ladder Test

We used the horizontal ladder test to assess walking ability on day 3 post-ICH (Metz & Whishaw, 2002; Fingas et al., 2009). We placed rats on a horizontal ladder (1 m) with variably spaced rungs (3-5 cm). We recorded rats crossing a distance of 0.5 m four times and analyzed percentage of foot slips for each limb. Rats that did not cross the ladder at least two times were excluded from analysis.

2.9 Experiment 3

We tested rats on NDS, cylinder, horizontal ladder, and corner turn tests on days 3, 11, and 32 post-ICH. In addition, we assessed skilled reaching using the single pellet reaching task on days 7-10 and 28-31 post-ICH. At the end of testing on day 32 post-ICH, we euthanized the rats to assess lesion volume.

2.10 Corner Turn Test

We used the corner turn test to assess turn preference (Schallert, 2006; Warkentin et al., 2010). We placed rats in front of two Plexiglas walls (41 cm in height; 30.5 cm in length), separated by 1 cm at a 30° angle. We recorded turning direction when they were exiting the corner. Rats will turn in the ipsilateral direction following ICH when facing a 30º corner (Hua et al., 2002; Hua et al., 2006). Two days of baseline testing (10 trials per day) were done before the ICH, and we excluded rats with a turn preference > 70 % in either direction. Rats were tested on days 3, 11, and 32 days post-ICH (10 trials per testing session).

2.11 Single Pellet Reaching

We also used the single pellet test to assess skilled reaching (Whishaw, 2000; MacLellan, Gyawali, & Colbourne, 2006). We restricted food access to maintain 90% body weight. Following two days of food restriction, we began reach training. Rats were placed in a clear Plexiglas box (60 cm in length, 14 cm in width, 35 cm in height), and trained them to reach through a 1 cm opening to grab a food pellet (45 mg; Bio-Serv, Frenchtown, NJ, USA) sitting in a well in front of the opening. We determined paw preference, and trained them to reach with their preferred paw. Rats were trained for 20 days (5 days a week for 4 weeks; 25 trials per day). A successful reach was achieved when the rat grabbed the pellet and brought it to its mouth in one motion. An unsuccessful reach occurred if the rat knocked the pellet from the well, failed to grasp the pellet, or failed to bring the grasped pellet to their mouth. The final four days of training were used for baseline analysis. We analyzed overall success rate [(# of

successful reaches/ trials) x 100]. Rats that did not successfully reach on 40 % of the trials were excluded from analysis. This was done to eliminate the "floor" effect. This effect refers to the situation where performance is so low that it is difficult to detect a reduction in performance. Also, if we used rats with poor performance, an improvement in score may be due to the added training. The 4-day average of each testing session was compared to their baseline average.

2.12 Histology

In experiment 3, we euthanized rats with an intraperitoneal injection of sodium pentobarbital (100 mg/kg) on day 32 post-ICH. Rats were transcardially perfused with 0.9% saline followed by 10% formalin. We took 40 µm coronal slices every 400 µm using a cryostat and stained the tissue with cresyl violet. We used Scion Image J 4.0 (Scion Corporation, Frederick, MD, USA) to trace around the tissue to calculate lesion volume (Auriat, Wowk, & Colbourne, 2010; Warkentin et al., 2010):

Volume of hemisphere = area of remaining tissue × distance between sections × number of sections analyzed

Volume of tissue lost = remaining volume of normal hemisphere – remaining volume of injured hemisphere

2.13 Statistical Analysis

All data are presented as mean ± SEM, for the exception of NDS data, which is represented by a median score and interquartile range. We analyzed

physiological variables and lesion volume with one-way ANOVA (SPSS v. 17.0; SPSS Inc., Chicago, IL, USA). We analyzed corner turn, single pellet, horizontal ladder, and forelimb asymmetry data with repeated measures ANOVA. We controlled for multiple comparisons with the Tukey HSD post-hoc test. We analyzed NDS data with Mann-Whitney U tests. We analyzed parametric correlations with the Pearson R correlation and non-parametric correlations with the Spearman Rho Coefficient.

3. Results

3.1 Temperature and Physiological Measurements

For each experiment, we measured body temperature before and after surgery. In each case, there was an initial decrease in temperature, but it quickly recovered to normothermia. Core body temperature did not differ between groups after ICH in experiment 1 ($F_{(1,37)} = 0.167$, $p = 0.685$; Figure 1a), 2 ($F_{(1,27)} = 1.593$, $p = 0.218$; Figure 1b) , or 3 ($F_{(1,21)} = 0.093$, $p = 0.764$; Figure 1c).

We also took physiological and blood pressure measurements during surgery for each experiment (Table 2). The values in the table represent an average of the measurements that were taken during hyperthermia (physiological variables = average of 6 measurements; blood pressure = average of 12 measurements). Hyperthermia increased the pH level of rats in experiment 1 ($F_{(1,37)} = 11.586$, $p = 0.002$), 2 ($F_{(1,25)} = 20.036$, $p < 0.001$), and 3 ($F_{(1,21)} = 8.748$, $p = 0.008$). Likewise, hyperthermia decreased pCO_2 in experiments 1 ($F_{(1,37)} = 6.351$, $p = 0.016$), 2 ($F_{(1,25)} = 14.462$, $p = 0.001$), and 3 ($F_{(1,21)} = 5.104$, $p = 0.035$).

Temperature is known to alter blood gas levels under normal circumstances (Bisson & Younker, 2006). Therefore, we also took temperature-corrected values for pH and pCO_2. Hyperthermia increased the temperature-corrected pH level in experiment 1 ($F_{(1,37)} = 5.04$, $p = 0.031$), but it did not significantly affect it in experiment 2 ($F_{(1,25)} = 3.361$, $p = 0.079$) or experiment 3 ($F_{(1,21)} = 1.697$, $p = 0.207$). Hyperthermia did not affect the temperature-corrected

pCO_2 in experiment 1 ($F_{(1,37)} = 3.412$, $p = 0.073$), 2 ($F_{(1,25)} = 2.875$, $p = 0.102$), or 3 ($F_{(1,21)} = 1.754$, $p = 0.008$).

Hyperthermia reduced pO_2 in experiment 2 ($F_{(1,25)} = 5.696$, $p = 0.025$), but it did not affect outcome in either experiment 1($F_{(1,37)} = 2.\ 500$, $p = 0.122$) or experiment 3 ($F_{(1,21)} = 0.110$, $p = 0.744$). Hyperthermia reduced hemoglobin levels in experiment 1 ($F_{(1,37)} = 4.567$, $p = 0.039$), but did not affect hemoglobin in experiment 2 ($F_{(1,25)} = 0.071$, $p = 0.793$) or experiment 3 ($F_{(1,21)} = 0.220$, $p = 0.644$). Hyperthermia did not affect glucose levels in experiment 1 ($F_{(1,37)} = 0.275$, $p = 0.603$), 2 ($F_{(1,25)} = 2.391$, $p = 0.135$), or 3 ($F_{(1,21)} = 0.068$, $p = 0.797$). Hyperthermia also had no effect on blood pressure in experiment 1 ($F_{(1,37)} = 0.147$, $p = 0.704$), 2 ($F_{(1,25)} = 2.674$, $p = 0.115$), or 3 ($F_{(1,21)} = 0.861$, $p = 0.364$).

However, when we combine the data from the three experiments, the pH ($F_{(1,87)} = 26.443$, $p < 0.001$), pCO_2 ($F_{(1,87)} = 14.51$, $p < 0.001$), temperature-corrected pH ($F_{(1,87)} = 7.865$, $p = 0.006$), temperature-corrected pCO_2 ($F_{(1,87)} = 5.778$, $p = 0.018$), and pO_2 ($F_{(1,87)} = 4.187$, $p = 0.044$) were all altered by hyperthermia. Whereas, glucose ($F_{(1,87)} = .509$, $p = 0.477$), hemoglobin ($F_{(1,87)} = 0.639$, $p = 0.426$), and blood pressure ($F_{(1,87)} = 0.837$, $p = 0.363$) were unaltered by hyperthermia.

3.2 Experiment 1

One rat (NORMO) died during surgery. There were no group differences in edema in the ipsilateral striatum ($F_{(1,38)} = 0.881$, $p = 0.354$), ipsilateral cortex

($F_{(1,38)} = 0.104$, $p = 0.748$), or cerebellum ($F_{(1,38)} = 0.046$, $p = 0.832$; Figure 2a) at 24 hours post-ICH, as analyzed by ANOVA.

3.3 Experiment 2

One rat died during surgery and one rat was excluded due to processing error (HYPER group). These exclusions reduced group sizes (HYPER = 13; NORMO = 15) for edema measurement. Group sizes were reduced further for the horizontal ladder test (NORMO = 14, HYPER= 12), and cylinder test (NORMO = 10, HYPER = 8) due to failure of some rats to reach criterion.

Hyperthermia reduced edema in the ipsilateral striatum ($F_{(1,26)} = 4.43$, $p = 0.045$; Figure 2b), but it had no effect on edema in the ipsilateral cortex ($F_{(1,26)} = 3.381$, $p = 0.077$) or cerebellum ($F_{(1,26)} = 1.547$, $p = 0.225$) at 72 hours post-ICH.

Mann-Whitney U tests revealed that hyperthermia reduced neurologic deficits at day 2 ($U_{(27)} = -2.05$, $p = 0.040$) and day 3 ($U_{(27)} = -2.776$, $p = 0.006$; Figure 3a), but not at day 1 ($U_{(27)} = -1.913$, $p = 0.056$). Baseline testing did not reveal any neurological differences between groups ($U_{(27)} = -0.961$, $p = 0.336$). The scores and inter-quartile ranges are presented in Table 3.

Using repeated measures ANOVA, we found a significant Time × Group interaction in contralateral forelimb error rate ($F_{(1,24)} = 11.268$, $p = 0.003$), with the hyperthermia group making less errors on day 3 post-ICH than normothermic

rats ($p = 0.002$; Figure 3b). There were no baseline differences between groups ($p = 0.187$).

Repeated measures ANOVA revealed that main effect of Time, where each group displayed significant forelimb asymmetry on day 3 post-ICH ($F_{(1,16)} = 13.472$, $p = 0.002$; Figure 3c) relative to baseline. However, we did not find a Time × Group interaction ($F_{(1,16)} = 4.267$, $p = 0.055$) or Group main effect ($F_{(1,16)} = 2.189$, $p = 0.158$).

3.4 Experiment 3

Three rats were excluded due to unexpected mortality (HYPER = 2, NORMO = 1) either during surgery or in the days following ICH. Additional rats were excluded from the ladder test (1 per group), cylinder test (NORMO = 2), and single pellet test (2 per group) due to failure to reach criterion.

We found a significant Time × Group interaction ($F_{(3,57)} = 3.938$, $p = 0.013$; Figure 4a) for the contralateral forelimb in the ladder test. Rats in the hyperthermia group made fewer contralateral forelimb errors on day 3 post-ICH than the rats in normothermia group ($p = 0.045$). Error rate did not differ on the other testing days ($p \geq 0.129$).

Neurological deficit score did not differ between groups at baseline ($U_{(21)} = -1.512$, $p = 0.131$), day 3 ($U_{(21)} = -1.280$, $p = 0.200$), day 11 ($U_{(21)} = -1.550$, $p = 0.121$), or day 32 post-ICH ($U_{(21)} = -1.008$, $p = 0.313$; Figure 4b). The scores and inter-quartile ranges are presented in Table 4.

Both groups displayed time-dependent deficits in the corner turn test ($F_{(3,54)} = 8.161$, $p < 0.001$; Figure 4c). Rats were significantly impaired on day 3 ($p = 0.003$) and day 11 post-ICH ($p = 0.010$) relative to baseline. Rats were no longer impaired on day 32 post-ICH ($p = 0.324$), and turning preference did not differ between post-ICH time points ($p \geq 0.221$). There was no Time × Group interaction ($F_{(3,54)} = 1.351$, $p = 0.268$) or Group main effect ($F_{(1,18)} = 0.120$, $p = 0.733$).

There were also time-dependent deficits in the cylinder test ($F_{(3,57)} = 7.844$, $p < 0.001$; Figure 4d). Rats were impaired on days 3 ($p = 0.006$) and 11 post-ICH ($p = 0.009$) relative to baseline. Rats were not impaired on day 32 post-ICH ($p = 0.096$), and forelimb asymmetry did not differ between the post-ICH testing days ($p \geq 0.717$). There was no Time × Group interaction ($F_{(3,57)} = 1.348$, $p = 0.268$) or Group main effect ($F_{(1,19)} = 0.307$, $p = 0.586$).

Rats from both groups had time-dependent reaching impairments in the single pellet task ($F_{(2,32)} = 15.436$, $p < 0.001$; Figure 4e). Rats were significantly impaired during days 7 – 10 ($p = 0.004$) and days 28 – 31 post-ICH ($p = 0.026$) compared to baseline. Skilled reaching did not differ between post-ICH testing sessions ($p = 0.773$). There was no Time × Group interaction ($F_{(2,32)} = 2.037$, $p = 0.147$) or Group main effect ($F_{(1,16)} = 0.625$, $p = 0.441$).

Hyperthermia did not alter lesion volume at 32 days post-ICH ($F_{(1,21)} = 0.008$, $p = 0.929$; Figure 5).

3.5 Correlations

Correlations between physiological variables and multiple outcome measures (edema and lesion volume) are presented in Table 3. The pH ($r_{(25)}$ = 0.343, p = 0.035), temperature-corrected pH ($r_{(37)}$ = 0.333, p = 0.041), and glucose ($r_{(37)}$ = 0.594, p < 0.001) were correlated with edema at 24 hours. Hemoglobin ($r_{(25)}$ = -0.477, p = 0.012) was the only physiological variable that correlated with edema at 72 hours. No physiological variables were correlated with lesion volume.

We also correlated the behaviour scores with the outcome measures. In experiment 2, BWC at 72 hours post-ICH was significantly correlated with day 1 ($r_{(27)}$ = 0.568, p = 0.001), day 2 ($r_{(27)}$ = 0.648, p < 0.001), and day 3 post- ICH ($r_{(27)}$ = 0.617, p < 0.001) of NDS testing. In addition, contralateral forelimb error rate ($r_{(24)}$ = 0.608, p = 0.001) was significantly correlated with brain-water content. Brain-water content did not correlate with any of the baseline measurements, or with day 3 post-ICH forelimb asymmetry (Table 4).

In experiment 3, lesion volume at 32 days post-ICH significantly correlated with turning preference on day 11($r_{(18)}$ = 0.507, p = 0.022) and day 32 post-ICH ($r_{(18)}$ = 0.500, p = 0.025), and NDS on day 32 post-ICH (r = 0.424, p = 0.044). Lesion volume did not significantly correlate with any of the other behavioural measurements (Table 5).

3.6 Combining Behavioural Data

In both experiment 2 and 3 we tested rats on NDS, the cylinder test, and the ladder test at baseline and on day 3 post-ICH. Hyperthermia reduced deficits in the ladder test in both experiments. Hyperthermia had no effect on forelimb asymmetry in either experiment. Hyperthermia reduced neurological deficits in experiment 1 but failed to reduce deficits in experiment 2. Therefore, we combined the data for the two experiments to better assess whether hyperthermia affected these behaviours. We found a Time × Group interaction ($F_{(1,45)} = 14.050$, $p = 0.001$) in contralateral forelimb error rate, where the hyperthermia group made less errors than the normothermia group on day 3 post-ICH ($p < 0.001$). Baseline error rate did not differ between groups ($p = 0.183$). Hyperthermia also reduced neurological deficits on day 3 post-ICH ($U_{(50)} = -2.913$, $p = 0.004$). Neurological deficits did not differ between groups at baseline ($U_{(50)} = -1.458$, $p = 0.145$). There was a Time main effect ($F_{(1,37)} = 24.159$, $p = 0.001$) in forelimb asymmetry, indicating that both groups were impaired on day 3 post-ICH relative to baseline. There was no Time × Group interaction ($F_{(1,37)} = 24.159$, $p = 0.001$) or Group main effect ($F_{(1,37)} = 0.103$, $p = 0.750$), however.

4. Discussion

In this thesis, we assessed whether hyperthermia has a detrimental effect on functional and histological outcome in a rat model of ICH. While there is significant evidence that fever is detrimental for ischemic patients, there is no conclusive evidence to indicate that fever is detrimental for ICH patients. There have been no previous animal studies assessing the impact of fever on ICH. In addition, there has only been one previous study assessing the impact of hyperthermia on ICH in an experimental rat model (bacterial collagenase). This thesis examines the impact of hyperthermia using the whole blood model of ICH, which had not been previously studied.

4.1 Impact of Hyperthermia on Edema Formation

Edema is a significant consequence of ICH, and is a major contributor to mortality following ICH. Therefore, it is important to determine which consequences of ICH will worsen edema. None of our data indicate that hyperthermia significantly increases edema. In fact, we found that hyperthermia reduced edema in the striatum at 72 hours, which is surprising considering that hypothermia reduces edema after ICH (Fingas, Clark, & Colbourne, 2007) and many mechanisms of injury in ischemia are aggravated by hyperthermia (Ginsberg & Busto, 1998) and attenuated by hypothermia (Polderman, 2009). It is important to point out that hyperthermia reduced edema at only one time point. At 24 hours, there was no statistical difference, and there was actually a small trend towards hyperthermia increasing edema at 72 hours post-ICH. Our data indicates

that hyperthermia altered the time course of edema formation in some way. However, it is unclear exactly how edema changes the time course. A larger study with more time points would be needed to determine exactly how hyperthermia alters the time course of edema in ICH. It is possible that hyperthermia speeds up the edema process, where edema rises to the same level but is initiated and resolves much quicker than normal. It is also possible that edema occurs in the same type of pattern but is consistently lower following hyperthermia. A third possibility is that hyperthermia prevents the rise in edema following ICH. Since containing edema is extremely important clinically, it is essential to determine the time course of edema in patients that develop fever. Our data does not support the idea that hyperthermia aggravates edema over the course of the normal period where edema occurs (Fingas, Clark, & Colbourne, 2007). The time course of edema may be different between patients that develop fever and patients that develop central hyperthermia. However, discovering the time course of edema in hyperthermic animals may give us an idea of the time course of edema in patients that develop a fever after ICH.

4.2 Hyperthermia Transiently Improves Functional Outcome

Hyperthermia reduced behavioural deficits in some of the tasks, but the deficits did not last past 3 days post-ICH. These data are supported by a previous study showing that hyperthermia did not affect outcome in a bacterial collagenase model of ICH (MacLellan & Colbourne, 2005). Surprisingly, we found that hyperthermia transiently improved general neurological impairment (NDS) and

walking ability (ladder test). We assessed general impairment and walking ability at day 3 post-ICH in experiments 2 and 3. In both experiments, hyperthermia reduced contralateral forelimb errors. In experiment 2, neurological deficits were reduced at day 3 post-ICH. In experiment 3, however, there was no significant difference at day 3. When data from the two experiments is combined, hyperthermia reduces errors for the contralateral forelimb in the ladder test and reduces neurological deficit. In contrast, there was no difference in forelimb asymmetry over the two experiments. In experiment 3, none of the tests revealed group differences from day 7 to day 32 post-ICH. Overall, there may be a small transient behavioural improvement from hyperthermia, but this improvement quickly disappears. One explanation for this result may be that the difference in edema between groups at day 3 is accounting for the difference in behavioural outcome at the same time point. If this is true, it is likely that edema is similar between groups at the later time points.

Hyperthermia seems to alter one or more processes that impacts outcome in the first few days of ICH, but does not have long-term consequences. One such process is edema formation, which is reduced at day 3 post-ICH in the hyperthermia group. This could explain the reduction of deficits at that particular time point. This is the simplest explanation, given the data that we collected, but it is certainly possible that hyperthermia alters some other process that we did not measure. One of the processes that would be most likely to be affected by hyperthermia is inflammation. MacLellan & Colbourne (2005) used Perl's Prussian Blue to stain for ferric iron and Leder's stain to assess chloracetate

esterase activity at 2 and 4 days post-ICH. These were used as markers of components of the inflammatory reaction after ICH, but were not affected by hyperthermia. Nevertheless, further work such as measuring cytokines levels, is needed to rule out effects of inflammation.

4.3 Hyperthermia Does Not Alter Long-Term Outcome

None of our assessments indicated a worsening of outcome due to hyperthermia (39 ºC). This is in agreement with MacLellan & Colbourne (2005) who found that several hyperthermia protocols failed to worsen outcome on any of the measures they assessed. Together, these two studies indicate that hyperthermia does not worsen skilled reaching, forelimb asymmetry, walking ability, general neurological impairment, turning preference, or forepaw inhibition. In addition, lesion assessment, neutrophil and macrophage accumulation, and hematoma volume assessment are unaffected by hyperthermia. No other experimental animal studies have investigated the impact of hyperthermia following ICH. Since there have been two comprehensive studies done using two different ICH models, and there is no evidence that experimental hyperthermia worsens outcome, it is unlikely that hyperthermia is detrimental in animal models of ICH. This is somewhat surprising considering the vast evidence that hyperthermia worsens outcome in experimental ischemia models (Busto et al., 1987; Hickey et al., 2003; Morikawa et al., 1992). However, there is not overwhelming evidence that fever causes greater impairment among ICH patients (Morgenstern et al., 2009). The conflict between ICH and ischemia studies

suggests that the two types of stroke have different sensitivities to temperature-manipulation.

4.4 Hyperthermia Alters Physiological Variables

Hyperthermia alters pH and pCO_2 following intracerebral hemorrhage. When we combine the data for the experiments, hyperthermia alters both the uncorrected and temperature-corrected values for pH and pCO_2 as well as the pO_2. The pH does correlate with edema at 24 hours post-ICH but does not correlate with edema at 72 hours or with lesion volume. The pCO_2 did not correlate with any of the outcome measures. In addition, hyperthermia did not cause values to fluctuate widely from the normal range (pH = 7.35 to 7.45, pCO_2 = 35 to 45). It is possible that pH affects early outcome (day 1 post-ICH edema), however, it does not seem to influence outcome at later time points (day 3 and day 32 post-ICH). Therefore, the early effect hyperthermia has on outcome may be due to its effect on these variables. In contrast, the influence of these variables may be explained by the fact that they are correlated with temperature and temperature has an independent effect on early outcome. Determining whether these variables are more important than an independent effect of temperature is a very difficult task. It is most likely that the effect on physiological variables contributes to the impact on short-term functional outcome but does not fully explain it. In any event, it seems apparent that neither temperature nor minor alterations in other physiological variables (pH, pCO_2, pO_2, glucose, hemoglobin, and MABP) have a long-term effect on outcome in this model of ICH. It may be

useful to monitor physiological variables in the first few days after intracerebral hemorrhage, but it does not seem like it would have a long-term impact.

4.5 Differences between Intracerebral Hemorrhage and Ischemia

Contrary to our ICH findings, the research on hyperthermia after experimental ischemia suggests that hyperthermia worsens injury (Busto et al., 1987; Hickey et al., 2003; Kim et al., 1996). In addition, hypothermia treatments in experimental ICH studies have been far less successful than in ischemia studies (MacLellan et al., 2009). The mechanisms of how hypothermia protects the brain after ischemia have been reviewed in several places (Colbourne, Sutherland, & Corbett, 1997; Zhao, Steinberg, & Sapolsky, 2007). The difference in the pathophysiology of the two conditions may explain the difference in sensitivity to temperature.

Ischemia and ICH share several characteristics. Both cause immediate destruction of tissue, and both are affected by the secondary consequences of injury due to processes such as free radical production, excitatory amino acid release, BBB breakdown, necrosis, and apoptosis. The blood degradation products also contribute to injury after ICH. One important aspect of ischemia that does not appear to exist in ICH is the existence of a penumbra surrounding the area of major tissue destruction. This area contains cells that do not die immediately, but are susceptible to death. When cerebral blood flow (CBF) falls below approximately 40 to 50 % of baseline, the brain tissue is at risk of dying (Astrup, Siesjö, & Symon, 1977). Once blood flow drops below this level, several

processes begin including acidosis, edema, potassium and calcium influx, and protein synthesis inhibition. These processes compromise the energy level of the cells, making the area vulnerable. If these processes continue, electrical and membrane failure will occur, which will result in cell death. However, the tissue in this area is salvageable if treatment to combat these processes is initiated quickly. This area is likely the most impacted by hypothermia treatment. Hypothermia slows down the process of cell death, allowing normal function to return once normal blood flow is restored. While hypothermia acts to salvage the penumbra, hyperthermia speeds up the process of destroying tissue in the penumbra. Hyperthermia does this by enhancing neurotransmitter release, free radical production, BBB opening, and ischemic depolarizations (Axelrod & Diringer, 2007; Ginsberg & Busto, 1998).

While there has been much debate surrounding the issue of whether or not there is a similar penumbral zone surrounding the ICH (Herweh et al., 2007; Kidwell et al., 2001), most have concluded that there is not a penumbral zone surrounding the hematoma (Schellinger et al., 2003; Zazulia et al., 2001). This provides a critical difference between ICH and ischemia. The lack of a penumbral zone in ICH may explain why outcome seems to be resistant to temperature change in the early hours after ICH.

4.6 Use of Anti-Pyretics in the Clinic

Despite differences between ischemia and ICH, some treatments for ICH are conducted based on evidence from ischemia trials. Even though, there are no

specific recommendations related to ICH and anti-pyretics, many physicians treat ICH patients with anti-pyretics (Morgenstern et al., 2010). However, clinical studies relating increased temperature with worsening of outcome after ICH have been inconclusive (Leira et al., 2004; Wang et al., 2000).

Based on the inconclusive data on fever and outcome in ICH patients and failure of hyperthermia to worsen outcome in experimental models of ICH, routinely giving anti-pyretics to ICH patients regardless of the situation may not be a very effective strategy. Even in ischemic stroke, merely using acetaminophen to control temperature in clinical trials as a treatment have been unsuccessful (den Hertog et al., 2009). However, the study group does suggest that it may be useful for patients that have a body temperature of 37 ºC to 39 ºC upon admission. Likewise, it may be important to identify specific circumstances that could be problematic. For instance, hyperthermia of infectious origin correlates with higher body temperature and worse mortality for ischemic patients (Wang et al., 2009).

Overall, there does not seem to be much evidence to support routine use of anti-pyretics for ICH patients. It is also important to consider that an increase in temperature may not be harmful. Fever helps the body fight against infection, and lowering body temperature can sometimes delay recovery from infection (Aiyagari and Diringer, 2007; Hasday and Garrison, 2000). Currently there have been no large RCTs evaluating the usage of anti-pyretics for ICH patients. Therefore, it is unknown whether or not anti-pyretics are helpful for ICH patients.

This needs to be done in order to establish whether ICH patients should be given anti-pyretics.

4.7 Future Research

There are several ways to examine the relationship between hyperthermia and outcome following intracerebral hemorrhage. One of the most interesting findings of this thesis was the reduction in edema at day 3 in the hyperthermia group. Determining exactly how hyperthermia alters the time course of edema would help the understanding of how hyperthermia affects the pathophysiology of ICH. It is most likely that hyperthermia alters edema surrounding the first day of ICH. The rationale for this would be that hyperthermia increases the speed of edema formation, as well as edema resolution.

In our lab, we are able to use a method to measure ICP continuously in free moving rats (Silasi, MacLellan, & Colbourne, 2009). This allows us to determine when ICP begins to rise after induction of stroke and how long it remains elevated. Since ICP is attributed to an increase in edema after ICH, this can provide an indirect measure of edema. Therefore, a future study could involve monitoring ICP after ICH in rats maintained under hyperthermic or normothermic conditions. This would give us an idea of progression of edema in each of these conditions.

Both of the experimental studies on outcome after ICH have used similar parameters as has been used in experimental ischemia studies. These studies

indicate that ICH is less sensitive to hyperthermia than ischemia. However, that does not necessarily mean that hyperthermia does not affect outcome under any circumstances. Both the timing of hyperthermia (how long after ICH is hyperthermia initiated) and the degree of hyperthermia (how much is temperature raised) could affect the degree to which hyperthermia alters outcome after ICH.

The majority of the experimental ischemia studies with hyperthermia use 39ºC (Dietrich et al., 1990; Morimoto et al., 1997; Noor, Wang, & Shuaib, 2003), although some studies have used 40ºC (Hickey et al., 2003; Kim et al., 1996). The experimental studies investigating the effect of hyperthermia on outcome after ischemia indicate that 40ºC hyperthermia causes more injury and impairment than 39ºC hyperthermia (Kim et al., 1996). Due to this finding, MacLellan & Colbourne (2005) included a group that was heated to 40ºC for 3 hours. This group of rats was no more impaired than the other hyperthermia groups, or the normothermia group. It is possible that increasing temperature to a higher degree (i.e. 42ºC) for a specific time (3 hours or longer) would influence outcome. If extremely high hyperthermia does worsen outcome following ICH, clinicians could monitor temperature of patients and normalize temperature when it gets very high.

The duration of the hyperthermia could also be investigated. MacLellan & Colbourne (2005) found that maintaining hyperthermia (38.5 ºC) for 24 hours did not affect outcome. However, some patients develop fever for many days (Schwarz et al., 2000). Therefore, studies could be conducted where hyperthermia

is initiated shortly after ICH and is maintained for several days. If hyperthermia of long duration affects outcome, clinicians could start to normalize body temperature for patients that develop fever for a long duration of time.

Finally, a future direction could be to initiate a controlled fever in animals after ICH by injecting them with lipopolysaccharide (LPS). In the current study, we chose to induce hyperthermia instead of fever for several reasons. Primarily, we were interested in maintaining consistency between our study and the previous ischemia studies. This also allowed us to isolate the rise in body temperature as a factor in worsening of outcome. In addition, LPS injection typically causes a rather modest temperature increase (Langdon, MacLellan, & Corbett, 2010). So in order to match the temperature increase in previous studies (39 to 40ºC), we needed to use a more proficient heating method.

Simply raising temperature does not seem to have a negative effect on outcome in rat models of ICH. However, it is possible that other aspects of fever affect outcome in the clinical population. Therefore, it may be useful to conduct some studies where rats are injected with LPS after ICH to identify if fever affects outcome in animal models of ICH.

4.8 Scope of Thesis

This thesis suggests that hyperthermia protocols similar to those used in experimental ischemia studies are not effective at worsening injury following ICH. This indicates that ICH is less sensitive to hyperthermia than ischemia. It

also appears that ICH is less sensitive to hypothermia than ischemia (MacLellan et al., 2009), suggesting that ICH is not as temperature-sensitive as ischemia. It does not necessarily mean that there are no parameters for which hyperthermia could alter functional outcome, but outcome may only be affected by very high temperatures or long durations of hyperthermia.

Our research does not support the prevailing hypothesis that since hyperthermia is detrimental after ischemia that it is also detrimental after ICH. It does not appear that moderate hyperthermia (39 ºC) has a substantial impact on outcome after ICH. However, fever does differ from hyperthermia in many ways. Therefore, it is possible that fever is detrimental to ICH patients, whereas hyperthermia is not. It is important to determine whether fever of infectious origin is detrimental to ICH patients. Unfortunately, in many cases giving anti-pyretics does not improve outcome, so patients may not be helped by this treatment. Clinical studies should look at which types of patients, if any, are helped by anti-pyretic treatment.

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Table 1: Criteria For Neurological Deficit Scale

Behaviour	**Score**	**Impairment**
Beam Walking	0	Rat crosses beam without slipping
	1	Rat crosses and < half steps are slips
	2	Rat crosses and > half steps are slips; Rat does not cross; Rat falls in > 10 s
	3	Rat falls in 10 s or less
Spontaneous Circling	0	No circling
	1	Some ipsilateral circling (towards lesioned side), mostly in first few minutes
	2	Mostly ipsilateral circling; not much circling in the contralateral direction; usually wider circles
	3	Continuous ipsilateral circling; usually tight circles; very little or no movement in the contralateral movement
Bilateral Forepaw Grasp	0	Grasps completely with both paws (all digits wrapped around bar)
	1	Grasps normally with ipsilateral forepaw, uses some contralateral digits
	2	Grasps normally with ipsilateral forepaw, uses contralateral wrist or arm
	3	Cannot grasp with either paw
Hindlimb Replacement	0	Immediate replacement
	1	Replacement in 0 to 1 minute
	2	Replacement in 1 to 2 minutes
	3	Replacement in > 2 minutes
Forelimb Flexion	0	Both forelimbs are extended when lifted up
	1	Shoulder adduction of contralateral forelimb (arm moves toward midline)
	2	Shoulder adduction with forelimb flexion (arm bends upwards)
Forelimb Placing	0	10/10 correct placements
	1	6 to 9 correct placements
	2	1 to 5 correct placements
	3	0 correct placements

Table 2: **Physiological and Blood Pressure Measurements**

	Experiment 1		Experiment 2		Experiment 3	
	HYPER	NORMO	HYPER	NORMO	HYPER	NORMO
pH	7.428 ± 0.033*	7.365 ± 0.076	7.453 ± 0.024*	7.411 ± 0.024	7.424 ± 0.011*	7.380 ± 0.010
pCO_2	34.75 ± 7.3*	44.29 ± 15.18	34.43 ± 3.18*	39.51 ± 3.71	31.91 ± 1.65*	37.58 ± 1.86
pO_2	129.97 ± 10.03	136.00 ± 13.61	126.25 ± 7.65*	132.06 ± 4.79	131.31 ± 5.47	133.29 ± 2.77
Glucose	8.88 ± 1.83	9.15 ± 1.41	9.16 ± 1.34	10.04 ± 1.59	8.95 ± 0.58	8.62 ± 1.08
ctHb	12.90 ± 1.00*	13.46 ± 0.59	14.38 ± 0.91	14.29 ± 0.87	13.80 ± 0.25	13.56 ± 0.44
pH (Corrected)	7.402 ± 0.031*	7.362 ± 0.074	7.424 ± 0.024	7.408 ± 0.022	7.395 ± 0.010	7.377 ± 0.010
pCO_2 (Corrected)	37.58 ± 7.83	44.33 ± 14.95	37.77 ± 3.06	39.94 ± 3.55	34.62 ± 1.71	37.91 ± 1.84
MABP	86.68 ± 10.69	88.47 ± 17.78	93.89 ± 8.08	87.40 ± 12.01	89.86 ± 10.44	84.31 ± 17.13

Table 3: **NDS Scores For Experiment 2**

	HYPER				NORMO			
	Baseline	Day 1	Day 2	Day 3	Baseline	Day 1	Day 2	Day 3
Median	0	3	1.5*	1*	0	5	4	3
25th Percentile	0	1	0	0	0	3	2	2
75th Percentile	1	5	4	2	1	8	5	5

Table 4: NDS Scores For Experiment 3

	HYPER				NORMO			
	Baseline	Day 3	Day 11	Day 32	Baseline	Day 3	Day 11	Day 32
Median	0	2	1	1	0	3.5	2	0
25th Percentile	0	1	0	0	0	2	1	0
75th Percentile	0	4	2	2	0	7.25	3.75	1.75

Table 5: **Correlations between Physiological Variables and Edema**

	Correlation with 24 hr edema		Correlation with 72 hr edema		Correlation with lesion volume	
	r-value	p-value	r-value	p-value	r-value	p-value
pH	0.343*	0.035	-0.287	0.146	0.096	0.662
pCO_2	-0.022	0.896	0.269	0.175	-0.115	0.603
pO_2	0.100	0.551	0.034	0.866	0.173	0.430
Glucose	0.594*	< 0.001	0.033	0.870	-0.020	0.928
ctHb	0.063	0.707	-0.477*	0.012	-0.156	0.478
pH (Corrected)	0.333*	0.041	-0.144	0.473	0.036	0.871
pCO_2 (Corrected)	0.015	0.929	0.142	0.479	-0.087	0.691
MABP	0.062	0.710	-0.276	0.164	0.050	0.821

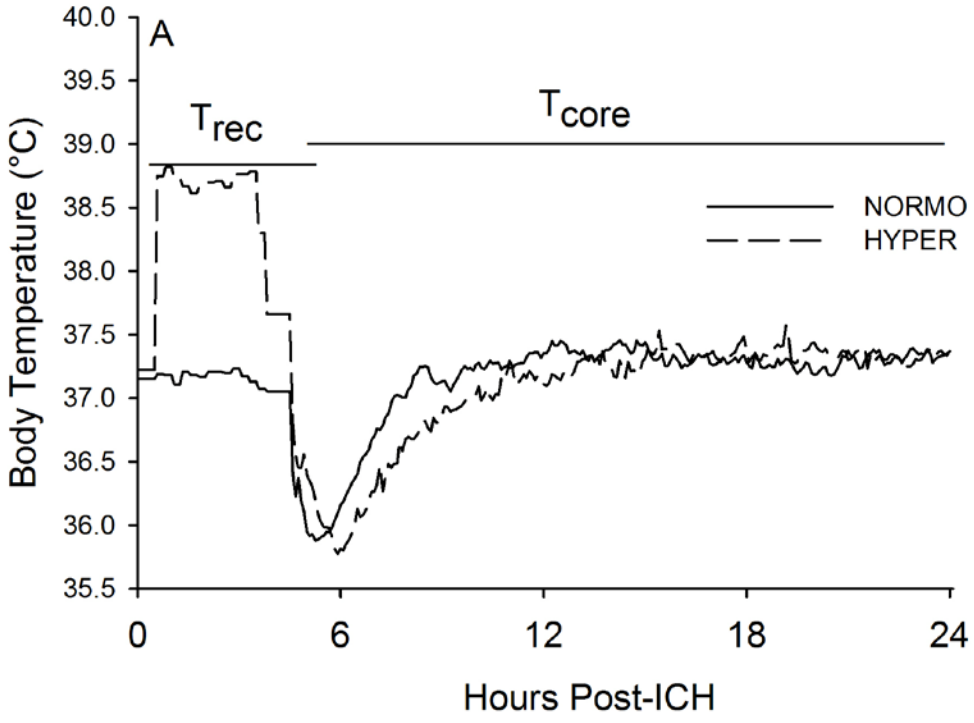
Table 6: Correlations between Behaviour and Edema in Experiment 2

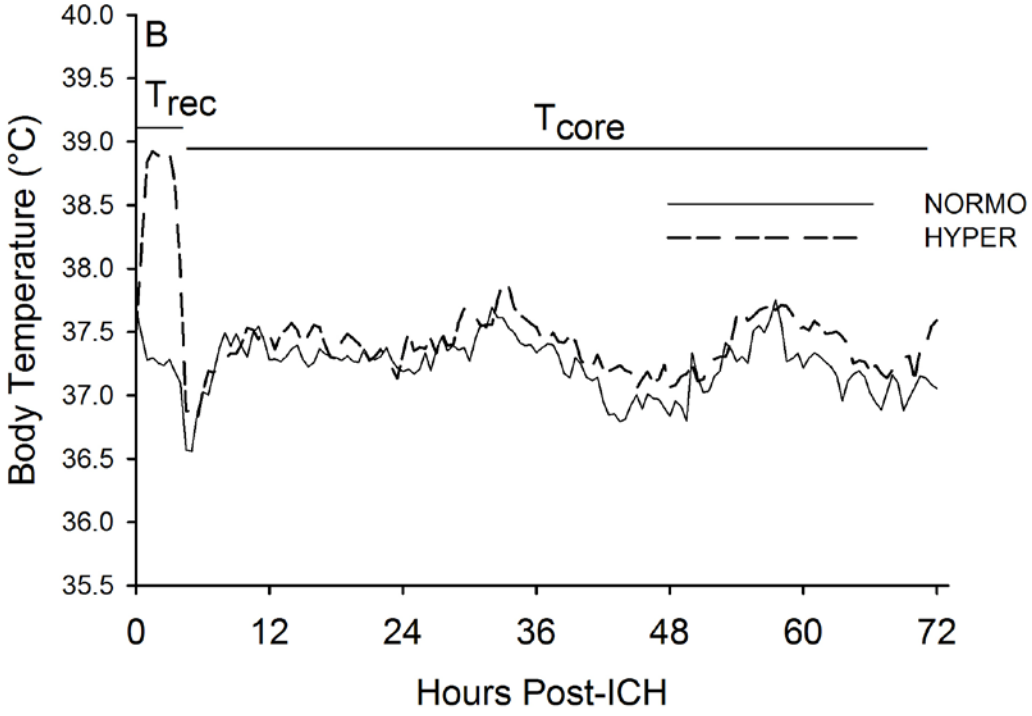
	R Value	P Value
Baseline NDS	-0.019	0.922
Day 1 NDS	0.568*	0.001
Day 2 NDS	0.648*	< 0.001
Day 3 NDS	0.617*	< 0.001
Baseline Forelimb Asymmetry	0.004	0.986
Day 3 Forelimb Asymmetry	-0.460	0.055
Baseline Contralateral Forelimb Error Rate	0.104	0.612
Day 3 Contralateral Forelimb Error Rate	0.608*	0.001

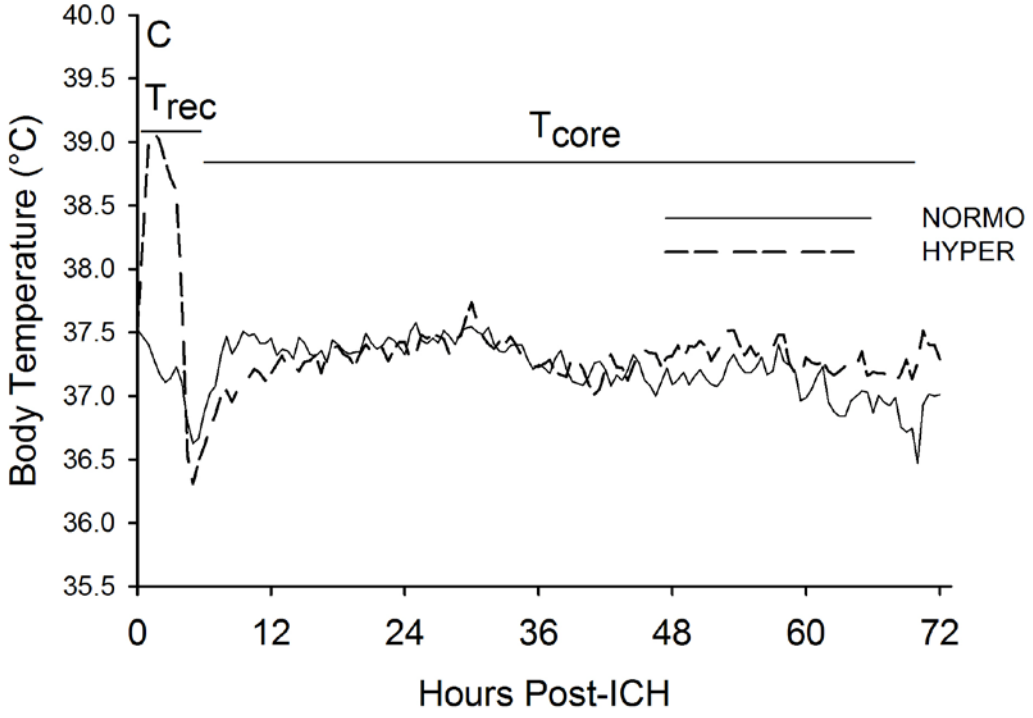
Table 7: Correlat**ions between Behaviour and Lesion Volume in Experiment 3**

	R Value	P Value
Baseline NDS	0.093	0.673
Day 3 NDS	0.238	0.274
Day 11 NDS	0.135	0.540
Day 32 NDS	0.424*	0.044
Baseline Forelimb Asymmetry	0.023	0.920
Day 3 Forelimb Asymmetry	0.146	0.527
Day 11 Forelimb Asymmetry	-0.378	0.091
Day 32 Forelimb Asymmetry	-0.206	0.371
Baseline Contralateral Forelimb Error Rate	N/A	N/A
Day 3 Contralateral Forelimb Error Rate	0.269	0.238
Day 11 Contralateral Forelimb Error Rate	0.173	0.454
Day 32 Contralateral Forelimb Error Rate	0.405	0.068
Baseline Turning Preference	-0.138	0.561
Day 3 Turning Preference	0.427	0.060
Day 11 Turning Preference	0.507*	0.022
Day 32 Turning Preference	0.500*	0.025
Baseline Reaching Success	0.117	0.633
Reaching Success on Days 7 – 10	-0.396	0.104
Reaching Success on Days 28 – 31	-0.487	0.04

Figure 1


A
Trec
Tcore
NORMO
HYPER
Body Temperature (°C)
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
0
6
12
18
24
Hours Post-ICH


B
Trec
Tcore
NORMO
HYPER
Body Temperature (°C)
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
0
12
24
36
48
60
72
Hours Post-ICH


40.0
39.5
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38.5
38.0
37.5
37.0
36.5
36.0
35.5
Body Temperature (°C)
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Trec
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NORMO
HYPER
0
12
24
36
48
60
72
Hours Post-ICH

Figure 2

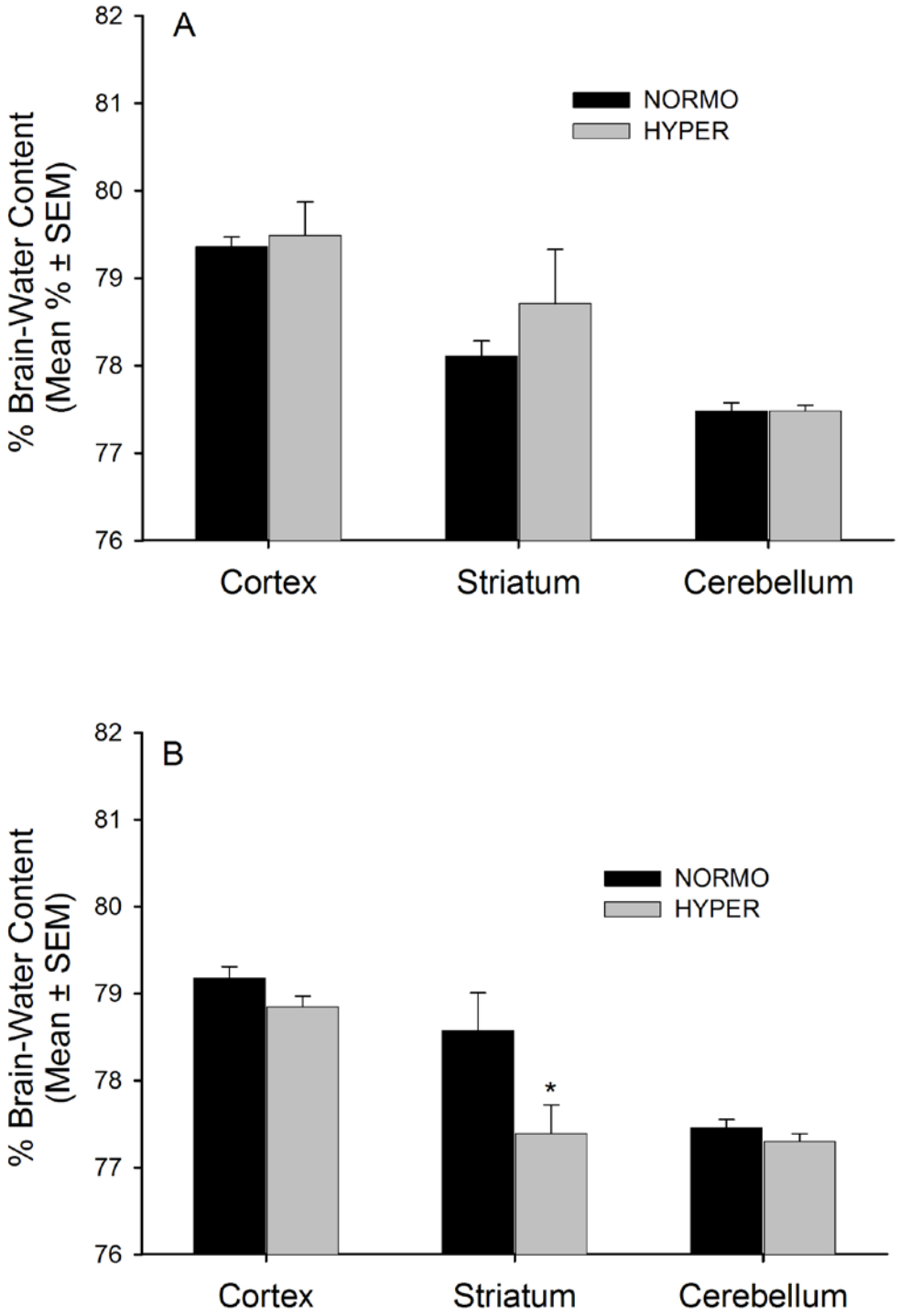
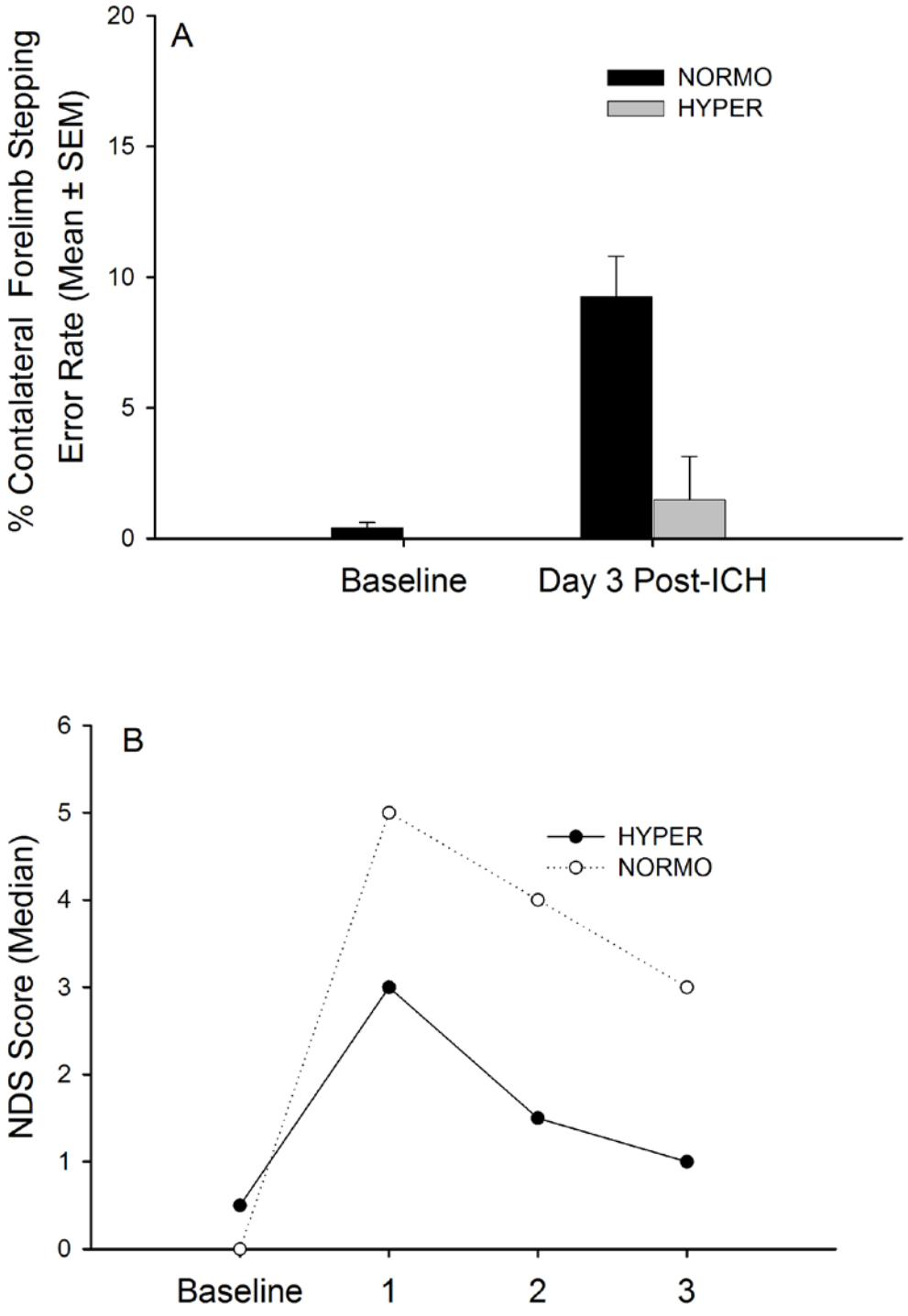

A
82
81
80
79
78
77
76
% Brain-Water Content
(Mean % ± SEM)
NORMO
HYPER
Cortex
Striatum
Cerebellum
B
82
81
80
79
78
77
76
% Brain-Water Content
(Mean ± SEM)
NORMO
HYPER
*
Cortex
Striatum
Cerebellum

Figure 3


A
% Contalateral Forelimb Stepping
Error Rate (Mean ± SEM)
20
15
10
5
0
NORMO
HYPER
Baseline
Day 3 Post-ICH
B
NDS Score (Median)
6
5
4
3
2
1
0
HYPER
NORMO
Baseline
1
2
3

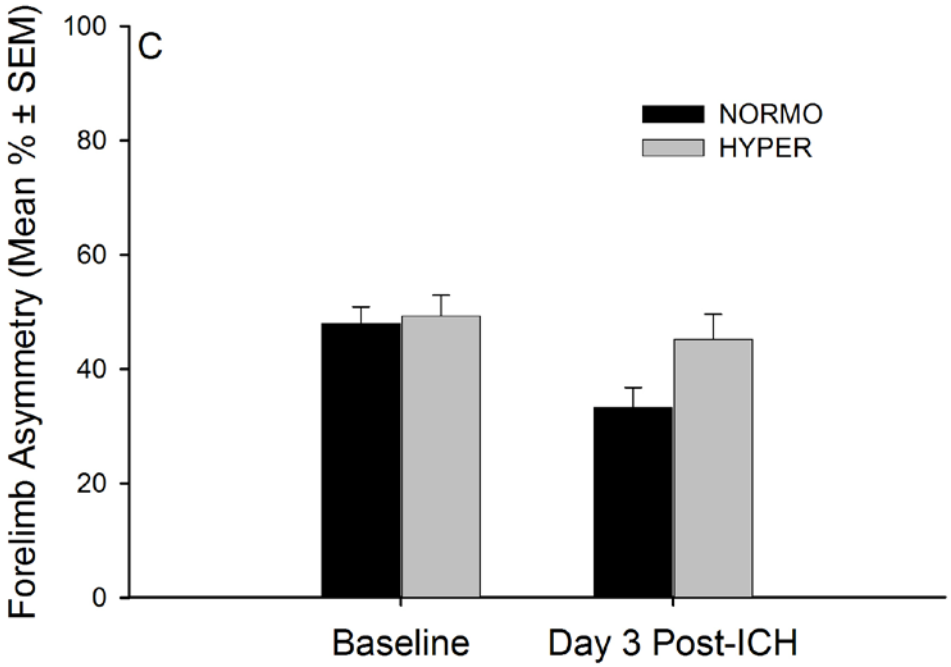
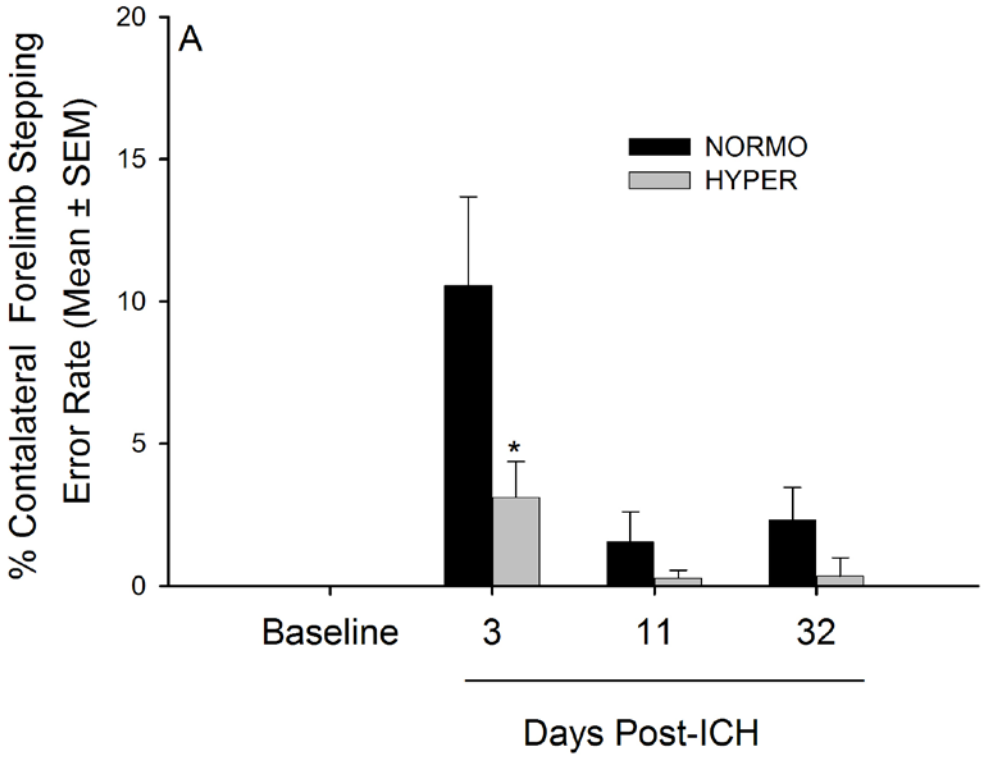
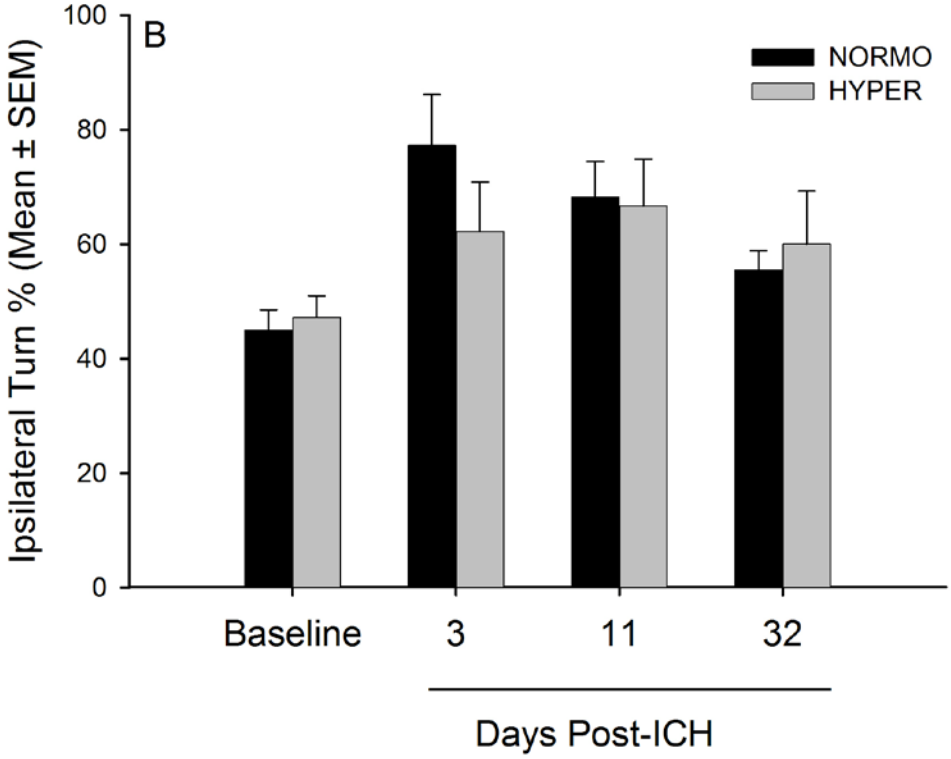
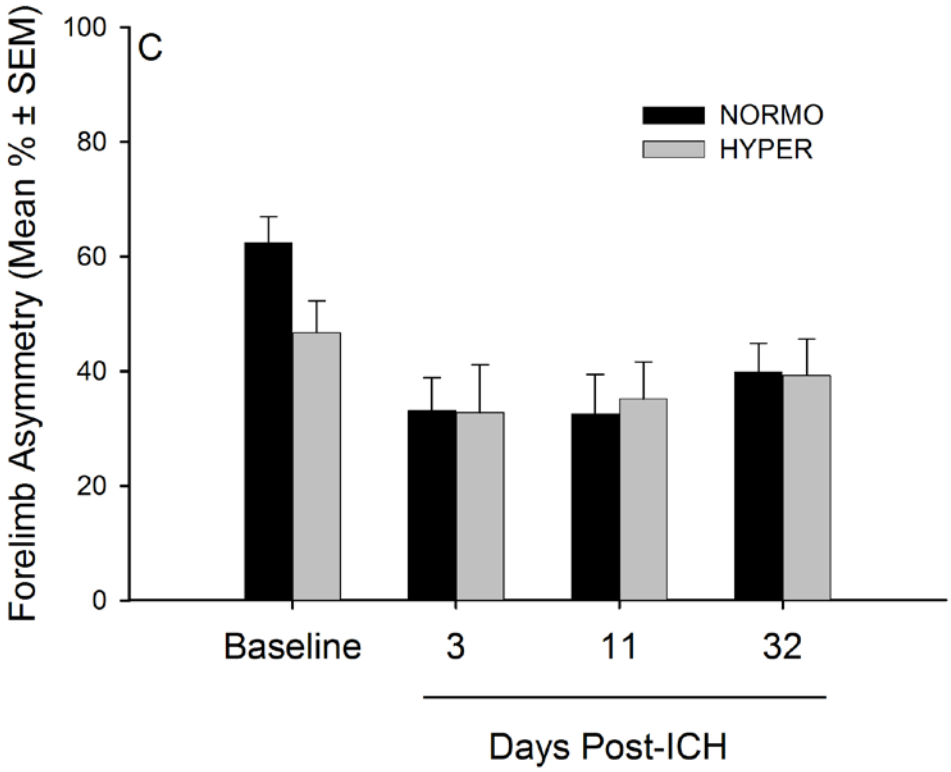
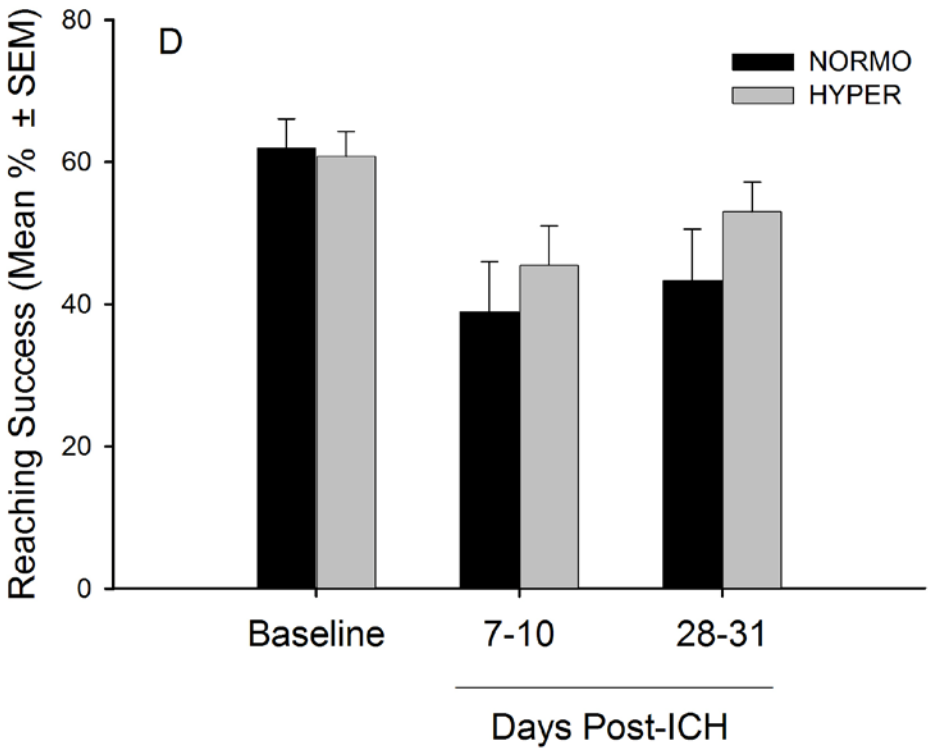

C
Forelimb Asymmetry (Mean % ± SEM)
100
80
60
40
20
0
NORMO
HYPER
Baseline
Day 3 Post-ICH

Figure 4


A
% Contalateral Forelimb Stepping
Error Rate (Mean ± SEM)
20
15
10
5
0
NORMO
HYPER
*
Baseline
3
11
32
Days Post-ICH


B
NORMO
HYPER
Ipsilateral Turn % (Mean ± SEM)
100
80
60
40
20
0
Baseline
3
11
32
Days Post-ICH


C
NORMO
HYPER
Forelimb Asymmetry (Mean % ± SEM)
100
80
60
40
20
0
Baseline
3
11
32
Days Post-ICH


D
NORMO
HYPER
Reaching Success (Mean % ± SEM)
80
60
40
20
0
Baseline
7-10
28-31
Days Post-ICH

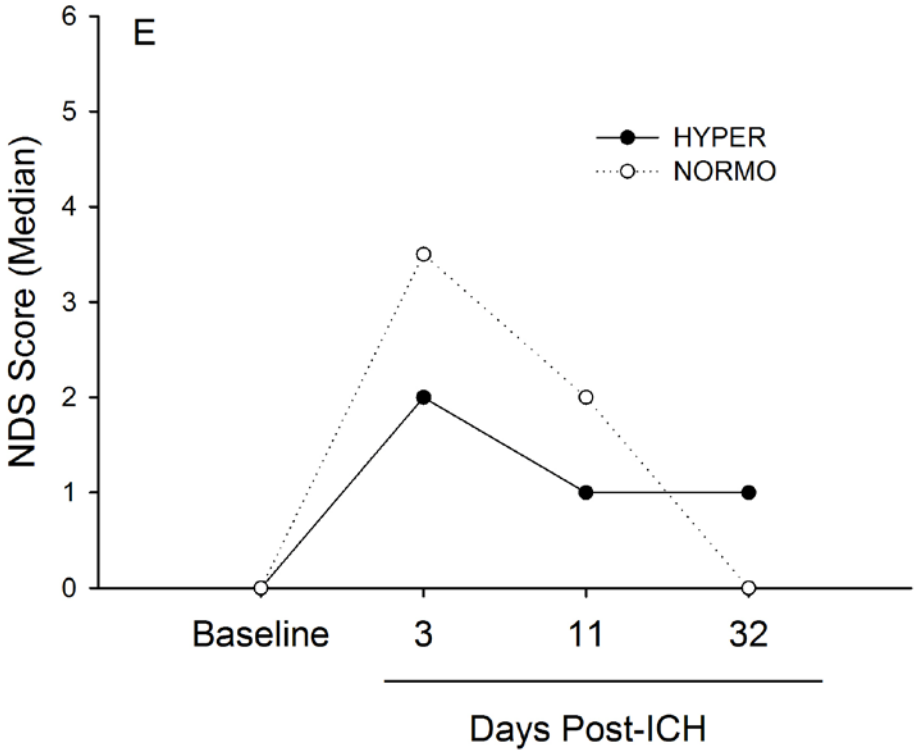

E
HYPER
NORMO
NDS Score (Median)
6
5
4
3
2
1
0
Baseline
3
11
32
Days Post-ICH

Figure 5